



# European Annals of Allergy and Clinical Immunology

THE OFFICIAL JOURNAL OF AAITO | ASSOCIAZIONE ITALIANA ALLERGOLOGI IMMUNOLOGI TERRITORIALI E OSPEDALIERI



Sublingual immunotherapy: certainties, unmet needs and future directions

Pre-lethal anaphylaxis to carboxymethylcellulose confirmed by identification of specific IgE – review of the literature

Efficacy, safety and tolerability of sublingual monomeric allergoid in tablets given without up-dosing to pediatric patients with allergic rhinitis and/or asthma due to grass pollen

Antihistamines do not inhibit the flare induced by the intradermal injection of autologous plasma in chronic urticaria patients

Paradoxical exacerbation of chronic urticaria by H1-antihistamines and montelukast



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G. PASSALACQUA<sup>1</sup>, C. LOMBARDI<sup>2</sup>, C. TROISE<sup>3</sup>, G.W. CANONICA<sup>1</sup>

## Sublingual immunotherapy: certainties, unmet needs and future directions

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### Introduction

The subcutaneous modality of immunotherapy injections (SCIT) remained for several decades the only available administration route. SCIT is effective and safe, when properly prescribed and administered, but a remote risk of severe side effects is present (1), and the occurrence of technical errors is still not negligible (2). The problem of the risk/benefit ratio prompted the search for safer administration routes (nasal, bronchial, oral)(3), including the sublingual one (SLIT) that was described in 1986 (4). In less than 20 years, due to the large amount of clinical data, SLIT achieved credibility, and was introduced in the official documents as a viable alternative to the classic injection route (5, 6) for both adults and children (Fig. 1). To date SLIT is commercialized and routinely used in many European countries.

Despite the increasing optimism, it must be acknowledged that some aspects still need to be clarified, and that there is room for improvement. The unmet needs repre-

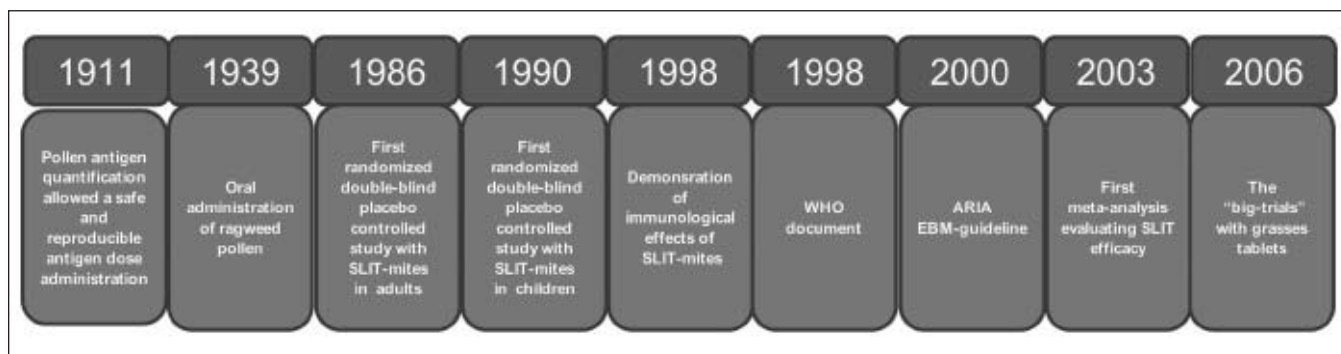
sent the basis for future research, whereas the clinical hypotheses would open the search for new indications and modalities.

### SLIT: where do we stand?

#### *Efficacy*

To date, there are 60 randomized double blind placebo controlled trials performed with SLIT. Due to the number of the trials available, meta-analyses could be carried out (Tab. 1), with various inclusion criteria such as rhinitis only (7), asthma only (8), asthma and rhinitis in children (9, 10) (no pediatric meta analysis is available for SCIT). All the meta-analyses concluded for a significant effect of SLIT versus placebo (11). The reliability of the meta-analyses has recently been questioned by Nieto et al (12) especially on the basis of possible publication biases and incorrect reporting of the data. Nonetheless, due to the

**Figure 1** - Cronology of sublingual immunotherapy



**Table 1** - Meta-analyses on SLIT

Author	Patients	Disease	Trials	Effect size on symptoms	Comment
Calamita, 2006	303 adults + children	Asthma	5 pollens 4 mite	-0.38 (p= 0.07)	No change in symptoms score Significant reduction medication score
Olaguibel 2005	256 children	Asthma/ Rhinitis	3 pollens 4 mite	-1.42 (p=0.01)	Decreased symptoms and medications for asthma, rhinitis and conj
Wilson 2005	959 adults + children	Rhinitis	16 pollens 6 mite	-0.42 (p=0.002)	Decreased symptoms and medications for rhinitis. Asthma not evaluable
Penagos 2006	484 children	Rhinitis	5 pollens 4 mite	-0.56 (p=0.02)	Decreased symptoms and medications for rhinitis. No sub analysis feasible
Penagos 2008	441 children	Asthma	3 pollen 3 mite	-1.42 (p=0.02)	Decreased symptoms and medications for asthma

poor performance of the funnel-plot analysis, these negative aspects must be interpreted with great caution and without inappropriate generalizations. Moreover, the mentioned meta-analyses pooled together the studies with all allergenic extracts, whereas differences may exist among allergens. In this regard, there is so far one single meta-analysis restricted to house dust mite SLIT, showing a significant effect on symptom and medication scores in allergy due to mite (13). The problem of heterogeneity has been repeatedly highlighted as a drawback, but it is also true that meta-analyses are intended to summarize the results of studies when they are not directly comparable each other. Of particular interest are the recent so called "big trials" (14-19), all conducted with grass pollen extracts (Tab. 2). Those trials enrolled more than 200 patients each, reaching in some cases up to 600 patients. Of note, only one study with a similar number of patients exists for SCIT (20). The big trials invariably showed an effect of SLIT versus placebo ranging from 25% to more than 50%. The cut-off of 20% is unanimously considered the threshold for a clinically relevant effect (21), since antihistamines and nasal steroids hardly reach a 15% improvement versus placebo (22). In addition, those big trials with a dose-ranging design, clearly showed that the clinical effect is dose-dependent, and this is a robust proof of the efficacy according to the GRADE rules (23). On the other hand, the effects on QoL were always statistically significant in the big trials, but a "clinically relevant" difference (0.5 points in the RQLQ) was not always achieved (14, 16). The effect in asthma is still a matter of debate, since some studies (24-25) reported marginal or no effect on

asthma symptoms. Nevertheless, in those studies, all the patients (active and controls) had no symptom of asthma at baseline and during the trial, therefore no effect could be seen. When patients have measurable asthma symptoms, the effect of SLIT is apparent, as recently shown in a pediatric trial (26). In addition, it has been shown that SLIT is capable of reducing the grade of bronchial hyperresponsiveness in adults and children (27, 28). Finally, the comparison of the efficacy of SLIT versus medications is still an open problem, because the effects of immunotherapy can be appreciated only in the long term (months). One head-to-head open randomized trial of SLIT versus inhaled budesonide in asthmatic patients, showed in the long term an overall superiority of immunotherapy (29). Another trial (30) in asthmatic children demonstrated that the clinical efficacy of SLIT plus fluticasone is equal to that of fluticasone alone, but the addition of SLIT improves also on non-bronchial symptoms.

### Safety

The safety of SLIT is unanimously recognized to be superior to that of SCIT (31). An apparent datum is that no fatality has been ever reported with SLIT in 23 years of trials and clinical use. In addition, the reports of anaphylaxis with SLIT so far available in the literature are only four (32-35), being one of them questionable (32). On the other hand, the report of an anaphylactic reaction at the first grass tablet SLIT (35), would suggest the opportunity to give the first dose under medical supervi-

**Table 2** - The “big trials” with grass extracts

Author, year	Age range	Patients A/P *	Allergen	Durat.	Dose Preparation	Main positive results over placebo
Durham 2006	18-66	569/286	Grass 3 doses	6 m	15 µg (136 pts) 150 µg (139 pts) 450 µg (294 pts) Phl p 5/month Tablets	Drug score -28% (0.012) Symptoms -21% (0.002) only with the highest dose QoL improved No clinical change with the 2 low doses
Dahl, 2006	23-35	316/318	Grass	6 m	450 µg Phl p 5/month. Cumulat. 2.7 mg Tablets	RC symptoms -30% (.001); RC drugs -38% (.001); Well days -52% (.004)
Didier 2007	25-47	472/156	Grass 3 doses	6 m	240 µg (157 pt) 750 µg (155 pt) 1.2 mg (160 pt) /month Tablets	For 300 and 500IR Total and individual symptom and drug scores (<.001); RQLQ improved
Wahn, 2009	4-17	139/139	Grass	8 m	600 µg major allergen/month. Tablets	Rhinitis score -28% (.01) Medications -24% (.006) Medication free days (.01)
Ott, 2009	20-50	142/67	Grass	5 y 4 seas	Cumulative 1.5 mg major allerg/season	Combined score and symptom score significantly reduced since 1st season. Symptoms decrease from -33% to 47% (3rd seas) No change med.scores
Bufe, 2009	5-16	126/127	Grass	6 m	450 µg Phl p 5/month	Significant reduction in RC sympt score (-24%), asthma score (-64%), RC medications (-34%), well days (+28%). All p<.03

sion. A great attention has been paid to the safety in children (36). In fact, the age of 5 years is considered as a relative contraindication for SCIT, mainly because in young children any reaction may be more severe and more difficult to treat than in adults. Some of the post marketing surveys involved also children aged between 3 and 5 years (37-39), and confirmed that the safety is not impaired in the younger ages. A controlled dose finding study of safety (40) involved 48 grass-allergic patients outside pollen season. They received SLIT for 28-day periods at progressively increasing doses, up to 200 mcg Phl p 5 allergen that is about 40 times the amount given with one injection. The overall incidence of side effects was 74%, all of mild or moderate intensity. The most frequently reported events were irritation of the throat and oral itching. According to the recent data, the number of

side effects seems to be dose-dependent, as happens with SCIT.

Since the majority of allergic patients are polysensitized, it is often necessary to prescribe immunotherapy with multiple allergens and it is crucial to know if the administration of different allergens with SLIT increases the risk of side-effects. Two post-marketing surveys performed in adults and children consistently suggested that the use of multiple allergens for SLIT does not increase the rate of side-effects (41, 42).

