A PILOT STUDY OF FEASIBILITY OF ULTRA-RUSH (20-25 MINUTES) SUBLINGUAL-SWALLOW IMMUNOTHERAPY IN 679 PATIENTS (699 SESSIONS) WITH ALLERGIC RHINITIS AND/OR ASTHMA

R. E. ROSSI and G. MONASTEROLO[1]

Allergy Unit, National Health Service, Regione Piemonte, Italy and [1]Laboratorio Analisi Chimico-Cliniche e Microbiologia, Ospedale S.S.Trinità, Fossano, Italy

Received July 30, 2004 – Accepted February 24, 2005

Few studies have evaluated the occurrence of immediate adverse reactions in allergic patients after an ultra-rush regimen of different commercial allergen extracts for sublingual immunotherapy (SLIT) Methods: 679 patients took part in trials of specific ultra-rush SLIT for the treatment of IgE - mediated rhinitis and/or IgE - mediated asthma. 14 patients received two different sublingual allergen vaccines during two distinct SLIT sessions. On the whole, 699 SLIT sessions were performed. The build up ultra-rush phase involved the administration every five minutes of increasing doses of either different allergen extracts. The cumulative allergen extract solution after half an hour was several times the dose administered at the start of subcutaneous immunotherapy (range 4.7-525μg of major allergens). All patients tolerated the treatment very well. 122 (17.96%) had mild local symptoms (pruritus of the buccal cavity) that spontaneously disappeared with increasing dose. Two patients allergic to *Parietaria* had urticaria about three hours after the last sublingual *Parietaria* -extract intake. A subject allergic to Artemisia vulgaris pollen had urticaria and rhinitis two hours later than the last dose of vaccine. As reported in our previous study, no immediate severe adverse reactions were observed after that rapidly increasing doses of allergen extract were administered in a very short period to a large number of patients, showing the excellent safety profile of ultra-rush SLIT.

Allergen extracts administered via the subcutaneous route induce systemic tolerance, including the induction of blocking antibodies specific for the sensitizing allergen (1-4). Alternatively, routes for the delivery of immunotherapies, such as nasal and sublingual routes, are currently being investigated for improved patient compliance and reduced risk of systemic reactions. The role of sublingual immunotherpy (SLIT) as viable alternative to subcutaneous immunotherapy is based on well-documented experimental evidence as noted in the consensus statement of the World Health Organization (5) and in the new ARIA position paper ("Allergic Rhinitis and its Impact on Asthma") (6-7). The safety of SLIT was confirmed in large population trials (8-12). Current therapeutic approach consist of a 11-30 day incremental dose period. This may represent a problem for some patients. The aim of the present study is to evaluate the possible occurrence of immediate and delayed adverse reactions in allergic patients treated by an ultra-rush SLIT regimen of administrations of either rapidly increasing doses of native allergen extracts, or a chemically modified allergen extract (sublingual monomeric allergoid) during 20-25 minutes updosing period with consecutive oral vaccine administrations every five

Key words: side-effects, sublingual, ultra rush sublingual-swallow immunotherapy, allergen

Mailing address: Renato E. Rossi, M.D.
Allergy Unit
National Health Service
Regione Piemonte, Cuneo
Tel. +390171450407 - Fax +39114366404
e-mail: immunoway@libero.it

0394-6320 (2005)

minutes, alternatively to the conventional several days up-dosing period.

MATERIALS AND METHODS

Patients

Six-hundred-seventy-nine patients with an history of rhino-conjunctivitis and/or moderate asthma due to birch-, timothy-grass-, *Parietaria* ssp-, mugwort-, olive-pollen, house - dust mites and other common allergen sources (cat and dog dander, *Alternaria alternata*, latex) participated in this study (Table I). Twenty patients received two different sublingual allergen vaccines during two distinct ultra-rush SLIT sessions. On the whole, 699 ultra-rush SLIT sessions were performed. All the patients (or a parent of minors) freely gave their informed consent. The diagnosis of allergy to these allergen sources was based on clinical history, positive skin tests using standardized allergen extract (Stallergenes SA, Antony, France), and the presence of specific IgE towards eleven recombinanant allergens (rPhl p 1, 2, 5, 6, 7, 11, 12; rBet v 1, 2, 4; rPar j 2) and natural Phl p 4; or against natural *D. pteronissinus- D. Farinae-, Alternaria alternata-*, cat dander-, olive tree-, *Parietaria judaica-*, and latex-extracts, measured by CAP-System(Pharmacia Diagnostics, Uppsala, Sweden).

