

# Coseasonal sublingual immunotherapy reduces the development of asthma in children with allergic rhinoconjunctivitis

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**Background:** We wondered whether short-term coseasonal sublingual immunotherapy (SLIT) can reduce the development of asthma in children with hay fever in an open randomized study.

**Objective:** We sought to determine whether SLIT is as effective as subcutaneous immunotherapy in reducing hay fever symptoms and the development of asthma in children with hay fever.

**Methods:** One hundred thirteen children aged 5 to 14 years (mean age, 7.7 years) with hay fever limited to grass pollen and no other clinically important allergies were randomized in an open study involving 6 Italian pediatric allergy centers to receive specific SLIT for 3 years or standard symptomatic therapy. All of the subjects had hay fever symptoms, but at the time of study entry, none reported seasonal asthma with more than 3 episodes per season. Symptomatic treatment was limited to cetirizine, loratadine, nasal budesonide, and salbutamol on demand. The hay fever and asthma symptoms were quantified clinically.

**Results:** The actively treated children used less medication in the second and third years of therapy, and their symptom scores tended to be lower. From the second year of immunotherapy, subjective evaluation of overall allergy symptoms was favorable in the actively treated children. Development of asthma after 3 years was 3.8 times more frequent (95% confidence limits, 1.5-10.0) in the control subjects.

**Conclusions:** Three years of coseasonal SLIT improves seasonal allergic rhinitis symptoms and reduces the development of seasonal asthma in children with hay fever. (*J Allergy Clin Immunol* 2004;114:851-7.)

**Key words:** Prevention, specific immunotherapy, sublingual, asthma, rhinitis

## Abbreviations used

SIT: Subcutaneous immunotherapy

SLIT: Sublingual immunotherapy

Approximately 20% of all patients with hay fever have asthma later in life,<sup>1</sup> particularly those with higher levels of bronchial hyperreactivity.<sup>2,3</sup> The efficacy of injective immunotherapy in controlling symptoms and drug use in allergic patients has been confirmed in various studies.<sup>4-8</sup> In 1967, Johnstone and Crump<sup>9</sup> reported that subcutaneous immunotherapy (SIT) prevented the progression and induced the remission of asthma in an open study of a pediatric cohort. Subsequently, Jacobsen et al<sup>10</sup> found that SIT prevented the development of asthma in treated patients followed up for more than 8 years in a randomized controlled trial. These results were confirmed by Moller et al<sup>11</sup> in patients allergic to birch pollen, timothy pollen, or both.

Sublingual immunotherapy (SLIT) is an efficacious form of immunotherapy for allergic disease.<sup>12-21</sup> SLIT is safer and easier to administer than SIT, and might be an equally efficient alternative.<sup>22</sup> The possibility that, like SIT, it can prevent the development of asthma has been suggested by Moller.<sup>23</sup>

The aim of this study was to investigate whether SLIT with grass pollen allergen vaccines administered in a short-term coseasonal protocol to children with hay fever could reduce the development of asthma.

## METHODS

### Study design

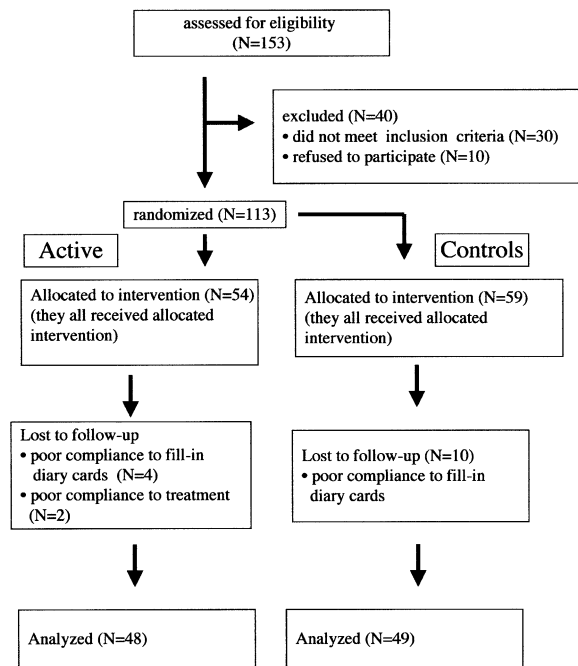
This was a 3-year, multicenter, randomized, open-controlled study involving 6 centers located in North-Central Italy (Emilia, Tuscany, and Lazio). A first cohort of 74 selected children underwent a baseline assessment, and members were randomized to the active or control group in January 1999. The active group received SLIT for 4 months (first administration on February 15 and last administration on June 15) in 1999, 2000, and 2001. Both groups were prescribed and instructed to use symptomatic drugs (cetirizine, loratadine, nasal budesonide, and salbutamol) during the grass pollen season.