#### *Mechanisms*

Although the traditional effects on IgE and IgG4 are less pronounced with SLIT than with SCIT, several observations have recently begun to clarify the mechanism of ac-



tion (Tab. 3). Some studies reported an increase of production of the regulatory cytokine IL-10 (43-46) and another study showed a reduction of the Th2 cytokine IL-13 (47). Savolainen et al demonstrated in vitro that SLIT reduces the expression of IL-5 and enhances the expression of IL-10 in PBMC stimulated with the allergen (48). Overall, the clinical effects of SLIT resemble those of SCIT, and the data available suggest that the mechanisms of action of the two routes are partially similar.

Additional mechanisms operating at the level of the sublingual mucosa and regional lymph-nodes may also be involved. During SLIT, allergens are captured within the oral mucosa by Langerhans-like dendritic cells expressing high-affinity IgE-receptors, producing IL-10 and TGF- $\beta$ , and upregulating indoleamine dioxygenase (IDO), this suggesting that such cells are prone to induce tolerance (49). Finally, unique data on biodistribution in humans are available for SLIT, showing a long-lasting persistence

of the allergen in the mouth, with an absent or negligible absorption through the mucosa (50, 51).

#### *Additional effects*

Recently, it was demonstrated that SLIT, similarly to SCIT can prevent the onset of new sensitizations. In a study involving more than 500 patients, the rate of occurrence of new sensitizations was 5.8% in the active group and 38% in the control group ( $p < 0,001$ ) (52). An open controlled study by Novembre et al (53), performed in children, demonstrated that SLIT is capable of reducing the risk of asthma onset. These results were replicated in a larger randomized open study, involving more than 200 children followed up for three years (54). The occurrence of persistent asthma after 3 years was 38% of the controls and 2% of the SLIT. Certainly, the evidence of a preventative effect is still weak and relies on small numbers of patients: 151 for SCIT (55) and 340 for SLIT. In addition, this study confirmed the prevention of the onset of new sensitizations. On the other hand, there are so far only two studies, one non randomized (56) and the other randomized and double blind (57) that demonstrated a long-lasting effect of SLIT after discontinuation.

#### *Adherence*

In the case of SLIT, the adherence has been always considered a major concern, since the treatment is self-administered. The problem of the adherence was systematically addressed in three studies with method of the random telephonic interviews.

In the first study (58), involving 126 patients the adherence was 95% for pollen immunotherapy and 97% for mite immunotherapy. In the second study (59), conducted in more than 400 patients, the adherence rate at 3 and 6 months was greater than 90% in about 75% of the patients. The third study was conducted in children (60), and the results on compliance did not differ from those in adults. On the contrary, one retrospective study by Pajno et al. showed that the adherence was slightly greater with SCIT than with SLIT, but no quantitative assessment was provided in this study (61). Finally, a priori subgroup analysis was conducted in an open-label European study where adult patients received once-daily SLIT grass tablets with or without a device to aid compliance. Eighty-two patients reported using the device sometimes or always, and rated it easy to use (62).

**Table 3** - Modification of the parameters of immune system after SLIT

#### **Local immune responses:**

- no differences in CD3+, CD1a+, CD68+ cell counts (Lima et al., 2002)
- significant decrease of sublingual salivary ECP levels (Marcucci et al., 2001)

#### **Systemic immune responses:**

- significant decrease of serum ECP levels (Passalacqua et al., 1998; Sanchez Palacios et al., 2001; Vourdas et al., 1998)
- serum ICAM-1, IL-2 receptor, E-selectin, IL-12 levels unchanged (Reich et al., 2003)
- reduction of IL-13 and Th2 related hormone prolactin (Ippoliti et al., 2003)
- increase of IL-10 production (Ciprandi et al., 2005)
- changes in IgE levels ??
- significant increase in serum IgG4 levels (dose-response depending) (Tari et al., 1990; La Rosa et al., 1999; Bufe et al., 2004; Lima et al., 2002)
- increase in serum IgG1 levels (Tari et al., 1990; Bufe et al., 2004)
- no significant changes in CD40+, CD3+, CD4+, CD8+ cell counts (Ippoliti et al., 2003)
- significant increase in peripheral blood CD8+ T cells (Tari et al., 1990)
- induction of a specific T-reg response (Ciprandi et al., 2007)
- urinary leukotrienes: conflicting results (rhinitis: yes; asthma: no) (Yuksel et al., 1999)
- nasal eosinophils: significant decrease (La Grutta et al., 2007)



## Unmet needs and critical aspects

Despite the official positions and the relatively wide clinical use, some aspects of SLIT need urgently to be elucidated, in order to provide clinicians with clear and evidence-based recommendation for the clinical use of the treatment.

One of the critical aspects is that some studies provided totally negative results, although conducted with a rigorous methodology (63-65). No clear explanation has been provided for those results, but a generic "incorrect patient's selection". This underlines the need for univocal criteria or parameters that help identifying the best candidates to SLIT. Another relevant problem is the large variability of the doses used in clinical trials. Indeed, both positive and negative results have been obtained with both low and high doses of allergens, and the dose interval for efficacy is reported to range between 2 and 375 times the amount given with SCIT. A clear dose response relationship has been formally demonstrated only for grass extracts, where the optimal dose has been identified in 15 to 25 mcg major allergen per day, that is roughly 50 times the monthly dose of SCIT. Thus, dose-response trials and the identification of the optimal maintenance dose are needed at least for the more relevant allergens. The variability of the study design, patients' selection, duration and regimen among the trials is another major problem that importantly affects the interpretation of the meta-analyses. Concerning this latter point, it should be remembered that meta-analyses put together the results obtained with various allergens and conclude for the efficacy of all allergens, that is not true. This underlines the need for a separate analysis of each single allergen, and this has been so far done only for dust mite (14).

From a clinical point of view, there is no consensus on which is the best administration regimen among the pre-seasonal, coseasonal, pre-coseasonal or continuous. It is true that for pollen allergens, the vast majority of the trials have utilized a pre-coseasonal regimen (66), but this cannot be immediately extrapolated to all extracts and to all patients. Similarly, the usefulness of a build-up phase is still a matter of debate. The no-updosing has been shown to be safe enough (67, 68), and some of the big trials have used a no-updosing regimen, but the applicability of this concept to all allergens and patients is not unanimously accepted. Also the best maintenance dosing (once daily, on alternate days, once weekly) has not yet been defined (66), as well as the optimal duration of a SLIT course. Another critical point is the the allergen and pro-

tein content of commercial extracts is highly variable among manufacturers (69). The use of multiple extracts have been shown to be safe (41, 42), but few and sparse data are available on its efficacy (70). A recent RDBPC trial was conducted to assess the efficacy of SLIT with a grass extract alone or in combination (71). This study showed that an immunological response is achieved with the thimothy extract over a period of 10 months, but the same dose combined with 9 other pollen extracts produced only a limited response.

Concerning the safety, it would be crucial to identify risk factors for systemic side effects, if any. In addition, the safety in subjects with previous reactions to injections has not been studied, as well as the risk for adverse events after a temporary suspension of SLIT.

## Working hypotheses for the future

The efficacy and safety of SLIT can be, in principle, improved by the chemical modification of allergens, by using adjuvants or by enhancing the contact with the oral mucosa. This latter aspect has been recently addressed with the use of mucoadhesive substances, which have been demonstrated to improve the immunological effects in an animal model (72). More realistically, it can be expected that the good safety profile of SLIT would allow to expand its indications, especially for conditions different than respiratory allergy.

There are, in fact, two studies in food allergy, one with hazelnuts (73) and one with the Pru p 3 allergen of peach (74), reporting positive results. In both studies a significant reduction of the oral provocation threshold was described, this suggesting the possible use of SLIT in food allergy. The use of SLIT has been also proposed in the past for the treatment of extrinsic atopic dermatitis (75). A randomized double blind placebo controlled trial, conducted in 30 children with mite allergy (76), reported a significant effect of mite-SLIT in reducing the SCORAD in mild to moderate atopic eczema. Those data substantially replicated which obtained in another open non-controlled, non-randomized pilot trial in 86 adult HDM-sensitized patients with mild-moderate atopic dermatitis (77). Surprisingly, a randomized controlled trial showed that SLIT with honeybee venom (maintenance 525 mcg) reduced the diameter of the large local reactions after sting challenge (78). This, although appealing, was only a proof of concept study, and SLIT cannot be presently recommended for the treatment of hymenoptera venom allergy (79). On the

other hand, several studies strongly suggest the applicability of SLIT for treating latex allergy (80–82). Other possible fields of investigation are the desensitization for nickel allergy, but in this case there are only basic studies in animal models (83), and baker's asthma (84).

## Conclusion

SLIT represents a significant advance because of the efficacy, safety and convenience, and it appears particularly suitable in paediatric patients, where an optimal safety profile is required. Despite the general optimism, more studies are needed about the mechanisms of action, the pharmacoeconomics, the optimal doses for each allergen and on the ideal patients. The available data and the results of meta-analyses confirm the official positions on SLIT and justify the tangible change in the general opinion, which considers SLIT a more acceptable treatment. It is essential to remember that SLIT prescription must be made only by a specialist, after a detailed diagnosis has been established and the expected benefit/cost ratio has been carefully evaluated (85).

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# Pre-lethal anaphylaxis to carboxymethylcellulose confirmed by identification of specific IgE – review of the literature

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## KEY WORDS

*Carboxymethylcellulose, Anaphylaxis, Barium suspension, IgE*

## SUMMARY

**Background:** Carboxymethylcellulose (CMC) is used extensively in the pharmaceutical and food industries on account of its various properties. Anaphylactic reactions are rare. It has been reported principally after intra-articular infiltration of sustained-release corticosteroids containing CMC and, very rarely, after barium enema. **Methods:** A case of pre-lethal anaphylactic shock after barium enema was studied by prick-test, intra-dermal reaction (IDR), leukocyte histamine release test (LHRT), basophil activation test (BAT), cystein-leukotriene release test (CAST) and dot-blot analysis. **Results:** IDR to CMC was positive at a concentration of 10 µg/ml. BAT and CAST were positive. Specific IgE were identified using dot-blot analysis. **Discussion:** This is the third report of CMC-specific IgE and the second of anaphylaxis to CMC associated with a barium suspension in contact with GI tract mucosa. CMC as an excipient in medicinal products may therefore be a risk factor for severe anaphylaxis after injection or following contact with GI tract mucosa. Sensitization and allergic reactions by CMC in food additives have to be considered.