Eighty-nine (13.1%) patients had previously received subcutaneous immunotherapy (mean: 27.3 months) with an extract from the same allergen source utilized in the ultra-rush SLIT protocol. These patients shifted to ultra-rush SLIT 30-45 days after stopping subcutaneous allergen vaccine. The aim of this choice was to evaluate the possible protective effect of previous subcutaneous immunotherapy in terms of immediate adverse reactions, in comparison to patients that did not received allergen immunotherapy. Before oral administration the sublingual allergen solution selected was tested in patients of Group I and Group II (see below) by prick test. Skin flare reactions towards sublingual allergen solution were measured as mean diameter, calculated as (D+d)/2, where D is the largest diameter and d the perpendicular diameter in its midpoint.

The method of applying allergen solution drops or tablets was to put it under the tongue and keep it for 2 minutes, then swallow it. This procedure was applied every five minutes until the maintenance dose was reached. Group I, was composed by 272 subjects. In this group, SLIT was performed using different standardized allergen extracts containing timothy grass pollen-, birch pollen-, mite-, and latex extracts (Pangramin, ALK - Abello, Madrid, Spain); the concentrations of the solutions were 2-5 µg/ml of Phl p 5; 22 µg/ml of Bet v 1; and 4 µg/ml of Der p 1, respectively.

Group II composed by 238 patients was treated with a standardized timothy grass pollen-, birch pollen- *Parietaria* spp pollen-, mite-extracts and other common inhalant allergen extracts (Stallergenes SA, Antony, France). As declared by manufacturer, the amount of timothy major allergen Phl p 5 in 300 IR standardized extract was 24 µg/ml; the mite major allergen Der p 1 in 300 IR standardized extract was 25 µg/ml; the birch major allergen Bet v 1 in 300 IR solution was 25 µg/ml.and the *Parietaria spp* major allergen in 300 IR solution was 210 µg/ml determined as Par j 1 (the amount of *Parietaria* spp major allergen content was taken from the study of La Rosa) (13). The in-house reference extract (called 100 IR) is defined as the concentration eliciting a wheal with a mean diameter of 7 mm in 30 skin-tested patients with allergies.

Group III was composed by 62 patients. SLIT was administered using two different standardized allergen extracts containing timothy grass pollen - or mite - extracts (Anallergo, Florence, Italy); the concentrations of the oral preparations were 5 µg/ml of Phl p 1, 12 µg/ml of Der p 1, respectively.

Group IV was composed by 45 subjects. All patients were prescribed a commercial SLIT with a monomeric allergoid in orosoluble tablets (Lais) (Lofarma, SpA, Milan, Italy). The product was titrated in allergen units (AU) and standardized according to the in-house reference-preparation. No information was available from the manufacturer about the content of major allergens in these preparations because the active principle is chemically modified (monomeric allergoid).

Thirty-nine patients of Group V received allergen extracts (Allergy Therapeutics Italia s.r.l.) in which the amount of grass-pollen and mite major allergen were 45µg of Phl p 5 and 21 µg of Der p 1, respectively.

Patients of Group VI (n=23) received allergen extracts in which the amount of major allergens not declared by manufacturer(Merck, Milan, Italy). The immunotherapy protocols and the cumulative doses administered during the build-up phase are shown in table 2.

In the present study, the start dose was 300-1250 times higher than that recommended in the standard protocol. This choice derived from anedoctical observations that some patients erroneously assuming, at home, higher oral vaccine dose intake respect to doses recommended by allergologist and manufacturer, they did not suffered from local or systemic symptoms. Moreover, it is well known that some subjects allergic to pollen eat large amounts of pollen for dietetic purposes without adverse reactions.