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**FIG 1.** CONSORT diagram showing the flow of participants through each stage of the randomized trial.

A second cohort of 39 children entered the study in 2000 and followed the same procedure, ending the treatment period in 2002.

The study design is shown in Fig 1 as a CONSORT diagram.<sup>24</sup>

The parents provided informed consent before the children started the trial.

## Patients

Of the 153 children assessed for eligibility, a total of 113 were enrolled (74 in 1999 and 39 in 2000). On the basis of a randomization list given to each recruiting center, 54 patients were assigned to the SLIT (active) group, and 59 were assigned to the control group (Fig 1).

The inclusion criteria were as follows:

- A history of rhinoconjunctivitis caused by grass pollen.
- Clinical monosensitization to grass pollen, defined as clinical symptoms limited to the grass pollen season, together with a positive grass extract skin test response. In most of the children, skin test responses for other allergens were negative; in individual cases results were positive, but this was not associated with any relevant symptom outside the grass pollen season. These criteria were met throughout the study by all of the children.

The first allergic symptoms in all of the study children appeared in the year they entered the study or in the previous pollen season (ie, no allergic symptoms had been observed previously in the children included in this study).

The exclusion criteria were as follows:

- asthma (defined as at least 3 episodes of wheezing-breathing difficulty, cough, or both separated by at least 1 week during one of the previous grass pollen seasons that required bronchodilator therapy for symptom relief and conditions other than allergy have been excluded)<sup>25</sup>;
- clinical sensitization to other inhalant allergens that could interfere with the assessment of the trial outcomes;
- previous immunotherapy for grass pollen allergy in the preceding 3 years; or

- any other standard contraindication, such as fever or upper respiratory tract infection.<sup>26</sup>

## Diagnosis

The *in vivo* diagnosis was made with biologically standardized extracts of mixed grass pollen (*Dactylis glomerata*, *Lolium perenne*, *Festuca pratensis*, *Phleum pratense*, and *Poa pratensis*), cat epithelium, *Parietaria judaica*, and tree mix (*Alnus glutinosa*, *Betula alba*, *Corylus avellana*, *Cupressus sempervirens*, and *Olea europaea*; 100 BU/mL; ALK-Abellò, Madrid, Spain), with a known content of major allergens.<sup>27-29</sup> Wheals of greater than 3 mm in diameter were considered positive<sup>30</sup> if skin reactivity to a positive and negative control was as expected (negative control yielding a wheal with a maximum diameter of  $\leq 2$  mm and 10 mg/mL histamine hydrochloride yielding a wheal with a maximum diameter  $\geq 5$  mm).

## Treatment

The active treatment was prepared from an extract of mixed grass pollens (*Dactylis glomerata*, *Lolium perenne*, *Festuca pratensis*, *Phleum pratense*, and *Poa pratensis*) that had been standardized by means of RAST inhibition in comparison with a biologically standardized in-house reference<sup>27,28</sup> (ALK-Abellò); the content of the major grass allergen group 5 was expressed in micrograms.<sup>29</sup> The extract was prepared in 5 increasing concentrations (0.04, 0.2, 1.0, 5, and 25 BU/mL) in a glycerinated and phenolated (0.3% wt/vol) aqueous solution. The highest concentration (25 BU/mL) contained 2.5  $\mu$ g/mL of the major grass allergen group 5.

During the build-up phase, the subjects received 2 daily administrations (morning and evening), starting with one drop from the most diluted vial and ending on day 15 with 5 drops from the most concentrated vial, as recommended by the manufacturer. The maintenance dose of 5 drops of the 25 BU/mL concentration (corresponding to 0.5  $\mu$ g of the major grass allergen group 5) was thereafter administered once daily in the morning 5 times a week (Monday to Friday) until the end of June, without any changes during the pollen season. Both the build-up and the maintenance phases were repeated for each of the 3 years of treatment. The drops were taken at least 15 minutes before eating and kept under the tongue for at least 2 minutes before swallowing.