## Introduction

Carboxymethylcellulose (CMC) is used in the pharmaceutical and food industries. It is a vegetable cellulose derivative obtained by the action of chloracetic acid on cellulose in an alkaline medium, whereby hydroxyl functions are substituted by carboxymethyl groups. The basic structure is a (1-4) D glucopyranosyl polymer. The degree of substitution varies according to preparation, but is usually between 0.6 and 0.95. The molecular weight may range from 90 000 to 700 000 kDa. Anaphylaxis to CMC is known, but is rare.

Specific IgEs against CMC were first identified by Patterson in 1995 (1) using immunoblot analysis, then by Muroi using ELISA (2). We have confirmed the existence of CMC-specific IgE using a dot-blot analysis.

## Material and methods

### Case report

In March 2003, a 62 year-old man underwent barium enema with Micropaque Colon® during follow-up for

colonic polyposis. He suffered from long-standing asthma, complicated by chronic obstructive pulmonary disease (COPD). There was no personal or family history of atopy. Micropaque Colon<sup>®</sup>, a barium sulfate suspension, was used as an enema. Thirty minutes later, the patient presented with generalized urticaria, severe bronchospasm, cardiovascular collapse and respiratory arrest. Immediate management of shock consisted of intubation, intravenous epinephrine and vascular filling. He recovered completely after 24 hours.

Allergological testing was performed two months later. The interview revealed two episodes of malaise after intra-articular infiltrations with sustained-release corticosteroids containing CMC for arthrosis-related joint pain. Micropaque Colon<sup>®</sup> also contains 2.77% CMC. The amount injected by enema was estimated at 2.7 g.

Prick-tests were performed on a skin reactive to codeine. They were positive to Micropaque Colon<sup>®</sup> (2.5 mm). CMC powder from Coopérative Pharmaceutique Française (Melun 77000 France) was diluted in 5% phenolated saline at 1mg/ml, then diluted for skin tests. The prick-test to CMC was negative but the intra-dermal test (IDT) to CMC was positive at 10 µg/ml (8 mm edema, 15 mm erythema, 15 mn after a 4 mm injection papule). In 6 controls, IDT was negative to 10 µg/ml, 100 µg/ml and 1 mg/ml. The basophil activation test (BAT) by flow cytometry was positive to CMC with 15% activation (0.5% spontaneous activation, positive control to anti-IgE: 17 %). The cystein-leukotriene release test (CAST) to CMC was positive: 1,700 pg/ml (control test to anti-IgE: 3,000 pg/ml). Leukocyte histamine release test (LHRT) to CMC was negative. Serum tryptase assay was normal: 5.9 µg/l.

A diagnosis of anaphylaxis to CMC was made. The patient was given a list of injectable medicinal products containing CMC.

## Identification of specific IgE

### *Samples*

Several solutions were prepared from sodium CMC (Pharmaceutical Cooperation, Melun, France): a 5 mg/mL aqueous solution of bovine serum albumin (BSA), a 5 µg/µL sodium CMC solution obtained from a 20µg/µL aqueous mother solution of sodium CMC in a phosphate buffer.

A CMC-HCl solution was made by diluting the 20µg/µL sodium CMC solution 1:4 in an acid solution (1N HCl).

The mixture was put into a water bath at 37°C for 3 hours.

A CMC-BSA solution was made by mixing equal volumes of the CMC-HCl solution and the 5mg/mL BSA solution. The mixture was kept at boiling point for 10 minutes.

### *Dot-blot*

PVDF (Sequi-Blot<sup>™</sup> PVDF membrane for protein sequencing 0.2 µm BIO-RAD) membranes were soaked in a methanol bath for 1 minute, then rinsed with distilled water for 2 to 3 minutes.

One micro-liter of each solution was placed on the PVDF membrane. After drying in air for 15 minutes, the membrane was saturated for 1 hour in a 5% BSA solution and 0.05% Tween in phosphate buffer (PBS). It was then rinsed for 2 minutes in PBS and 0.05% Tween. The membrane was then incubated overnight at +4°C in the serum diluted 1:5 in a 1% BSA solution, PBS and 0.05% Tween. The control membrane was incubated in a 1% BSA solution, PBS and 0.05% Tween. The membrane was rinsed 4 times in PBS buffer and 0.05% Tween, then incubated for 1 hour at room temperature in a 1:1500 dilution of human IgE secondary antibodies labelled with peroxidase (Dako). The membrane was rinsed 4 times, then immersed in luminol in the presence of H<sub>2</sub>O<sub>2</sub> directly on the image analyzer (Kodak digital Science 1 Digital Science 1D image analyzer). Image acquisition was performed after an exposure time of 6.6 minutes (20 uptakes).

The dot-blots showed binding of specific IgE to CMC and CMC-HCl samples. Binding was lower to the CMC-BSA mixture. There was no binding of specific IgE to BSA alone. The control membrane did not show non-specific binding to secondary antibodies (Figure 1).

## Discussion

CMC (also known as carmellose or E466) is physiologically inert. It is a white to off-white, odorless powder and is slightly hygroscopic. It is soluble in water at all temperatures but practically insoluble in organic solvents. It has several properties: it is a stabilizing, emulsifying, thickening, binding, hydrophilic agent that retains water and can form a protective film. It increases viscosity when dissolved or dispersed in water. It helps form suspensions (from fluids to gels). In the pharmaceutical industry, it is used in topical skin products, eye drops, tablets, solutions for injection, such as corticosteroids for intra-articular in-

jection and other injectable hormones (LHRH and somatostatin). In injectable preparations, CMC is used as a suspension agent for poorly hydrosoluble components. It is a component of barium preparations, hydrocolloidal dressings (because of its absorbent properties) and also adhesive stoma bags. It is also used extensively in the food industry: ice creams, cakes, etc... (table 1) (3). CMC was long thought to lack toxicity and have only laxative effects after oral administration to animals. As a food additive, CMC is considered to be safe in quantities up to 25mg/kg/day since it is inert and not absorbed. Despite its wide spread use, allergy to CMC is rare (table 2). A case of contact dermatitis to CMC was described by Hamada in 1978 in a baker who used CMC to make cakes (4). CMC was also incriminated in the onset of chronic urticaria after using hydrocolloidal dressings (5). A case of reaction to CMC in a lidocaine gel used to lubricate a gastroscope to facilitate its passage has also been described (6) (Table 2), with onset of upper and lower limb weakness lasting for several hours. A nasal provocation test to CMC triggered ipsilateral nasal congestion and dysesthesia of the tongue and temporal region for 30 minutes.

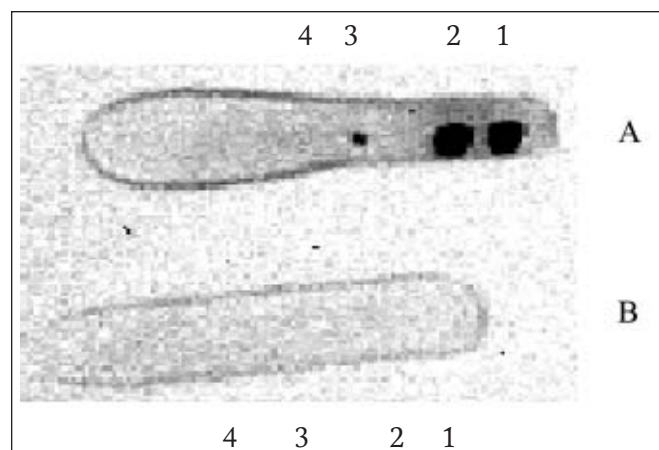
Anaphylactic shock to CMC was first documented with veterinary products in cattle (penicillin, vaccines, steroids) (7)(8)(9). In 1972, De Weck drew attention to the potential risk of accidents in man due to CMC in medicinal products (10). Anaphylaxis was later described after intra-

articular injections of sustained-release corticosteroids containing CMC (11-21) (Table 2). Reactions generally consist of pruritus and urticaria followed by hypotension and anaphylactic shock.

Allergic reactions after oral administration of CMC are not documented (Table 2). One team carried out oral challenge tests in 3 patients, demonstrating tolerance up to 136 - 250 mg (18). Two oral challenges to 62 mg were negative (22). The rectal administration of a barium enema containing CMC has elicited an anaphylactic shock (23); the adverse accident occurred after insufflation. In our case, mastocytosis was excluded and the anaphylactic shock could be related to the amount of CMC in the Micropaque Colon<sup>®</sup> introduced into the intestine: 2.7 g (estimated at 3 g in the case reported by Muroi), whereas intra-articular infiltration usually contains about 15 to 30 mg. Similarly, the reaction could be potentiated by insufflation, which may increase passage into the blood by rupturing tight inter-cellular junctions so that the product enters the sub-epithelial space (24). Gastro-enterological examinations with barium are frequent, and the incidence of anaphylactic reactions occurring during these examinations is estimated at 1 in several thousand. However, the true number may be higher. (2).

The immunological nature of these reactions has been shown by skin tests (prick-tests, IDR, patch-tests and occasionally scratch-tests) (Table 2), by leukocyte histamine release test (LHRT) to CMC (12, 22), and also by lymphocyte stimulation tests (LST) (6). When the concentration of CMC for positive intradermal tests was specified in the published cases, the range was from 0.075 to

**Figure 1** - Dot-blot: One micro-liter of each solution was placed on a PVDF membrane: (1) CMC (5µg/µl), (2) CMC-HCl, (3) CMC-BSA, (4) BSA. After drying and saturation in BSA, the membrane was incubated with (A) the test serum (1:5), or (B) a BSA solution for one night. The IgE bound to CMC was revealed by a human IgE secondary antibody labelled with peroxidase. Chemoluminescence was read directly on an image analyzer (Kodak).