After the last dose of ultra-rush SLIT protocol patient was maintained under observation for three hours. At home, patients were instructed to auto-administration a tablet of cetirizine and immediately to consult the allergologist which performed the ultra-rush SLIT protocol, in case of relevant oral or systemic reactions. Finally, all patients were instructed to follow maintenance doses recommended by producer.

Statistics

To evaluate the difference of mite-and timothy-pollen-oral vaccine potency in term of skin reactivity a non-parametric test (Mann-Withney-*U* test) was chosen because data were not normally distributed.

RESULTS

A significant difference of skin reactivity was observed utilizing two different oral allergen solutions indicating various allergen extract potency (Fig. 1 and Fig. 2). Previous SIT did not apparently confer protection in term of oral symptoms. In fact, 115/590 (19.5%) patients without previous SIT had roughly the same prevalence of mild oral symptoms of patients previously treated with subcutaneous allergen vaccine (16/89, 19.1%). Mild systemic reactions after ultra-rush SLIT involved four patients which did not previously receive any form of allergen specific immunotherapy.

During the build up ultra-rush phase with the allergen extracts, local itching of the mouth was observed in 138 of 679 patients (20.3%), twenty-seven (19.6%) of them suffering from oral allergy syndrome (OAS) to apple. However, the oral symptoms disappeared after few minutes with successive increasing vaccine doses. Remaining eleven patients with OAS did not experienced oral symptoms after allergen solution intake.

One patient, male, aged 48 years, mono-sensitized to *Parietaria* had generalized itchiness and a single episode of diarrhoea, three hours after the last sublingual dose administration of Staloral 300 vaccine. The patient was cautionally treated with subcutaneous injection of 300 µl of 1/1000 adrenaline and oral administration of cetirizine (10 mg). Notably, in this patient, SLIT was performed during the pollen season. The same patient was resubmitted to another ultra-rush SLIT session three weeks later employing a lower cumulative dose of Staloral 300 vaccine (300 IR instead of 750 IR, of the current protocol during 25 minutes). After the second ultra-rush SLIT regimen, this patient had mild transitory pruritus of the mouth. Another patient, female, aged 51 years, with 10.4 mg of serum specific IgE to Par j 2, had generalized urticaria three hours after the intake of *Parietaria* oral solution (cumulative dose 750 IR, about 525 µg of Par j 2). Symptoms disappeared with subcutaneous injection of adrenaline and cetirizine. In this patient itchiness persisted for about one week.

One patient 20 years old, allergic to mites,

Fig.1. *Comparison between two mite-oral solution potency by prick test in term of skin reaction.*