Because the treatments were self-managed, the children and their parents were instructed to keep a record on diary cards and immediately notify the center of any relevant local or systemic side effects possibly related to the therapy. During the build-up phase, the subjects were instructed to avoid any therapy that could modify treatment tolerance (antihistamines and systemic steroids).

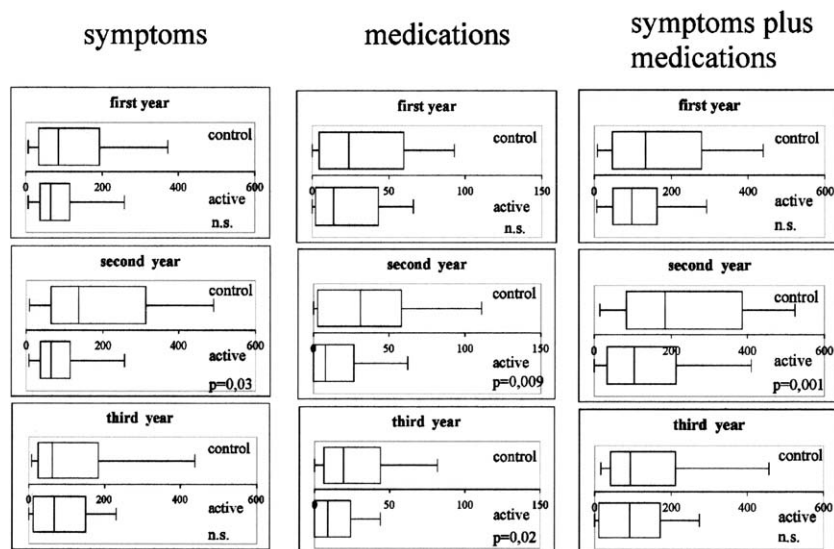
## Pollen counts

From 1999 through 2002, daily pollen counts for grass (grains per cubic meter) were made with a Burkard pollen trap in 2 centers (Parma and Florence) belonging to the Italian Aerobiology Network.

## Efficacy parameters

During the peak of the grass pollen season each year (May and June), the children and their parents were asked to record symptoms and medications on a diary card. Symptoms were graded as present or absent without any estimate of severity. Four symptoms were considered for the nose (itching, runniness, blockage, and sneezing), 3 for the eyes (itching, lacrimation, and redness), and 2 for asthma (cough and wheezing-breathing difficulty); the recurrence of any one was given a score of 1.

The following rescue drugs were allowed as needed to control symptoms during the pollen season: oral antihistamines (cetirizine and loratadine, score 1 per tablet), nasal corticosteroids (flunisolide,



**FIG 2.** Symptom, medication, and symptom-plus-medication scores during the 3 years of treatment. The data are shown as whisker plots: the box indicates the lower and upper quartile, and the central line is the median; the points at the ends of the whiskers indicate the 5th and the 95th percentiles. Mann-Whitney test probability values (*P*) are shown.

score 1 per 2 actuations; ie, one per nostril), bronchodilators (salbutamol, score 1 per actuation), and clobetasone (eye drops, score one per 2 actuations; ie, one per eye).

### Subjective evaluation

At the end of the second and third years of the trial, the patients (or their parents) and the investigators were asked to judge disease evolution by using a 5-point scale: 0, much better; 1, better; 2, unchanged; 3, worse; and 4, much worse. The mean of the patient and investigator scores were used to quantify the subjective evaluation.

### Skin reactivity

The evolution of skin reactivity to the sensitizing allergen (grass pollen) or the development of new sensitizations was evaluated by comparing the results of the skin prick tests. These were repeated at the same time of day in the same month every year by using the same technique and standard panel as that used for the baseline assessment. The size of the wheal elicited by each allergen was related to the wheal diameter elicited by histamine hydrochloride (10 mg/mL) as follows:

- allergen-induced wheal from one fourth to one half the size of the histamine-induced wheal: 1;
- allergen-induced wheal from one half to equal the size of the histamine-induced wheal: 2;
- allergen-induced wheal from equal to double the size of the histamine-induced wheal: 3;
- allergen-induced wheal more than double the size of the histamine-induced wheal: 4.