**Table 1** - Typical products containing CMC (3)

Packed cheesecake and cake mixes	Ice cream
Icings	Milk shake
Bakery fillings	Frozen mousse
Fruit bar filling	Tomato sauces
Meringues	Salad dressings
Dips and spreads	Frozen chips
Tinned potato salad	Frozen fish sticks
Tinned cream soups	Batter coatings
Frozen whipped toppings	Low-calorie orange squash
Whipped topping basis	Low-calorie orange squash
Sterilized whipping cream	Cottage cheese

**Table 2** - Allergy to CMC: cases described in the literature.

Reference	Patient	Pathology	Medication	Administration route	Time to reaction	Type of reaction	Prick test, IDT, HLRT, BAT, CAST, LTT and NPT
Müller et al. 1973	Man 29 y.o.	Shoulder Arthralgia	Volon® (triamcinolone acetonide)	Infiltration	Immediate	Anaphylactic reaction	- Prick-test Volon® - - Scratch-test Volon® - - IDT Volon® + - IDT CMC (7.5mg/ml) + and 0.75mg/l +
Bourgeois et al. 1989	Woman 51 y.o.	Carpal tunnel syndrome	Altim® (cortivazol)	Infiltration	30 min	-Localized and then generalized urticaria Treatment with antihistamine and corticosteroids	- Prick test Altim® (1:10) +: localized urticaria spreading to the arm and the hemi-thorax - Prick test CMC (10-7) +: localized urticaria with spreading to the arm
Beaudouin et al. 1992	Woman 49 y.o.	Sciatalgia	Hydrocortancyl® (hydrocortisone)	Infiltration	2 min	Anaphylactic reaction	- IDT Hydrocortancyl+ (10µg/ml) (13 mm) - IDT CMC+ (10µg/ml) (9 mm) - LHRT + hydrocortancyl® and CMC
Beaudouin et al. 1992	Woman	Epicondylitis	Altim® (cortivazol)			Anaphylactic reaction	- Prick and IDT Altim®+ (1µg/ml) -IDT CMC + (1µg/ml) - BAT CMC- LHRT CMC-
Murietta - Aguttas et al. 1991	Woman 30 y.o.	Nonallergic Rhinitis	Kenalog® (triamcinolone acetonide)	Injection	5 min	Cough, localized and then generalized urticaria	- IDT CMC (1µg/ml) + (wheal 11 mm / flare 30 mm)
Patterson et al. 1995	Man 26 y.o.	Sturge-Weber skin lesion of the face	Kenalog® (triamcinolone acetonide)	Injection	15 min	Anaphylactic reaction	-Skin testing Kenalog® (1µg/l) + (>10mm) -Skin testing CMC (0,1µg/ml) + (>10mm) -Immunoblot +
Muroi et al. 1997	Woman 63 y.o.		Balgin S Solution number 3® (Suspension of barium sulphate)	Double-contrast upper gastrointestinal Examination	30 min	Anaphylactic reaction	-Skin testing barium sulphate suspension + (wheal 25 by 20 mm/flare 57 by 50mm) -Skin testing CMC + (wheal 21 by 21 mm/ flare 57 by 50 mm) - LHRT by CMC +
Johnsson et al. 1999	Woman 77 y.o.	Chronic leg ulcer	Comfeel® (hydrocolloidal dressing)	Topical treatment	30 min 2 hours	-Itching in the ulcer area -Generalized urticarial rash and slight nausea	-1x1cm piece of the hydrocolloid dressing applied to her forearm for 20 min: stinging at the test site -Scratch test with the dressing: itchy wheal and flare reaction -Prick test CMC (100µg/ml) +
Kakuyama et al. 1999	Woman 69 y.o.		Lidocaine jelly	For local anesthetic and lubricant in gastroscopic examination		Paresia of the limbs lasting several hours	-IDT lidocaine and CMC - -NPT lidocaine- -NPT CMC + (ipsilateral nasal congestion and dyesthesia of the tongue and the ipsilateral temporal region during 30 min - LTT CMC +
Caduff et al. 2000	Man 41 y.o.	Lumbago	Kenacort® (triamcinolone acetonide)	Infiltration		Anaphylactic reaction	-Prick test CMC - -IDT CMC +
Caduff et al. 2000	Woman 53 y.o.	Calcaneodynia	Kenatocort® (triamcinolone acetonide)	Infiltration	2-3 hours	Anaphylactic reaction	-Scratch test CMC+



Montoro et al. 2000	Man 47 y.o.	Recurrent arthritis in the left shoulder	Trigon Depot® (triamcinolone acetone)	Infiltration	15 min	Anaphylactic reaction	- Prick Trigon® (40mg/ml) - IDT Trigon® (4mg/ml) + (11 x 12mm) -Prick CMC (8mg/l) - IDT CMC (8mg/l) + (9x8mm)
Schuster et al. 2000	Woman 54 y.o.	Achillodynia	Kenacort® (triamcinolone acetone)	Infiltration	5 min	Anaphylactic reaction	-Prick Kenacort® (7,5mg/ml)+ (>3mm) -IDT Kenacort® (1µg/ml)+ (>15mm) and (10µg/ml)+ (>20mm) -Scratch-test CMC (7,5mg/ml)+ (>5mm)
Bigliardi et al. 2003	Woman 76 y.o.	Sciatalgia	Kenacort® (triamcinolone acetone)	Infiltration	30 min	Anaphylactic reaction	-Prick test Kenacort® - IDT Kenacort® (100µg/ml) + -Prick test CMC (7.5µg/ml)- IDT CMC (0.075µg/ml) + -LHRT CMC - CAST CMC - Immunoblot - OC 136 mg-
Bigliardi et al. 2003	Man 37 y.o.	Shoulder arthralgia	Triamcort- Depot® (triamcinolone acetone)	Infiltration	30 min	Anaphylactic reaction	-Prick Triamcort®-(10 mg/ml)- IDT Triamcort®(100µg/ml) + -Prick CMC (10ug/ml)- IDT CMC (1ug/ml)+ -CAST CMC + Immunoblot -OC 250 mg -
Bigliardi et al. 2003	Man 59 y.o.	Elbow arthralgia	Diprophos® (bethamethasone)	Infiltration	30 min	Anaphylactic reaction	- Prick test Diprophos® + IDT Diprophos® + -Prick test CMC (7.5µg/ml) equivocal -IDT CMC (0.075µg/ml) + -CAST CMC - -Immunoblot - -OC 250 mg
Garcia-Ortega et al. 2003	Man 48 y.o.		Trigon Depot® (triamcinolone acetone)	Intra-articular injection	2 hours	Anaphylactic reaction	- Prick test Trigon® + -Prick test CMC (1mg/l)+ wheal-and-flare local reaction with severe forearm itching and erythema lasting 30 min
Bircher et al. 2004	Man 52 y.o.		Kenacort® (Triamcinolone acetone)	Paravertebral infiltration		Anaphylactic reaction	-Prick-test Kenacort® - -IDT CMC (7.5mg/l, 1:10) +
Opplinger et al. 2004	Woman 20 y.o.	Lichen planus	Kenacort® (triamcinolone acetone)	Infiltration	1 hour	Generalized urticaria	-Prick test Kenacort® - -Prick test CMC - -IDT Kentocort® (0.1µ/l) + - IDT CMC (0.1µg/l) +
Opplinger et al. 2004	Woman 55 y.o.	Epicondylitis	Diprophos® (bethamethasone)	Infiltration	20 min	Anaphylactic reaction	-Prick test Diprophos® + -Prick test CMC + - IDT Diprophos® and CMC-
Venturini et al. 2006	Woman 69 y.o.		Trigon depot® (triamcinolone acetone)	Infiltration		Anaphylactic reaction	-Prick test triamcinolone with CMC + -Prick test CMC +
Venturini et al. 2006	Man 38 y.o.		Trigon depot® (triamcinolone acetone)	Infiltration		Anaphylactic reaction	-Prick test triamcinolone with CMC + -Prick test CMC +
Rival 2008	Man 58 y.o. Woman	Fibrotic nodule Calcaneum osteophytose	Altim® (cortivazol) Altim®	Infiltration infiltration	Unknown <1min	Anaphylaxis anaphylaxis	IDT:+ oral challenge (62 mg):: negative Prick test :++ oral challenge (62 mg):: negative

IDT: intradermal test, BAT: basophil activation test, LHRT: Leucocyte Histamine Release Test, NPT: Nasal Provocation Test, LTT: Lymphocyte Transformation Test, CAST: Cellular Antigen Stimulation Test.

10 microgram/ml. In this study, skin tests with immediate results were negative with up to 1 mg/ml in 6 controls, but positive in our patient at 10 µg/ml. BAT and CAST were also positive to CMC. The presence of specific IgE was first demonstrated by Patterson in 1995 by an immunoblot analysis performed after anaphylactic shock following intra-articular injection of sustained-release corticosteroids (1). Muroi reported finding CMC-specific IgE, using the ELISA technique, in a case of anaphylactic shock following barium enema (2). We present here a third case where CMC-specific IgEs were identified. The identification of specific IgE using a dot-blot analysis shows that CMC is indeed an allergen.

With this technique, the CMC bound non-covalently to the PVDF membrane. Heat-induced binding to a protein gives a weaker result, suggesting that the CMC epitopes are masked by the protein binding. Processing to obtain the free acid form did not modify the allergenicity of CMC.

Muroi et al. searched for specific IgE using ELISA in 387 healthy subjects (25). They showed an incidence of 9%. In their opinion, the combination of CMC-specific IgE and a positive LHRT could identify subjects at high risk of anaphylactic reaction.

In our case, sensitization to CMC probably occurred during intra-articular injections of sustained-release corticosteroids carried out months or years before the barium enema. However, questions remain as to the role of dietary CMC as a long-term sensitizing factor, especially in people with impaired GI tract mucosa. So far allergy to CMC in food has not been reported. Further studies should be however carried out

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# Efficacy, safety and tolerability of sublingual monomeric allergoid in tablets given without up-dosing to pediatric patients with allergic rhinitis and/or asthma due to grass pollen

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## KEY WORDS

*Asthma, carbamylated allergoid, pediatric patients, sublingual immunotherapy, rhinitis*

## SUMMARY

*The efficacy and safety of monomeric allergoid (Lofarma, Milan) have been demonstrated in adults but very few studies have examined it in children. This study therefore investigated the efficacy and safety of this sublingual immunotherapy (SLIT) at the dosage of 1000 AU five times a week without any up-dosing. Forty allergic children (17 M and 23 F, mean age 7 years, range 4-16 years), 16 with rhinitis and 24 with rhinitis and asthma, were randomized to SLIT or drug therapy. All the patients were sensitized to grass; some were also sensitized, though to a lesser extent, to Parietaria, Olea and Betulaceae. The patients were treated pre-/co-seasonally for two years. A visual analogue scale (VAS) was used at baseline and at the end of the first and second pollen seasons to rate the patients' well-being. The VAS score was significantly higher after both the first and the second year of treatment in the SLIT group than in the controls ( $p < 0.05$ ). It improved in comparison to baseline only in the active group. All 40 children tolerated the therapy very well. The monomeric allergoid at the dosage of 5000 AU/week thus appears to have a good efficacy and safety profile in children.*

## Introduction

Specific immunotherapy (IT) is important in the prevention and treatment of respiratory allergy and its clinical value is acknowledged today (1-3). In the last few years new routes of administration have been investigated and developed. The sublingual route (sublingual immunotherapy - SLIT) appeared the most promising alternative to the traditional IT (3-6). Some randomized clinical trials have demonstrated the efficacy and safety of SLIT in the management of respiratory allergy due to grass pollen, at least in adults (7-9).