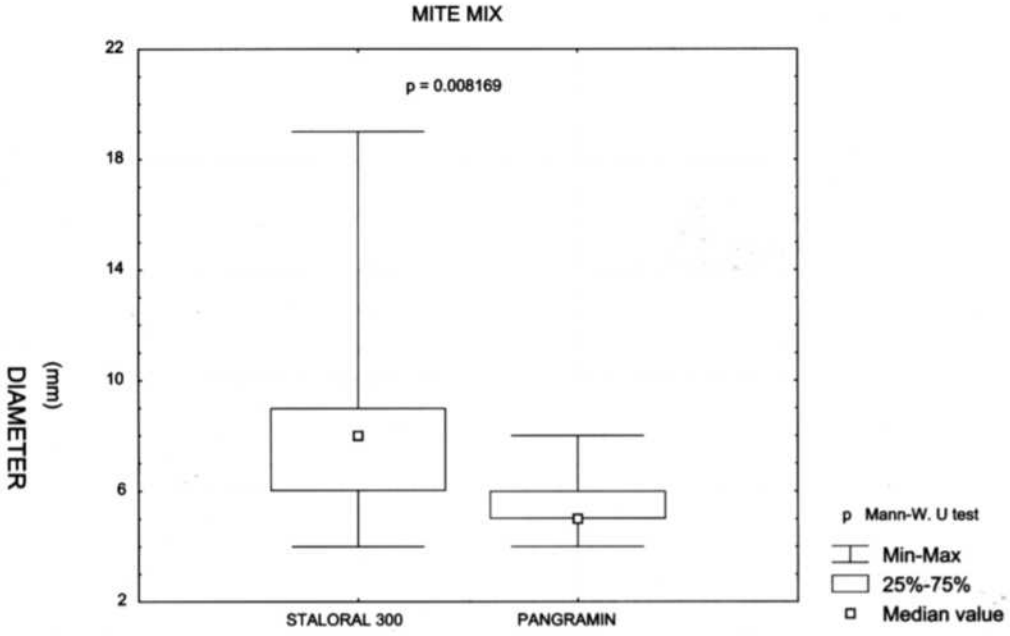
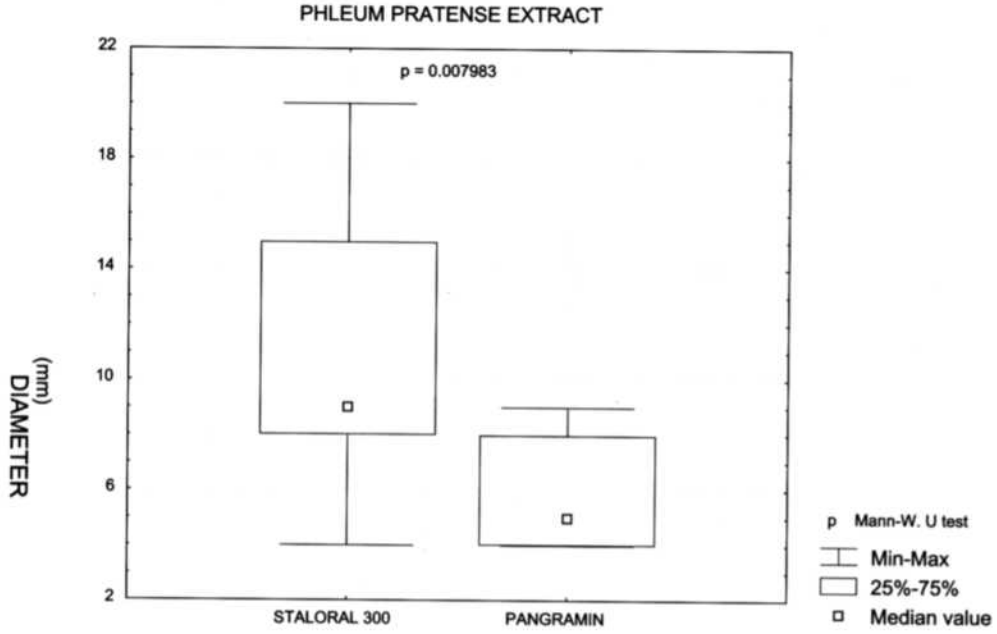


Fig. 2. *Comparison of two timothy-pollen oral solution potency by prick test in term of skin reactivity.*



symptomless during 25 minutes updosing period, 14 days later had occurrence of oral tickling few minutes after mite-solution intake. These oro-pharingeal effects were punctually observed after each vaccine administration. Therefore, this patient decided to stop the SLIT. Finally, a patient allergic to mugwort pollen suffered from rhino-conjunctivitis and urticaria three hours later the last dose of oral vaccine.

Fifty-six out of 238 patients (23.5%) had mild oral symptoms during the ultra-rush protocol with Staloral® 300 while 53/272 patients (19.5%) belonging to the Pangramin® group reported mild and self-resolving oral symptoms. Eleven out of 62 subjects (17.7%) had mild oral itching with Anallergo allergen solutions. Forty-five patients treated with the sublingual monomeric allergoid (Lais®) did not report symptoms during the treatment but one (2.2%) (mild oral pruritus). Similar results were observed in patients under treatment with Oralvac and Merck extracts (data not shown). Interestingly, two out of 25 patients (8%) allergic to *Parietaria*, had systemic reactions, whereas, of the remaining 254 patients allergic to other allergen sources, two subjects (0.08%) had systemic reactions. Finally, of seven patients allergic to latex three patients (42.9%) reported mild oral itching 2-3 minutes after sublingual latex solution administration. Such symptoms gradually disappeared increasing vaccine doses.