This scoring system is used in Italian clinical practice and was based on the findings of Malling.<sup>31,32</sup>

### Development of asthma

At the end of each pollen season, the onset of asthma (as defined above)<sup>25,30</sup> was assessed on the basis of the investigators' reports, the symptom-medication diary cards, or both.

### Statistics

The intergroup nonparametric data were compared by using the Mann-Whitney test, whereas the  $\chi^2$  test was used to compare the

categorical variables between the active treatment and control groups. The data were expressed graphically by means of box-and-whisker plots.

A logistic model (including age and other factors possibly affecting the development of asthma in children, such as sex, in-house pets, at least one allergic parent, and exposure to passive smoking) was used to calculate the adjusted treatment odds ratio. The age-by-treatment interaction was also tested. *P* values of less than .05 were considered statistically significant.

The odds ratio (and 95% confidence limits) was calculated to estimate the relative risk of development of asthma in the control group against the actively treated group.

The mean odds ratio of clinically diagnosed asthma was calculated with the logistic procedure by analyzing maximum likelihood estimates with treatment, center, and treatment-by-center effects. The calculations were made with SAS System Software.

## RESULTS

### Treatment

In accordance with the trial design, the first cohort of 74 children entered the study in January 1999, and 65 completed the treatment period in June 2001; the second cohort of 39 children entered the study in January 2000, and 32 completed the treatment in June 2002 (Fig 1). A total of 97 children concluded the study (47 in the active group and 50 in the control group).

None of the 16 dropouts (14.6%) was clearly caused by treatment-related side effects. In particular, 2 children in the active group recruited in the first year dropped out in the second season because their symptoms had decreased and asthma had not developed, but their parents did not want them to continue immunotherapy. Furthermore, one of the treated children recruited in 1999 dropped out because of eye pruritus, which could not be clearly attributed to the immunotherapy but compelled the family

**TABLE I.** Characteristics of analyzed patients

|                        | Patients receiving SLIT (n = 47) | Control subjects (n = 50) | P value |
|------------------------|----------------------------------|---------------------------|---------|
| Male sex               | 33 (70.2%)                       | 35 (70.0%)                | .7      |
| Female sex             | 14 (29.8%)                       | 15 (30.0%)                |         |
| Age, y (range)         | 8.96 (5-14)                      | 7.74 (4-16)               | .049    |
| Parent with allergy    | 31 (65.9%)                       | 35 (70.0%)                | .8      |
| No parent with allergy | 16 (34.1%)                       | 15 (30.0%)                |         |
| Passive smoking        | 22 (46.8%)                       | 19 (38.0%)                | .4      |
| No passive smoking     | 25 (53.2%)                       | 31 (62.0%)                |         |
| Pet in the house       | 17 (36.2%)                       | 17 (34.0%)                | .8      |
| No pet in the house    | 30 (63.8%)                       | 33 (66.0%)                |         |

P values indicate results of the  $\chi^2$  test and (in the case of age) the *t* test (patients receiving SLIT vs control subjects). The nonsignificant values are shown in italics.

pediatrician to discontinue it. All of the other dropouts were due to poor compliance to treatment schedules or the completion of the diary cards (Fig 1).

The groups were well matched in terms of sex, the presence of at least one parent with known respiratory allergies, passive exposure to cigarette smoking, and the presence of one or more household pets (cats, dogs, or both; Table I). These results did not change if all of the patients initially allocated to treatment were considered. The children in the active treatment group were slightly younger than those in the control group ( $P = .049$ ).

The cumulative amount of the administered major allergen grass group 5 was about 40  $\mu\text{g}$  over 4 months, corresponding to the amount of the same allergen normally administered subcutaneously over 20 months. The children receiving active treatment were therefore administered about 120  $\mu\text{g}$  of the major allergen grass group 5 during the study. These doses are within the range of the recommended SLIT doses according to a recent thorough review of the published literature.<sup>20</sup>

### Tolerance

Among the actively treated children, 2 experienced mild side effects during the build-up phase: one case of gastrointestinal complaints during the administration of vial 0 and one case of itching in the throat during the administration of vial 3. The side effects spontaneously resolved without requiring treatment discontinuation in both of these cases. There was one case of cutaneous rash during the administration of vial 4 in the maintenance phase (during the pollen season), which spontaneously resolved without any intervention. One of the control children also experienced a cutaneous rash during the pollen season.