A decade ago the EAACI-ESPACI position paper (10)

did not recommend SLIT for normal use in pediatric practice, since only a few controlled clinical trials had evaluated its efficacy and safety in children. Many more trials have now been conducted in children, with rhinitis and asthma, and the efficacy and safety are good (11-16). However, some findings are still conflicting in terms of effectiveness, type of allergen and dose (17). The present study in a pediatric population allergic to grass pollen evaluated the efficacy, safety and tolerability of SLIT with a carbamylated monomeric allergoid during two consecutive pollen seasons, employing a dosage of 5000 allergenic units (AU) per week without any build-up phase.

## Materials and methods

### Study design

This prospective, open-label, randomized study included two parallel groups given either SLIT or standard pharmacotherapy, with a history of at least two years of intermittent or persistent rhinitis or rhinoconjunctivitis, and/or mild intermittent or mild persistent allergic asthma for at least one year (18). Both groups were allowed rescue medication on demand for a very short period (no more than a few days). There was no run-in period. All the patients had a baseline evaluation at the beginning of the study (Tab. 1). The endpoints were the occurrence of symptoms in the two groups and the differences between visual analogue scale (VAS) scores in the treated and control groups at baseline and after one and two years. The VAS rating system was used to assess the patients' well-being before and after therapy and thus, indirectly, the severity of symptoms during the SLIT. The best possible score for well-being was 10 and the worst 0.

### Patients

Forty allergic children were enrolled (17 M and 23 F, mean age 7 years, range 4-16 years), 16 with rhinitis only and 24 both rhinitis and asthma. The allergies were caused by grass pollen in most of the patients. All 40 were in fact sensitized to grass as confirmed by a positive (>3 mm) skin prick test response (Lofarma S.p.A., Milan) and positive CAP-RAST assay (class II or greater) (CAP System EIA, Pharmacia, Uppsala, Sweden). Twenty percent of the patients were sensitized to other seasonal allergens such as Parietaria, Olea and Betulaceae,

though to a lesser extent and without any associated symptoms.

Children with systemic or immunological diseases, major anatomical alterations of the upper airways, renal insufficiency, coronary heart disease, neurologic or psychiatric diseases, or requiring chronic corticosteroids were excluded from the study. The children's parents signed an informed consent form before the child entered the study.

### Investigational SLIT and concomitant pharmacotherapy

The SLIT consisted of a monomeric carbamylated allergoid (Lais®, Lofarma S.p.A., Milan) biologically standardized (19) in AU and prepared as soluble tablets for oral use (allergoid SLIT). The tablets were taken in the morning on an empty stomach and kept under the tongue for 1-2 minutes so they dissolved before swallowing. There was no build-up phase. Patients were treated pre/co-seasonally for 12 weeks/year for two consecutive years. The maintenance dosage was 1000 AU five times a week for 12 weeks in each pollen season (total amount of allergen 60,000 AU/year). Treatment started eight weeks before the pollen season and continued for four weeks during it.

Rescue medication, used as needed to control acute symptoms, was as follows: cetirizine or desloratadine tablets, inhaled salbutamol, intranasal fluticasone. A short course of systemic steroid was allowed (1 mg/kg daily for three days) for severe symptoms that did not respond to standard treatment.

### Clinical evaluation

The patient's parents were required to record the presence and severity of symptoms on a special diary form each day during the pollen season. The following symptoms were considered: sneezing, rhinorrhea, obstruction, tearing, cough, nocturnal and diurnal asthma. Each symptom was rated from 0 (absent) to 3 (severe). Parents were also asked to complete the VAS and record any adverse events (AE). AE were classified as local (oral itching, swelling of the tongue) and systemic (asthma, rhinitis, urticaria, abdominal pain/diarrhea, anaphylaxis).

### Statistical analysis

The Mann-Whitney U test for intergroup comparison was used to establish whether a particular variable differed significantly between the two populations (active and

**Table 1** - Patients characteristics at baseline

	5000 AU/Week (for 12 weeks)	Controls	P
Patients (n.)	20	20	NS
Sex (M/F)	9/11	8/12	NS
Mean Age (years ± SD)	9.1± 3.6	4.8±1.5	NS
Weight (KG)	36±14.8	19.6±5.6	NS
Height (cm)	139±22.0	113±11.3	NS
<i>Disease:</i>			
Rhinitis without asthma	9	7	NS
Rhinitis with asthma	11	13	NS



control) at baseline. The Wilcoxon Signed Ranks test was used to evaluate the differences in VAS scores within each group and the Mann-Whitney test to analyze these differences between cases and controls.

## Results

The VAS score was significantly higher throughout the treatment period in the allergoid SLIT group than the control group ( $p < 0.05$ ). It improved from baseline only in the SLIT group (Fig. 1). The global symptom score was slightly lower in the active group but the difference from controls was not significant (Fig. 2). All 40 children tolerated the therapy very well, with no systemic or local AE.

## Discussion

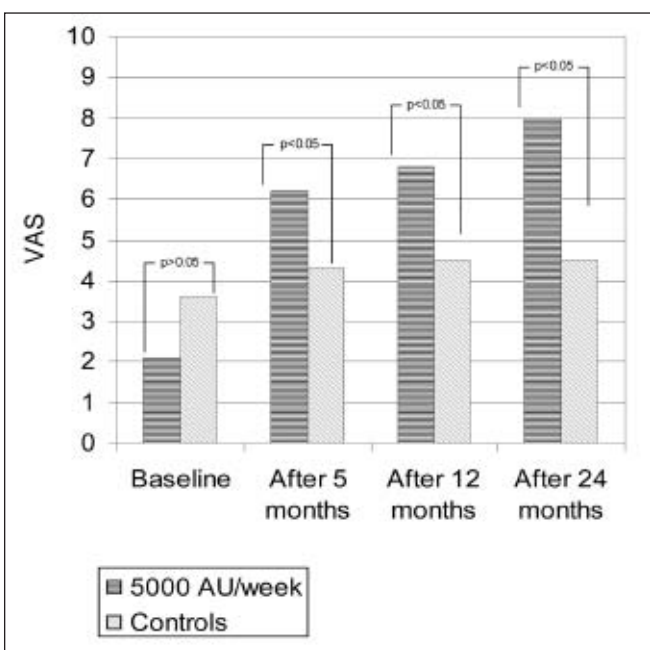
The clinical efficacy and safety of SLIT with oro-soluble tablets, with no up-dosing phase, has been demonstrated in recent placebo-controlled, randomized clinical trials in large numbers of adults allergic to grass pollen (7-9). SLIT improves the patients' quality of life (6) and can prevent the development of asthma in children with aller-

gic rhinoconjunctivitis (20). That SLIT is effective in pediatric patients was shown by the two meta-analyses of Penagos et al. in rhinitic and asthmatic children (15, 16), and by Whan et al. in 278 children and adolescents with grass-pollen induced rhinitis treated with SLIT in tablets; there was significant improvement of allergic symptoms during the pollen season and no serious AE (14).

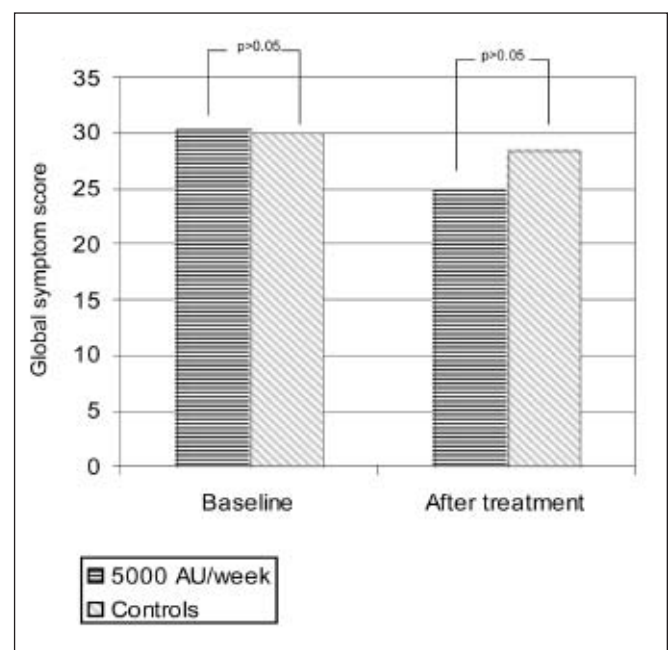
The present trial, though it did not show any dramatic improvement in the symptom score in the SLIT patients, found a significant increase in the VAS ratings whose reliability in assessing the efficacy of treatment for allergic rhinitis was recently reported by Bousquet et al. (21).

Side effects, always a deterrent to using SLIT in children, have been very few in most studies to date, particularly in those using the monomeric allergoid (5, 6, 8-12). This can probably be ascribed to the low IgE-binding activity of the active ingredient (19) which prevents the IgE-mediated allergen presentation by dendritic cells to  $T_H2$  cells, which is the key mechanism explaining the large increase of allergen-specific IgE observed during SLIT with native grass allergens (8-10). No AE were observed in present study despite the absence of a build-up phase. This allows us to suggest that in the future treatment might start even in children younger than five years old, with possible benefits for preventing the "allergic march" and new sensitizations.