DISCUSSION

This study validates the concept that high dose allergen SLIT with an ultra-rush regimen is safe treatment for type I allergy (14, 15). The significant difference of skin reactivity induced by two different sublingual vaccines may reflect the allergen contents of these allergen extracts, but may be in part due to a possible effect of previous subcutaneous immunotherapy on patient skin reactivity (Table I).

Recently, Vervloet et al. (16) demonstrated the efficacy and safety of rush sublingual immunotherapy administered during the pollen season. Whether other studies will confirm the present findings, the choice of ultra-rush regimen by clinicians, may allow immediate maintenance treatment to be proposed even to patients presenting at the time of appearance of their symptoms due to pollen. In our previous study (17), we observed a substantial lack of adverse reactions in 91 allergic patients treated by an ultra-rush SLIT regimen of administrations of either allergen extracts or chemically modified ones, during a two hours up-

	GROUP I		GROUP II		GROUP III		GROUP IV		GROUP V		GROUP VI	
Number of patients	**272**		**238**		**62**		**45**		**39**		**23**	
Age,ys,mean,*SD*	29.4	*14.11*	30.2	*12.79*	30.8	*15.8*	31.61	*13.8*	25.8	*14.9*	29.6	*14.7*
Males	139		125		33		28		22		12	
Females	133		113		29		17		17		11	
Diagnosis												
Rc	159		143		46		37		39		18	
Rc,BA	46		43		10		7		9		5	
Rc,OAS	23		4		1							
RC,BA,OAS	6		2									
Previous SIT	38		46		5		1		0		0	
IgE, mean, *SD* kU/l	488	*664*	524	*666*	472	*692*	413	*248*	416	*247*	489	*677*
	SPECIFIC IgE kUA/l											
	n	**mean**	**SD**	**n**	**mean**	**SD**	**n**	**mean**	**SD**	**n**	**mean**	**SD**	**n**	**mean**	**SD**	**n**	**mean**	**SD**
g6	112	49.4	*39.9*	105 (100%)	51.6	*42.7*	16	35.7	*32.6*	25	42.4	*44.18*	11	39.0	*41.3*	9	41.1	*32.9*
Phl p 5 *	92(82.1%)	29.7	*35.9*	83(79.0%)	27.9	*32.4*	12(75.0%)	12.7	*13.7*	19(76.0%)	22.2	*26.3*	10(9.9%)	19.8	*24.3*	9(75.0%)	24.4	*27.5*
t3	63	44.0	*34.6*	18	24.7	*40.1*	4	17.9	*2.5*	5	58.6	*20.0*	==	==	==	==	==	==
Bet v 1*	63(100%)	50.4	*34.4*	16(100%)	28.5	*42.4*	4(100%)	34.1	*27,9*	5(100%)	56.2	*28.14*	==	==	==	==	==	==
w21	==	==	==	25	26.5	*31.7*	2	6.0	*6.4*	==	==	==	==	==	==	==	==	==
Par j 2*	==	==	==	25(100%)	20.6	*35.3*	2(100%)	8.9	==	==	==	==	==	==	==	==	==	==
d1	67	38.2	*41.3*	66	45.7	*46.3*	35	21.5	*31.6*	29	28.1	*41.4*	20	37.3	*41.4*	7	22.9	*33.5*
k82	7	2.9	*3.4*	0	==	==	==	==	==	==	==	==	==	==	==	==	==	==
Other allergen extracts	23	7.9	*12.9*	24	13.3	*21.5*	==	==	==	==	==	==	9	24.5	*26.7*	3	4.6	*7.4*

Table I. *Demographic, clinical and serological characteristics of 679 patients with ultra-rush regimen(20-26 minute)*
Group I= Patients treated with Pangramin, Group II= Patients treated with Staloral 300, Group III= Patients treated with Anallergo Allergen Extract, Group IV= Patients treated with Lais, Group V= Patients treated with Oralvac, Group VI= Patients treated with Merck Allergen Extract.