The rate of side effects was therefore 2 per approximately 4500 administrations (ie, approximately 0.44/1000) during the build-up phase and 1 per approximately 12,000 administrations (ie, approximately 0.083/1000) during the maintenance phase.

### Pollen counts

Unlike the birch season, the grass season in Italy is relatively homogeneous from year to year in terms of amounts and timing; this was confirmed by means of pollen count analysis (not shown). The symptoms and

drug use scores paralleled pollen levels (not shown), thus indicating that the clinical scores were related to environmental exposure to grass pollen.

### Scores

*First year of treatment.* Diary cards were completed during the first grass season by 46 actively treated subjects and 50 control subjects. There were no statistically significant between-group differences in terms of symptoms, drug use, or the combined score of symptoms plus drugs (Fig 2).

*Second year of treatment.* Diary cards were completed by 46 actively treated subjects and 47 control subjects. The former had lower scores for symptoms ( $P = .03$ ), drugs ( $P = .009$ ), and symptoms plus drugs ( $P = .001$ , Fig 2).

*Third year of treatment.* Diary cards were completed by 41 actively treated subjects and 32 control subjects. The former had lower medication scores ( $P = .02$ ), but the reductions in the symptom and symptom-plus-drug scores were not significantly different from those in the control group (Fig 2).

### Subjective evaluation

The between-group differences in the changes from baseline of the subjective symptom evaluation scores were significant in both the second ( $P = .0004$ ) and third years of follow-up ( $P < .0001$ ).

### Skin tests

The grass skin prick test values remained quantitatively unchanged in both groups during the 3 years of observation (not shown), as did the number of sensitizations. In particular, there were no significant between-group differences in the number of individual sensitizations at the end of each year in comparison with baseline values (not shown).

### Development of asthma

None of the subjects had asthmatic symptoms at the time of randomization, but 6 of the actively treated patients had asthma after the first year, and this number increased to 7 after the second and 8 after the third years of treatment. The corresponding figures in the control group were 6, 16 ( $P = .058$ ), and 18 ( $P = .0412$ ).

The proportion of children who had asthma after 3 years varied between centers from 10% to 63% ( $P = .02$ ). However, the treatment-by-center interaction was not statistically significant ( $P = .8$ ).

The common relative risk of development of asthma in the control group was 3.80 (95% confidence limits, 1.5-10.0). Of the possible interacting factors considered (age, sex, house pets, at least one allergic parent, and exposure to passive smoking), only age significantly correlated with the development of asthma after 3 years, with the children in whom asthma developed being significantly younger than those in whom asthma did not develop ( $P = .02$ ). However, the effect of treatment on the development of asthma (ie, the age-by-treatment interaction) was not different in children of different ages (Fig 3). These results did not change when only the individuals recruited in the first year were considered (not shown).

It is worth noting that the development of asthma was not significantly associated with sex, the presence of one or more household pets, the presence of at least one allergic parent, or exposure to passive smoking.

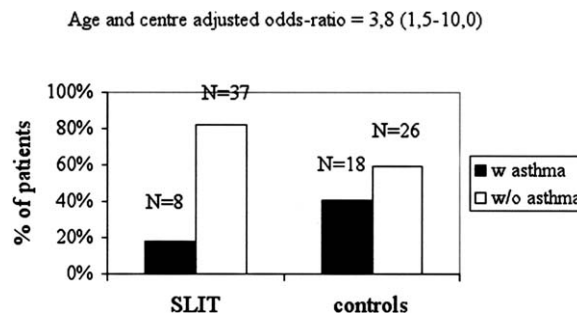
## DISCUSSION

SLIT is a safe and effective alternative to injection immunotherapy.<sup>12-21</sup> The results of this study show that it can prevent the onset of asthma in children with rhinoconjunctivitis and thus reflect similar results from a previous study of SIT.<sup>11</sup> The main limitation of our study is that it was not double-blind. Thus the report of symptoms, the diagnosis of asthma, and the use of medication might have been biased. However, in our experience it is very difficult to convince a sufficient number of parents to keep their children with allergic symptoms for years in a double-blind setting in which asthma has to be considered a possible outcome.

