Montelukast, a leukotriene receptor antagonist, reduces the concentration of leukotrienes in the respiratory tract of children with persistent asthma

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Background: Leukotrienes are bronchoactive mediators secreted by inflammatory cells in the respiratory mucosa on exposure to asthma triggers.
Objective: We investigated the effect of montelukast, a leukotriene receptor antagonist, on the release of leukotrienes in the respiratory mucosa of children with persistent asthma.
Method: Twenty-three children aged 6 to 11 years with moderately severe asthma were treated in a cross-over design starting, after a 2-week run in period, with either montelukast (n = 12) or cromolyn (n = 11) for 4 weeks with a 2-week washout period between treatments. Twelve of them were then treated with either montelukast or beclomethasone for 6 months. The use of β_2-agonists was recorded on a diary card. The concentration of leukotriene C_4 (LTC_4) was measured by HPLC in nasal washes obtained before and at the end of each treatment period. Eosinophilic cationic protein (ECP) was measured in the nasal washes by RIA.
Results: The LTC_4 concentration significantly decreased in the children treated for the first 4 weeks with montelukast, from 5.03 ± 1.17 to 1.42 ± 0.33 ng/mL ($P < .005$), and a nonsignificant increase was noted in children treated with cromolyn, from 3.37 ± 1.11 to 5.88 ± 2.17 ng/mL ($P = .17$). ECP concentration also decreased in the children receiving montelukast ($P = .12$). The concentration of LTC_4 remained low after 3 and 6 months of treatment with montelukast (0.8 ± 0.7 and 1.0 ± 0.3 μg/mL) and was lower than with beclomethasone. Children treated with montelukast required significantly fewer β_2-agonists ($P < .04$),
Conclusion: Montelukast reduces the concentration of leukotrienes in the respiratory tract of children with persistent asthma parallel to reduction in ECP and clinical improvement. This effect was not observed when the same children were treated with cromolyn. (J Allergy Clin Immunol 1999;104:1162-7.)

Key words: *Leukotrienes, nasal washes, asthma, children, montelukast, leukotriene modifiers*

Abbreviations used
BAL: Bronchoalveolar lavage
$CysLT_1$: Cysteinyl leukotriene 1 (receptor)
ECP: Eosinophilic cationic protein
LTC_4: Leukotriene C_4
LTD_4: Leukotriene D_4
LTE_4: Leukotriene E_4

Cysteinyl leukotrienes are potent proinflammatory mediators produced from a variety of inflammatory cells, including mast cells, eosinophils, basophils and macrophages. Leukotriene C_4 (LTC_4) is metabolized enzymatically to leukotriene D_4 (LTD_4) and subsequently to leukotriene E_4 (LTE_4), which is excreted in the urine.[1] The leukotrienes have been implicated in the pathophysiologic mechanisms of asthma.[2] LTE_4 has been detected mainly in the urine[3-5] but also in the blood[6,7] and nasal[8] and bronchoalveolar lavage (BAL) fluid[9] of patients with asthma,[9,10] especially after allergen challenge,[4,6,8] after exercise,[3,11] or during an acute asthma attack.[12] LTC_4[8,13-19] and, in lesser amounts, LTD_4[14] have also been detected in the blood[20] and in nasal,[8,15-18,21] tracheal,[18] and BAL fluid[13,14,19] of symptomatic patients with asthma[13,20] and after natural exposure to allergens,[17] viral infection,[15,16] and antigen challenge.[8,14]

Montelukast is a leukotriene receptor antagonist that has been found to be effective in the treatment of asthma in adults[22] and children.[23] There are no data on the effect of montelukast on the presence of leukotrienes in the human respiratory tract.

The current study compares the concentration of leukotrienes in nasal washes of children with persistent asthma treated in a cross-over design with montelukast and cromolyn. The concentration of eosinophilic cationic protein (ECP) in the nasal washes was also measured to determine the relationship of eosinophils to leukotriene concentration.

METHODS

Patient population

The study population consisted of 26 children with asthma aged 6 to 11 years, with FEV_1 between 60% to 85% predicted and improvement of ≥12% after inhaled β_2-agonist. During the 2 weeks period before inclusion in the run-in and 2 weeks during run-in the

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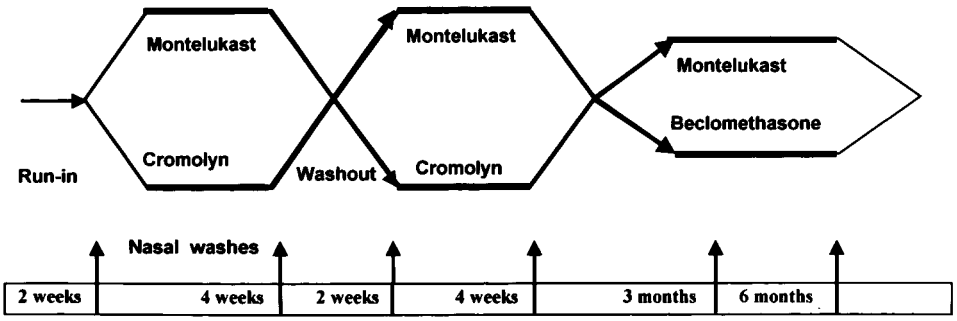

FIG 1. Study design.