**Figure 1** - VAS mean values at baseline and after 5, 12 and 24 months of treatment in the 2 groups of patients



**Figure 2** - Global symptom score in the 2 groups of patients before and after treatment



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# Antihistamines do not inhibit the flare induced by the intradermal injection of autologous plasma in chronic urticaria patients

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## KEY WORDS

*Chronic urticaria, autoreactivity, skin testing, histamine*

## SUMMARY

**Background:** There is some evidence suggesting that factors other than autoantibodies to FcεRI or IgE and histamine released from mast cells may play a role in skin autoreactivity that characterizes many patients with chronic urticaria (CU) and, possibly, in the pathogenesis of this disease. **Objective:** The effect of antihistamine treatment on autologous plasma skin test (APST) in patients with CU was assessed. **Methods:** 24 patients with CU underwent autologous plasma skin test (APST) as well as SPT with histamine 10 mg/ml while taking antihistamines. In 6 cases the same tests had been carried out also before the start of antihistamine treatment. Plasma levels of D-dimer, prothrombin F1+2 fragment, and vascular endothelial growth factor (VEGF) were measured in 21 patients. **Results:** 21/24 (87%) patients showed a large flare on APST while taking antihistamines while the skin reaction to histamine 10 mg/ml was abolished or negligible. Little difference in the autologous plasma-induced flare was seen before and after the start of cetirizine therapy in 6 cases, whereas the drug exerted a marked effect on the histamine SPT as well as on the autologous plasma-induced wheal. The APST-induced flare was not associated with patients' response to antihistamine. Plasma levels of VEGF, prothrombin F 1+2 fragment, and D-dimer were increased in plasmas from 8, 9, and 2 patients, respectively. **Conclusions:** Factors other than histamine are probably involved in the flare following APST in CU; such factors might play a pathogenic role particularly in patients not responding to standard antihistamine treatments.

## Introduction

Chronic urticaria (CU), defined as the recurrent occurrence of short-lived wheals with or without angioedema for more than 6 weeks, has remained an obscure disorder until Grattan and co-workers observed that the intradermal injection of autologous serum (ASST, autologous serum skin test) caused a wheal-and-flare reaction in a

proportion of patients (1). This prompted the presence of circulating histamine-releasing factors, which was confirmed by the following detection of functional IgG autoantibodies to IgE (2) and/or to the high affinity IgE receptor, FcεRI (3-5). This finding provided an immunological pathogenic basis for at least a proportion of patients with this disease. Although some scientists suspect that all CUs might be autoimmune in origin (6), autoantibodies

can be detected in less than 50% of sera from CU patients (5-12), and a number of observations seem to put their clinical role into question. FcεRI autoantibodies can be detected also in clinical conditions other than CU, such as autoimmune diseases and bullous dermatoses (13), and even in normal subjects (14, 15), although in these cases they seem not functional. Further, sera from CU patients containing FcεRI autoantibodies are still able to induce a wheal-and-flare reaction upon ASST after depletion of IgG (7). Finally, while CU sera causing histamine release from cultured human basophils in-vitro score regularly positive on ASST, only a proportion (about 50%) of ASST-positive sera induce histamine release in-vitro (12). All these observations suggest that skin autoreactivity occurs also in the absence of circulating autoantibodies and point to the possible involvement of factors other than autoantibodies in the pathogenesis of CU.

Recent observations that in CU patients the intradermal injection of autologous plasma anticoagulated with Na citrate (APST, autologous plasma skin test) produces a wheal and flare reaction much more frequently than ASST (16) led to detect an activation of the coagulation cascade via the extrinsic pathway in this disease (16-19). In view of these findings, factors other than histamine, such as thrombin, have been suggested as potential mediators of vasodilatation in CU. The present study adds further evidence to this concept showing that in CU the skin reaction produced by the intradermal injection of autologous plasma is only partially inhibited by histamine.

## Methods

### *Patients*

24 patients (M/F 5/19; mean age 51,8 years, range 27-85 years) with chronic urticaria seen at the allergy department of the Clinica San Carlo were studied. The diagnosis of CU was based on the presence of recurrent wheals with or without angioedema for more than 6 weeks. Eighteen patients were taking antihistamines (cetirizine 10 mg daily in all cases but 2 that were taking desloratadine 5 mg daily) at the time of the first visit and were unable to discontinue the treatment due to the immediate relapse of their disease. The remaining 6 patients were not taking antihistamines at the time of the first visit. Clinical activity of CU was assessed according to Sabroe et al.: 1-10 small (< 3 cm in diameter) wheals = grade 1 (slight); 10-50 small wheals or 1-10 large wheals = grade 2 (mod-

erate); > 50 small wheals or > 10 large wheals = grade 3 (severe) (20).

### *Skin tests*

All 24 patients underwent intradermal testing with 0.05 ml of fresh autologous plasma anticoagulated with Na citrate (APST, autologous plasma skin test) as previously described (16); an intradermal test with 0.05 ml of saline as well as a SPT with histamine 10 mg/ml were carried out in all cases as negative and positive control, respectively. The 18 patients who were unable to stop antihistamine treatment underwent skin tests while taking their therapies, whereas the remaining 6 underwent the skin tests both at the first visit and 7 days after the start of antihistamine treatment (cetirizine 10 mg daily). All patients gave an informed consent before the skin tests.

Readings were taken at 15 minutes when the wheal-and/or-flare skin reaction diameters were measured. Even though a 30 minutes reading has become a standard practice, the wheal-and-flare response usually appears within 10 minutes (21) and we have taken readings at 15 minutes also in previous studies with excellent results (12, 22). Further, although a wheal-and-flare reaction is generally needed to regard as positive the skin response following an autologous serum skin test (23), in view of the antihistamine treatment taken by our study patients, the clinical criteria for a positive skin test were slightly changed, and a clear-cut flare in absence of a palpable wheal was considered as a positive skin response if the intradermal injection of saline did not produce any appreciable skin reaction.

### *In-vitro tests*

Plasma levels of D-dimer, prothrombin F1+2 fragment, and vascular endothelial growth factor (VEGF) were measured in 21/24 patients.

D-dimer levels were measured by ELISA (Enzygnost D-dimer; Behring Diagnostics GmbH) according to manufacturer's instructions. The intra- and inter-assay coefficients of variation were 10% and 15%, respectively.

Prothrombin fragment F<sub>1+2</sub>, a marker of thrombin generation, was measured by a sandwich immunoenzymatic assay (Enzygnost F<sub>1+2</sub>; Behring Diagnostics GmbH, Frankfurt, Germany) according to manufacturer's instructions. Intra-assay and inter-assay coefficient of variations were 5% and 8% respectively. The measuring range of the assay is between 20 and 1200 pmol/L.



**Table 1** - Patients, skin tests without and with antihistamines, and response to treatment

Patient	Sex/age	APST (no therapy)	SPT H (no therapy)	APST (therapy)	SPT H (therapy)	Response
1	F/34	ND		flare 20 x 20 mm	Negative	Absent
2	F/37	flare 20 x 20 mm	12mm	flare 20 x 20 mm	1mm	Good
3	F/76	ND		flare 10 x 10 mm	Negative	Good
4	F/69	flare 15 x 15 mm	10 mm	flare 10 x 10 mm	2 mm	Good
5	F/62	flare 20 x 20 mm	12 mm	flare 15 x 10 mm	5 mm	Good
6	F/51	ND		flare 30 x 30 mm	2mm	Poor
7	F/29	ND		flare 40 x 40 mm	4 mm	Poor
8	F/41	ND		flare 20 x 20 mm	2 mm	Good
9	F/27	ND		flare 15 x 15 mm	2 mm	Good
10	F/51	ND		flare 12 x 12 mm	2 mm	Poor
11	M/54	ND		flare 8 x 8 mm	2 mm	Sufficient
12	F/32	ND		flare 10 x 10 mm	2 mm	Sufficient
13	F/64	ND		flare 10 x 10 mm	2 mm	Good
14	F/85	ND		flare 14 x 14 mm	4 mm	Sufficient
15	F/60	ND		flare 18 x 10 mm	3 mm	Good
16	F/27	flare 15 x 15 mm	11 mm	Negative	2 mm	Good
17	F/65	ND		flare 10 x 10 mm	3 mm	Good
18	M/36	ND		flare 12 x 12 mm	Negative	Good
19	F/47	flare 25 x 20 mm	14 mm	flare 20 x 20 mm	3 mm	Sufficient
20	F/62	ND		Negative	Negative	Poor
21	M/59	flare 20 x 20 mm	14 mm	flare 8 x 8 mm	5 mm	Poor
22	M/44	ND		flare 14 x 12 mm	4 mm	Poor
23	M/67	ND		Negative	2mm	Sufficient
24	F/64	ND		flare 12 x 10 mm	5 mm	Sufficient

The response to antihistamine treatment was considered good if the drug fully controlled the disease, sufficient in case of a significant reduction but not of complete disappearance of wheals, and poor in case of a lack of response.

VEGF concentration was measured by a sandwich enzyme immunoassay (R&D Systems, Inc., Minneapolis, MN, USA), according to manufacturer's instructions. Intra-assay and inter-assay coefficients of variation were 5% and 7%, respectively. The detection limit of the assay is less than 0.1 pmol/L and the upper limit is 22.2 pmol/L. The assay employs a monoclonal antibody, pre-coated onto a microplate, and an enzyme-linked polyclonal antibody conjugated to horseradish peroxidase, both specific for VEGF. After drawing venous blood from the subjects under examination, plasma was frozen at  $-80^{\circ}\text{C}$  until assayed for VEGF concentration. Mean plasma VEGF level in 53 normal subjects was  $0.54 \pm 0.08$  pmol/l (range 0.1-2.11).

#### Statistics

Proportions were compared by  $\chi^2$ -test with Yates' corrections. Probability (p) values less than 5% were considered statistically significant.

## Results

### Skin tests

At the time of skin testing and blood drawing, all patients were under antihistamine treatment and the clinical score ranged between 0 and 1. Twenty-one out of 24 (87%) patients showed a marked skin reaction on APST while taking antihistamines. Interestingly, the skin reaction induced by autologous plasma consisted of a large flare (diameter range 8 x 8 mm - 40 x 40 mm) with little or no wheal; in contrast, not surprisingly, the skin reaction to histamine 10 mg/ml was abolished or negligible (diameter < 3 mm) under antihistamine therapy in most cases (Tab. 1). A typical case is shown in figure 1.