*= Positive sera, RC Rino-conjunctivitis, BA Bronchial Asthma, OAS Oral Allergy Syndrome, SIT Subcutaneous immunotherapy, g6: *Phleum pratense,* t3: *Betula verrucosa,* w21: *Parietaria judaica,* d1: *Dermatophagoides pteronissinus,* d2: *Dermatophagoides farinae,*
k: 82 Latex, Other allergens extract: cat dander, *Alternaria alternata*, olive tree, mugwort; n=number of starting therapy.

Time	Stallergens[1] Microliter	Alk-Abello Drops[2]	Anallergo Drops[3]	Lofarma Tablets[4]	Allergy Therapeutic Drops[5]	Merck Drops[6]
0 minutes	100	1	1	100 AU	5 (3200 TU/ml)	5 (100 TU)
5 minutes	200	2	2	300 AU	5 (32000 TU/ml)	5 (1000 TU)
10 minutes	400	4	4	600 AU	1 (320000 TU/ml)	1 (10,000 TU)
15 minutes	800	8	8	1000 AU	2 (320000 TU/ml)	2 (10,000 TU)
20 minutes	1000	12	12	2000 AU	4 (320000 TU/ml)	4 (10,000 TU)
25 minutes	-	20	20	-	8 (320000 TU/ml)	8 (10,000 TU)

Table II. *Ultra-rush sublingual immunotherapy schedules.*

1 Cumulative dose administered : 750 IR containing 60 µg of Phl p 5 or 64 µg of Der p 1
2 Cumulative dose administered : 4.7 µg of Phl p 5 or 7.6 µg of Der p1 and 7.6 µg of Der f 1, or 41 µg of Bet v 1
3 Cumulative dose administered : 9.8 µg of Phl p 5; 23 µg of Der p 1
4 Cumulative dose administered : not available because the active principle is chemically modified (monomeric allergoid)
5 Cumulative dose administered : not available
6 Cumulative dose administered : not available

dosing period and the administration every twenty minutes of increasing doses. Another our study (18), showed the feasibility of ultra-rush SLIT in 30 minutes with an oral allergen load that exceed 300-1250-fold the standard protocol dose of conventional SLIT. In the present study, after 20-25 minutes, the cumulative dose was in some cases, depending to the allergen extract employed, more than 7500 times the dose administered at the start of conventional subcutaneous immunotherapy. Nevertheless, no increase in the adverse event rate was seen in a large number of patients. However, we observed a systemic adverse reaction involving gastro-intestinal tract and skin in two patients which underwent ultra-rush SLIT during the pollen season. This adverse event occurred three hours after the last administration of *Parietaria* extract (cumulative dose administered during up-dosing period: 750 IR= 525 µg of Par j 1), as reported in studies utilizing *Parietaria* extracts (13). As previously reported by André (19) these adverse reactions were not unexpected. It is well known that the major allergens of *Parietaria* (i. e. Par j 1 and Par j 2) belong to the family of nonspecific lipid transfer proteins (LTPs) showing sequence homology to fruit LPTs which have an extreme resistance to proteolysis, heat denaturation and pH changes. This enables them to survive the digestive tracts to elicit possible systemic reactions. Our clinical observations seem to indicate that the occurrence of symptoms induced by high allergen dose during the escalation phase of ultra-rush SLIT was a rare event (4/699, 0.6% of ultra-rush SLIT sessions). All the adverse reactions due to the allergen extracts were mild reactions localized only at oral mucosa that did not require any treatment and, curiously, disappeared with increasing doses within few minutes. Moreover, as documented with patient' follow-up, all subjects but one, did not drop out the treatment because of adverse events (follow-up mean period: 13.2 months). Interestingly, of 89 allergic patients under 14 years of age, no systemic event was observed during an ultra-rush regimen of administration. This suggest the excellent safety profile of SLIT also in children.

As previously shown by Lombardi (20), the sublingual monomeric allergoid resulted both safe and well tolerated. Similar results were obtained by Arena (21). In fact, no patients but one treated with the monomeric allergoid ultra-rush SLIT, reported oral side-effects during the induction and maintenance phase.