In clinical settings SLIT is not only safer than SIT but is also much easier to administer, particularly in pediatric patients. It is well tolerated and does not need to be administered in specialist centers with access to resuscitation measures.<sup>33-35</sup>

The main study end point was the development of asthma, which was defined very strictly on the basis of the position paper of the Global Initiative for Asthma,<sup>33</sup> which requires the recurrence of 3 core symptoms. According to these guidelines, symptoms are more predictive than methacholine bronchial provocation testing,<sup>34,35</sup> and the relief of symptoms with bronchodilator therapy is more diagnostically relevant than the presence of symptoms alone.<sup>33</sup> A limited number of our children (11 control subjects and 10 actively treated patients) performed an exercise test (data not shown),<sup>36</sup> and a significant difference was found in the third year (10/10 actively treated patients and 7/11 control subjects had negative exercise test results). Although anecdotal, this observation supports our conclusions.

It is worth noting that the development of asthma was not significantly associated with sex, the presence of one



**FIG 3.** The percentages of children with and without asthma after 3 years of immunotherapy. The absolute numbers are shown above the bars. All of the children were free of asthma before starting treatment. The odds ratios and 95% confidence limits of the common relative risk of developing asthma in the control group are shown, adjusted for age and center.

or more household pets, having an allergic parent, or exposure to passive smoking. This likely reflects the relatively small sample size of this study group. Although the children in whom asthma developed were younger than those in whom asthma did not develop, our statistical analysis indicated that age had no influence on the effect of treatment in the development of asthma.

Our patients experienced self-limited systemic reactions (one case of cutaneous rash in the pollen season) and local reactions (one case of gastrointestinal complaints and one case of itching of the throat). This is in line with the results of other published trials and a large-scale postmarketing surveillance study.<sup>37</sup>

The fact that drug use but not symptom scores significantly decreased in both the second and third years of treatment might be due to the fact that hay fever symptoms are tolerated to a certain extent. Hardly any published immunotherapy study has reported the resolution of symptoms, and the reduction in medication typically exceeds the reduction in symptoms.<sup>38</sup> On the contrary, the appearance of a severe and socially disabling symptom, such as asthma, is much more carefully monitored by patients and their parents.

We chose a 3-year coseasonal protocol (February 15 to June 15) for vaccine administration, which makes our results particularly innovative in pediatric terms because parents and children are reluctant to accept prolonged injective immunotherapy. Added value for pediatric patients is provided by the reduction in the development of seasonal asthma. However, although coseasonal treatment might enhance compliance in hesitant patients, the possibility that continuous SLIT is more effective than coseasonal SLIT cannot be excluded.

Our results further support the notion that allergic rhinoconjunctivitis and allergic asthma are different manifestations of the same systemic disease<sup>39,40</sup> insofar as treatment of the former can prevent the development of the latter.<sup>11</sup> It is conceivable that although it is improving allergic inflammation in one segment of the airways (ie, the nose), SLIT might also change the immunologic

response in another (ie, the lower respiratory tract). Although it has been said that SLIT modulates the immune response to allergens prevalently at the level of the immunologically privileged oral mucosa,<sup>41</sup> its effect on systemic immune response has been documented in terms of serum antibodies and peripheral blood T-cell responses.<sup>13,15,19,42,43</sup> However, most studies have failed to find any change in specific IgE, IgG, or T-cell cytokine balance.<sup>12,13,44-48</sup>

We did not find any change in the early skin test reaction to the allergen used in SLIT, and Lima et al<sup>49</sup> have reported that the late, but not the early, allergen reaction is modulated by SLIT. We also found that the number of sensitizations was similar in the treated and control children, which is in line with the results of a 10-year follow-up study of children receiving SLIT by Di Rienzo et al.<sup>50</sup> This contrasts with a previous report<sup>51</sup> showing that specific immunotherapy in children allergic to *Dermatophagoides pteronyssinus* prevents the onset of new sensitizations. It is possible that SIT and SLIT differentially influence the onset of new sensitizations.

In conclusion, our study shows that SLIT reduces the risk of development of clinical asthma in children with allergic rhinitis.

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