The 6 patients who were examined both before and after the start of cetirizine treatment showed a marked wheal-and-flare reaction upon intradermal injection of autologous plasma while off antihistamine treatment (the flare

**Figure 1** - A typical case of persistence of APST-induced flare in a patient treated with cetirizine 10 mg daily. Autologous plasma (P) induces a flare that largely exceeds that induced by a SPT with histamine 10 mg/ml (H). The intradermal injection of saline does not cause any visible skin reaction



diameter is shown in table 1); in these patients histamine SPT induced an intense skin reaction as well. Cetirizine treatment abolished or markedly reduced the skin response to histamine. Interestingly, the drug abolished the palpable wheal in all 6 patients but exerted a variable effect on the flare induced by autologous plasma which was abolished in 1 case (no. 16), slightly reduced in 3 patients (4, 5 and 21), and virtually unchanged in 2 cases (no. 2 and 19).

The intradermal injection of saline did not cause any skin reaction in all patients either taking or not taking antihistamines.

The flare response induced by autologous plasma was not significantly associated with patients' response to antihistamine treatment (Tab. 1).

#### *Plasma measurements*

Levels of VEGF, prothrombin F 1+2 fragment, and D-dimer were increased in plasmas from 8, 9, and 2 patients, respectively. Interestingly the two patients showing elevated D-dimer levels showed increased plasma levels of both VEGF and F 1+2 as well. Elevated plasma levels of both VEGF and F 1+2 were observed only in 2 further cases (Tab. 2).

**Table 3** - Plasma VEGF, F 1+2, and D-dimer levels in the 24 study patients

Patient	VEGF (pmol/l)	F 1+2 (pmol/L)	D-dimer (pmol/l)
1	5.390	119.82	0.17
2	0.37	152.24	0.63
3	2.9	138.82	0.69
4	0.33	173.61	0.88
5	8.11	145.25	0.75
6	0.1	121.53	1.62
7	6.97	134.31	0.34
8	0.66	247.84	1.49
9	0.1	233.72	0.85
10	43.1	422.13	10.39
12	0.32	280.42	0.69
13	1.55	214.69	2.02
14	5.66	358.43	5.19
15	2.45	304.65	2.01
16	0.80	387.25	0.24
19	0.30	83.14	0.78
20	4.43	309.98	1.79
21	1.09	163.82	1.14
22	2.39	110.22	0.38
23	5.46	179.86	0.37
24	0.1	293.82	0.50
Mean	4.88	217.88	1.56
SD	9.66	98.69	2.29
SEM	2.1	21.53	0.50
Median	1.55	179.86	0.78

Mean VEGF plasma level in 53 healthy subjects was 0.54±0.08 pmol/l (range 0.1-2.11).

F 1+2 normal range: 69 - 229 pmol/L.

D-dimer normal range: 0.5-4 pmol/l.

#### **Discussion**

In this study we found that in most patients with chronic urticaria treated with antihistamines the intradermal injection of autologous plasma still induces a clear-cut flare. The clinical effect of antihistamine therapy was shown by the markedly reduced or absent skin reaction to histamine 10 mg/ml. Further, although few patients were studied in this sense, cetirizine treatment caused the disappearance of the palpable wheal induced by the intradermal injection of autologous plasma, whereas the APST-induced flare persisted. This observation suggests that the flare

(vasodilation) elicited by the intradermal injection of autologous plasma is only partially dependent on histamine release from skin mast cells. The vasodilation induced by autologous plasma upon intradermal injection was not associated with patient's better or worse response to antihistamine treatment nor was associated with disease severity. This finding supports a role played by vasoactive factor(s) other than histamine which are present in plasma of most patients with chronic urticaria; it is possible that this substance plays a role in amplifying the histamine-induced vasodilation in CU and may be responsible for the limited response to antihistamine treatment that characterizes some CU patients. We recently found that both F 1+2 prothrombin fragment and D-dimer plasma levels may be increased in patients with CU as a result of a (sometimes) massive activation of the coagulation cascade by the extrinsic pathway (16-18), and that CU is frequently associated with elevated VEGF levels, possibly as a result of the activation of eosinophils (19, 24). All these phenomena are associated with disease severity. Thus, in view of their potential vasoactive properties, we investigated whether one of these 3 substances was associated with the flare induced by autologous plasma on intradermal injection in patients unable to stop antihistamine treatment. Our plasma measurements showed that VEGF and F 1+2 were frequently elevated, however it was not possible to detect a clear association with the skin reaction induced by autologous plasma. Other potential candidates responsible for histamine-independent vasodilation induced by the intradermal injection of autologous plasma include neuropeptides released from sensory nerves of the skin. However, in a previous study we were unable to detect any increase in circulating substance P in most patients with CU (25), and other groups have shown that the wheal-and-flare reaction induced by substance P is inhibited by cetirizine (26), although this effect was not observed with hydroxyzine (27). Interestingly, it has been shown that CU serum is able to induce de-novo synthesis of sulfidoleukotrienes (28), however, in the same study, such effect was inhibited by the anti-histamine mizolastine which does not seem to correspond to our present observations.

Previous studies showed that in CU patients positive on autologous serum skin test (ASST) the intradermal tests with heparin-anticoagulated plasma always scores negative, suggesting that heparin inhibits autoreactivity in-vivo (11, 12). Interestingly, heparin exerted its inhibitory effect in all ASST-positive patients irrespective of the ability of their sera to induce histamine release in-vitro on cul-

tured basophils, but did not inhibit the wheal-and-flare reaction induced by a SPT with histamine nor the skin reaction induced by a SPT with a specific allergen extract in an allergic subject (12). These observations prompted that histamine is not the target of heparin, at least in-vivo; in effect, besides its well-known anticoagulant activities, heparin is able to interact with a number of plasma proteins and mast cell surface components. These previous findings fit rather well with the recent observation of an activation of the coagulation cascade with generation of thrombin in CU patients (16-19) as, in experimental models, thrombin has been shown to induce edema through an increase in vascular permeability, to trigger mast cell degranulation, to activate protease activated receptor-1 on mast cells, and to generate C5a in the absence of C3, thus bypassing the whole first part of the complement cascade (29-33).

In conclusion, along with histamine, vasoactive substances other than histamine seem to be involved in the marked vasodilation induced by autologous plasma upon intradermal injection in CU; it is possible that such substances play a pathogenic role in the disease and may particularly relevant in patients showing a poor response to antihistamines.

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# Paradoxical exacerbation of chronic urticaria by H1-antihistamines and montelukast

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## KEY WORDS

*Chronic urticaria, H1-antihistamines, montelukast, ciclosporin, azathioprine*

## SUMMARY

*Histamine is the main mediator of urticaria and H1-receptor antagonists represent the treatment of choice in all patients with chronic urticaria. Leukotriene receptor antagonists as montelukast have also been used in patients with chronic urticaria unresponsive to H1-antihistamines alone. We report a patient with chronic urticaria whose disease was paradoxically exacerbated by H1-antihistamines and montelukast, and controlled by immunosuppressive drugs as ciclosporin and azathioprine. Urticaria exacerbations were caused by different molecules including either piperidine (fexofenadine, desloratadine, ebastine, rupatadine) or piperazine (hydroxyzine, cetirizine) derivatives as well as by montelukast suggesting that an IgE-mediated mechanism was not involved. A possible explanation of the observed urticaria exacerbation is that H1-antihistamines and montelukast may shift the H1 histamine receptor and the leukotriene receptor to the active conformation instead of the inactive state. The beneficial effects of ciclosporin and azathioprine confirm that immunosuppressive drugs have an important role in the treatment of refractory chronic urticaria and back the hypothesis that an autoimmune/autoreactive mechanism often underlies the disease.*

## Introduction

Histamine is recognized as the main mediator of urticaria, and the treatment of choice in all patients with chronic urticaria is represented by H1-receptor antagonists. In most cases chronic urticaria can be sufficiently controlled by the use of antihistamines at licensed doses or, in some cases, at higher than licensed doses, but this approach is not always effective (1-4). In these cases, all guidelines published so far recommend systemic corticosteroids as the second line treatment and immunosuppressive drugs, namely ciclosporin, as the third line treatment. In addition to the lack of effectiveness, a few cases of

multiple H1-antihistamine-induced urticaria have been reported (5-9).

We report a patient with chronic urticaria whose disease was exacerbated by H1-antihistamines and controlled only by immunosuppressive drugs including ciclosporin and azathioprine.

## Case report

In the late spring 2008, a 23-year-old man was seen at the Allergy outpatient clinic because of uncontrolled chronic urticaria. He reported recurrent urticaria with angioedema

since the age of 14 and daily urticaria symptoms in the last year. He had already undergone extensive investigations for chronic urticaria, including specific IgE determination for food allergens, serological test for B and C hepatitis and human immunodeficiency virus, complement C3 and C4 fractions and C1 inhibitor, search for *Helicobacter pylori* and stool parasites, thyroid function and thyroid autoantibodies, and antinuclear antibodies. All these tests were in the normal range or negative. Total IgE level was 9 kU/L. Because of continuous urticaria, the patient was prescribed on different occasions almost all H1-antihistamines available in Italy, including cetirizine, hydroxyzine, desloratadine, fexofenadine and ebastine. In all cases the H1-antihistamines not only failed to control the disease, but provoked a severe urticaria exacerbation within one-three hours after administration. Since continuous treatment with prednisone allowed only partial relief of the disease, ciclosporin was started in the fall 2007. This led to a complete control of the disease, but only with a relatively high dosage (6 mg/kg/daily). After a six months treatment, following the detection of raised ciclosporin plasma levels, the drug was gradually tapered, and the disease relapsed. At that time the patient sought advice at our Allergy Clinic. Autologous serum and plasma skin tests were performed as described (10, 11) and gave an unequivocal positive response (at 30 min reading the diameter of the serum-induced wheal was 8 mm and the diameter of the plasma-induced wheal was 11 mm). As a negative control skin test, saline solution (0.9% weight/volume NaCl) was injected intradermally, and caused no detectable wheal at 30 min reading. Skin prick test with 10 mg/ml histamine was performed as positive control (the wheal diameter at 30 min reading was 5 mm). Positivity of autologous serum and plasma skin tests supported the autoreactive origin of urticaria, since autologous serum skin test has been considered as a screening test for histamine-releasing autoantibodies (10, 12). The patient received continuous prednisone treatment at variable doses (10-37.5 mg daily) which allowed a partial control of the disease. A further attempt to reintroduce antihistamine therapy using the recently licensed rupatadine was again followed by urticaria exacerbation within few hours from drug intake. Similarly, the addition of the leukotriene receptor antagonist, montelukast, 10 mg/day was followed by worsening of urticaria symptoms. Then, in January 2009, following a report on the efficacy of azathioprine in the management of anti-histamine resistant urticaria (3), treatment with azathioprine 100 mg daily was started. The patient experienced a gradual improvement of the

disease that allowed steroid tapering until withdrawal (June 2009). Azathioprine has been well tolerated and the patient is no longer complaining of any urticaria symptom. The dosage has been gradually reduced and now (October 2009) the patient is assuming 50 mg daily.