Also the treatment of patients allergic to latex resulted safe. However, the occurrence of late local or systemic delayed reactions, must be taken into account. These undesirable effects may be due to enhanced specific IgE production towards relevant allergens induced by vaccine, with subsequent involvement of effector cells (22). In this case, determination of individual reactivity profile to relevant allergen (i.e. rPar j 2) may be useful during patients' follow-up. In fact, it was recently shown that oral administration of a major pollen allegen to naive mice can elicit the production of blocking and interspecific cross-reactive antibodies (23)

One of the main implications of our clinical observations is that with the ultra-rush SLIT protocol, adverse reactions, paradoxically, seem to decrease respect to adverse events reported in other conventional SLIT protocol studies (19, 23-25). However, one must take precaution against administration of large amount of oral *Parietaria* extract in patients with specific IgE to rPar j 2.

We can conclude that, as a whole, the safety/tolerability profile of ultra-rush SLIT is quite favorable. Moreover, it resulted well accepted by patients due to its capability of simplifying and shortening dramatically the SLIT initial phase.

ACKNOWLEDGEMENTS

This article is dedicated to the living memory of Dr Silvano Parmiani. The authors thank Dr Marco Bruno and Dr Paolo Falagiani for kindly reviewing the paper. We acknowledge the skilful nursing assistance of Mrs Silvia Bertolino, Mrs Irma Dao, Mrs Sara Gabelli, Mrs Daniela Matteodo, Mrs Silvia Monetto, Mrs Stefania Orgiu, Mrs Maria Teresa Torta.

REFERENCES

1. **Vrtala S., T. Ball, S. Spitzauer, B. Pandjaitan, C. Suphioglu, B. Knox, W. R. Sperr, P. Valent, D. Kraft and R. Valenta.** 1998. Immunization with purified natural and recombinant allergens induces mouse IgG1 antibodies that recognizes similar epitopes as human IgE and inhibit the human IgE-

allergen interaction and allergen-induced basophil degranulation. *J. Immunol. 160:6137.*