## Discussion

The case reported is peculiar in that chronic urticaria was exacerbated by H1-antihistamines that are commonly considered as the cornerstone of the treatment strategy. A few cases of urticaria induced by H1-antihistamines have been reported and in some cases an IgE-mediated mechanism has been suspected since positive skin prick tests have been found (8-9). However, in our case an IgE-mediated mechanism is unlikely since exacerbations were caused by different molecules including either piperidine (fexofenadine, desloratadine, ebastine, rupatadine) or piperazine (hydroxyzine, cetirizine) derivatives. Furthermore, the timing of urticaria worsening (one to three hours after administration) was slower than that observed in most IgE-mediated reactions. H1-antihistamines are inverse agonists of histamine at H1 binding sites, and combine to H1 receptors to shift the equilibrium toward the inactive state, preventing H1 response (13). An interesting explanation of the paradoxical effect of H1-antihistamines has been proposed by González de Olano et al. (6) who have suggested that in rare cases antihistamines may shift the H1 histamine receptor to the active conformation instead of the inactive state, causing urticaria exacerbation. It is interesting to note that our patient also experienced urticaria worsening after montelukast administration. De-novo synthesis of sulfidoleukotrienes has been detected in chronic urticaria (14) supporting their involvement in the disease pathomechanism. The exacerbation of urticaria symptoms that occurred in our patient following montelukast administration might be explained by a shift to the active state of leukotriene receptors, as it has been hypothesized for H1 histamine receptors. Finally, the disease control that was achieved in our patient firstly with ciclosporin and then with azathioprine is not surprising since immunosuppressive drugs have been largely used in recalcitrant chronic urticaria, and quite a large experience has been collected with ciclosporin (15). Tacrolimus, micofenolate and high- and low-dose intravenous immunoglobulin are among the other treatment options that have been considered (16-19); conversely, the experience with azathioprine is limit-

ed and deserves to be expanded. In the case reported, both serum and plasma skin tests were positive supporting the autoreactive origin of chronic urticaria. In fact, autologous serum skin test has been proposed as a screening test for histamine-releasing autoantibodies (12) and has been found positive in about 50% of chronic urticaria patients whose disease is considered of autoimmune/autoreactive origin (10). Notably, a positive autologous serum skin test has been also found in about 50% of patients with multiple drug hypersensitivities and in patients with chronic urticaria and nonallergic asthma (20, 21), disorders that may be at least in part sustained by an autoimmune/autoreactive mechanism. The meaning of autologous plasma skin test still needs to be investigated but appears to be related to circulating vasoactive factors and possibly to coagulation factors (11). The favorable response to ciclosporin and azathioprine observed in our patient can be explained by the suppressive effect on the autoimmune/autoreactive mechanism involved in the disease pathophysiology. When H1-antihistamines fail to control or even worsen chronic urticaria symptoms, immunosuppressive drugs still remain a good therapeutic option that can allow achieving disease remission.

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# News

ACAAI ANNUAL MEETING, NOV. 5-10, 2009

*Miami Beach Convention Center in Florida*

The latest developments in the treatment of allergic diseases including asthma and immunologic disorders presented at the American College of Allergy, Asthma and Immunology (ACAAI) Annual Meeting.

## **New Developments Improve Food Allergy Management**

MIAMI BEACH, Fla. – Less restrictive dietary options, better detection, targeted avoidance measures, educational directives and potential new therapies are improving food allergy management and giving hope to the more than 12 million Americans affected according to experts at the thirteenth international food allergy conference held during the annual meeting of the American College of Allergy, Asthma and Immunology (ACAAI) in Miami Beach, Fla.

“The management of food allergy relies primarily on avoidance of exposure to suspected or proven foods,” said Alessandro Fiocchi, M.D., director of the Pediatric Department at The Melloni University Hospital in Milan, Italy. “This can best be done if the specific foods responsible for the patient’s symptoms are identified by history and appropriate tests.”

Not all foods a patient is sensitized to should be eliminated, and not all sensitized patients should be on a diet, said Dr. Fiocchi. Patients may not need to avoid all in a specific food group, such as different kinds of fish for a person with fish allergy.

ACAAI President-Elect and Program Chair Sami L. Bahna, M.D., Dr.P.H., professor of pediatrics & medicine, and chief of allergy & immunology at Louisiana State University Health Sciences Center in Shreveport, La., said food allergy must sometimes be investigated even without an apparent relationship to eating.

## **Diagnosing Food Allergy**

“The allergist must be a good detective in discovering the cause of some reactions, often seeing a patient multiple times to com-

pile a detailed medical and environmental history. Food allergens can be hidden, very minute, or cross-reactive with other food allergens,” Dr. Bahna said.

“We have seen cases where food allergy is caused by skin contact or smell, such as an allergy to fish, shellfish, egg or milk. Even a touch can be risky to patients with severe food allergy, especially to peanuts,” he said.

An allergist, an expert in the diagnosis and treatment of allergies and asthma, can perform allergy testing to identify the specific food and additives that trigger allergic reactions and determine the most appropriate and effective food allergy management procedures.

## **Diet Restrictions**

Allergists may refer food allergy patients on restricted diets to a dietitian for a nutrition assessment to assure they are getting proper nutrition. Referrals to a dietitian with experience in food allergy may include patients in the following situations:

- Diagnosis of a food allergy at any age for education on allergen avoidance
- Mother of an allergic child who is breastfeeding and following a restricted diet
- Considering discontinuation of a nutrition formula to an alternative beverage
- Poor growth.

“Restricting common dietary staples creates potential for nutritionally suboptimal diets,” said Mandy Monty, R.D., L.D., Nutrition Therapy at Cincinnati Children’s Hospital Medical Center in Cincinnati, Ohio.

A dietitian will review age appropriate portion sizes in an elimination diet and explore alternative sources for calories, protein and nutrients, including calcium. Patients usually benefit from a sample allergen-free meal plan and a list of family resources, Ms. Monty said.

Patients with food allergy must also be educated about the label law titled “Food Allergen and Consumer Protection Act” effective Jan. 1, 2006. The law requires food manufacturers to identi-



fy eight major allergens, which are: egg, milk, peanut, tree nut, fish, shellfish, soybean and wheat. Flavorings, additives, colorings and spices are no longer exempt.

### Prevalence of Food Allergy

The prevalence of food allergy is 6 percent to 8 percent of young children, and 2 percent to 3 percent of adolescents and adults, and appears to be rising sharply according to Robert A. Wood, M.D., professor of pediatrics & international health, and director, pediatric allergy and immunology, at Johns Hopkins University in Baltimore, Md.

The prognoses for the resolution of milk, egg, wheat and soy allergy are worse in more recent studies than previously reported," said Dr. Wood. "Whether these findings represent a true change in the natural history of these allergies, or a unique, highly atopic population, remains to be determined. Peanut allergy is less often outgrown, but more often than previously thought," he said.

The loss of food allergy is complete tolerance to a food that previously caused a clinical reaction said Dr. Wesley Burks, MD, professor and chief, pediatric allergy and immunology at Duke University Medical Center in Durham, N.C. Peanut allergy is outgrown in 20 percent of young children, generally by school age, whereas 60 percent of children outgrow milk, egg, wheat and soy allergies.

### Potential Therapies

Food allergy is the most common cause of visits for anaphylaxis treated in Emergency Departments. Nearly 15 percent of patients per year have accidental reactions. "Investigations are being conducted on potential therapies for food allergy with the goal of developing an active treatment by means of desensitization or increased tolerance to protect patients from accidental exposures," Dr. Burks said. Treatment options under investigation include allergen non-

specific therapies that would be effective for any food allergy include anti-IgE and certain preparations of Chinese herbal medicine.

Studies indicate anti-IgE monoclonal antibody therapy may be effective in 75 percent of patients, but it must be given on a continuous basis, and there are concerns about its safety and cost. Future anti-IgE treatments for food allergy may be utilized in combination with other immunotherapy treatments.

"Herbal remedies used in Asia for centuries are under investigation in the United States. A study of Chinese medicine FAHF-2 used in a mouse model for peanut allergy worked to prevent symptoms of a reaction, and we are seeing favorable results in early human studies," Dr. Burks said.

Therapies that are allergen-specific include heat-denatured protein, sublingual immunotherapy (SLIT), engineered recombinant protein, and oral immunotherapy (OIT).

Investigations into the use of baked or extensively heated food for daily ingestion in certain patients are successfully promoting desensitization and or tolerance to foods, such as milk and egg products.

In food allergy, the risks of traditional immunotherapy (subcutaneous injections of intact allergen) have far outweighed the benefits, but new approaches under investigation look promising.

Several preliminary studies on oral or sublingual immunotherapy for food allergy have very encouraging results, with strong evidence of at least short term desensitization. Investigators are looking carefully at the safety of dosing and working on establishing initial, build-up and maintenance protocols for peanut allergy," Dr. Burks said.

Using an "engineered" peanut protein in a mouse model of peanut allergy, the "new" proteins worked to help prevent anaphylaxis in the peanut-allergic mice, he noted.

"The work on the development of a treatment for food allergy is progressing rapidly and is very encouraging," Dr. Burks said.

### Manuscript Reviewers 2009

*The Editors wish to thank the following colleagues for their help in reviewing the articles submitted to European Annals of Allergy and Clinical Immunology.*

Leonardo Antonicelli  
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# AUTHOR GUIDELINES

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**European Annals of Allergy and Clinical Immunology** also publishes solicited and unsolicited review articles on subjects of topical interest to clinical and experimental allergy.

## Manuscript

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For example:

Bodtger U, Linneberg A. Remission of allergic rhinitis: An 8-year observational study. *J Allergy Clin Immunol* 2004; 114(6): 1384-8.

- for books: name of the author/editor, title, publisher/institution, town where published, year of publication, first and last page numbers of the work.

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Paupé J, Scheinman P (Eds.). *Allergologie Pédiatrique*. Flammarion, Paris, 1988: 324-42.

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