2. **Van Neerven R.J., T. Wikborg, G. Lund, B. Jacobsen, A. Brinch-Nielsen, J. Arnved and H. Ipsen.** 1999. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4+ T cells by inhibiting serum-IgE-facilitated allergen presentation. *J. Immunol. 163:2944.*
3. **Vrtala S., C.A. Akdis, F. Budak, M.Akdis, K. Blaser, D. Kraft and R. Valenta**. 2000. T cell epitope-containing Hypoallergenic recombinant fragments of the major birch pollen allergen, Bet v 1, induce blocking antibodies. *J. Immunol. 165:6653.*
4. **Rolland J.M., J. Douglass and R.E. O'Hehir.** 2000. Allergen immunotherapy: Current and new therapeutics strategies. *Expert Opin. Investig. Drugs 9:515*
5. **Bousquet J., R.F. Lockey and H.-J. Malling**. 1998. Allergen immunotherapy: Specific vaccines for allergic disease. WHO position paper. *Allergy 53 (S):1*
6. **Lockey R.F.** 2001. "ARIA". Global guidelines and new forms of allergen immunotherapy. *J. Allergy Clin. Immunol. 108:497*
7. **Bousquet J. and P. van Cauwenberge.** 2001. Allergic rhinitis and its impact on asthma (ARIA). *J. Allergy Clin. Immunol. 108:S148*
8. **Sabbah A., S. Hassoun, J. Le Sellin, C. Andrè and H. Sicard.** 1994 A double-blind placebo-controlled trial by the sublingual route of immunotherapy with a standardized grass pollen extract. *Allergy 49:309*
9. **Feliziani V., G. Lattuada, S. Parmiani and P.P. Dall'Aglio.** 1995. Safety and efficacy of sublingual rush immunotherapy with grass allergen extracts: a double blind study. *Allergol. Immunopathol. 23:224*
10. **Troise C., S. Voltolini, A. Canessa, S. Pecora and A.C. Negrini.** 1995. Sublingual immunotherapy in *Parietaria* pollen induced rhinitis: a double-blind study. *J. Invest. Allergol. Clin. Immunol. 5:25*
11. **Bousquet S., P. Scheinmann, M. Perrin-Fayolle, J. Sauvaget, A.B. Tonnel, G. Pauli, D. Caillaud, R. Dubost, D. Vervloet, D. Herman, S. Galvain and C. André.** 1999. Sublingual-swallow immunotherapy (SLIT) in patients with asthma due to house-dust mites: a double-blind, placebo-controlled study. *Allergy 54:249*
12. **Mortemousque B., F. Bertel, J. De Casamayor, P. Verin and J. Colin.** 2003. House-dust mite sublingual immunotherapy in perennial conjunctivitis: a double-blind, placebo-controlled study. *Clin. Exp. Allergy 33:464*
13. **La Rosa M., C. Ranno, C. André, F. Carat, M.A. Tosca and G.W. Canonica.** 1999. Double-blind placebo-controlled evaluation of sublingual immunotherapy with standardized *Parietaria judaica* extract in children with allergic rhinoconjuctivitis. *J. Allergy Clin. Immunol. 104:425.*
14. **Di Rienzo V., A. Pagani, S. Parmiani, G. Passalacqua and G.W. Canonica.** 1999. Post-marketing surveillance study on the safety of sublingual immunotherapy in children. *Allergy 54:1110*
15. **Guez S., C. Vatrinet, R. Fadel and C. André.** 2000. House-dust mite sublingual immunotherapy in perennial rhinitis: a doble-blind, placebo controlled study. *Allergy 55:369*
16. **Vervloet D., J. Birnbaum, P. Laurent, B. Hugues, M.F. Fardeau, Y.P. Massabie- Bouchat, A.C. Sadania, A. Aferiat-Derome, C. Vatrinet and C. André.** 2002: Safety and clinical efficacy of rush sublingual cypress immunotherapy: preliminary results. *Allergy 73:57*
17. **Rossi R. E. and G. Monasterolo.** 2002. Safety of ultra-rush(two hours) sublingual-swallow immunotherapy in allergic patients. *Italian J. Allergy Clin. Immunol. 12:221*
18. **Rossi R.E. and G. Monasterolo.** 2003. Safety of ultra-rush (24-30 minutes) sublingual-swallow immunotherapy in patients with allergic rhinitis and asthma. *Proceedings of the XXII congress of the European Academy of Allergology and Clinical Immunology.*
19. **André C., C. Vatrinet, S. Galvain, F. Carat and H. Sicard.** 2000. Safety of sublingual-swallow immunotherapy in children and adults. *Int. Arch. Allergy Immunol. 121:229*

20. **Lombardi C., S. Gargioni, A. Melchiorre, A. Tiri, P. Falagiani, G.W. Canonica and G. Passalacqua.** 2001. Safety of sublingual immunotherapy with monomeric allergoid in adults: multicenter post-marketing surveillance study. *Allergy 56:989*

21. **Arena A., E. Barbatano, E. Gammeri., M. Bruno and G. Riva.** 2003 Specific immunotherapy of allergic diseases: A three years perspective observational study. *Int. J. Immunopathol. Pharmacol. 3:277*

22. **Kempuraj D., S. Frydas, E. Karagouni, M. Hatzistilianou, P. Boscolo, F. Ferro, M. Di Giannantonio, C.M.V. Conti, D. Merlitti R. Doyle, C. Petrarca, M.L. Castellani and T.C. Theoharides.** 2004. Cytokines and the brain. *Int. J. Immunopathol. Pharmacol. 17:229.*

23. **De Weerd N., P.L. Bhalla and M.B. Singh.** 2003. Oral immunization with a recombinant major grass pollen allergen induces blocking antibodies in mice. *Int. Arch. Allergy Immunol. 130:11*

24. **E. Madonini, F. Agostinis, R. Barra, A. Berra, D. Donadio, A. Pappacoda, E. Stefani and E. Tierno.** 2003. Long-term and preventive effects of sublingual allergen-specific immunotherapy: a retrospective, multicentric study. *Int. J. Immunopathol. Pharmacol. 16:73*

25. **Khinchi M.S., L.K. Poulsen, F. Carat, C. Andrè, A.B. Hansen and H.–J. Malling.** 2004. Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: a randomized, placebo-controlled, double-blind, double-dummy study. *Allergy 59:45.*