TABLE I. Study population: baseline data

Group	Montelukast→ cromolyn	Cromolyn→ montelukast	Statistical significance
No. of children	12	11	
Mean age	9 y 3 mo	8 y 8mo	NS*
Sex (male/female)	6/6 (50%)	8/3 (73%)	NS†
Asthma in family	10/2 (83%)	9/2 (82%)	NS†
Previous inhaled steroid therapy	10/2 (83%)	7/4 (64%)	NS†
FEV_1 (% of predicted) before run-in period	73	78	NS*
FEV_1 (% of predicted) after run-in period	71	75	NS*

NS, Not significant.
*One-way ANOVA.
†Fisher's exact test.

children were not allowed to use any antiasthma medication except for β_2-agonist on an as-needed basis. During the entire study the children were not permitted to use any nasal drugs. Only children who required β_2-agonists on at least 7 of the 14 days during the run-in period were included in the study. The Helsinki committee of our hospital improved the study. The goals and risks of the study were explained to the parents, and signed informed consent forms were obtained.

Study design

An open-label, 2-period cross-over design was used. After a 2-week run-in period patients received either montelukast (one 5-mg chewable tablet at bedtime) for 4 weeks followed by cromolyn (2 puffs of 1 mg cromolyn four times daily by metered-dose inhaler) for 4 weeks or cromolyn followed by montelukast, with a 2-week washout period between treatments (Fig 1). β_2-Agonists were used as needed, and their use was recorded on a daily diary card. The children were included in a 6-month extension study wherein they were randomly assigned to receive either montelukast or inhaled beclomethasone (100 μg 3 times daily) (Fig 1).

Sample collection

Nasal fluid washes were collected from each patient on 4 occasions: (1) before onset of the trial (end of run-in period), (2) at the end of the first 4-week treatment period with montelukast or cromolyn, (3) at the end of the 2-week washout period, and (4) at the end of the second 4-week treatment period with the other drug. They were also collected from the children in the extension study after 3 and 6 months of treatment. The samples were collected by suction catheter and a trap. A 4-mL volume of PBS was gently instilled into the nostrils in 8 consecutive 0.5-mL installations, followed each time by gentle suction. The suction catheter was rinsed with 0.5 mL of PBS. Samples were kept on ice during transport to the laboratory and were stored at –70°C until assayed.

Measurement of leukotrienes

The nasal washes containing the secretions in PBS were mixed with 4 volumes of methanol (HPLC grade). After incubation in ice for 2 hours, the samples were centrifuged at 2000 revolutions/min for 10 minutes at 4°C. The supernatant was applied to a Sep-Pak C_{18} cartridge (Millipore, Waters Associates, Milford, Mass) previously activated with 10 mL of 0.5% EDTA, 10 mL of methanol, and 10 mL of water and then eluted with 5 mL of 80% methanol and 20% water (both HPLC grade). This sample was subjected to vacuum extraction to dryness with use of a new small plastic flask (Azlon) for each sample, and the residue was redissolved in 0.75 mL of 30% methanol. A 0.5-mL sample was injected into a C_{18} reversed-phase column (Beckman, San Ramon, Calif) with 2 ng of purified PGB_2 (Sigma Chemical, St Louis, Mo) added as an internal standard. A second sample of 0.2 mL was tested whenever the result of the first sample was technically uncertain. LTC_4, LTB_4, and LTD_4 were isocratically eluted in an HPLC system (Waters Associates) with a mixture of 72% methanol, 28% water (both HPLC grade), and 0.5 mmol/L ammonium acetate; acetic acid was added to obtain a pH of 5.88. Purified preparations of, LTC_4, LTD_4, and LTB_4 (Merck Frost, Montreal, Canada) were used as standards and tested in parallel with the samples. The solvent for HPLC was pumped through an HPLC pump (501 HPLC, Waters), and the effluent was monitored at 280 nm with a spectrophotometer (Lambda MAX model 81 LC spectrophotometer, Waters). Data were processed with a Waters Baseline 810 chromatography computerized work station. The technician performing the leukotriene measurements was blinded to the type of treatment. The lower limit of detection of our assay was 0.5 ng/mL.

Our all-step recovery rates of standard leukotrienes include incubation with methanol/water, extraction with Sep-Pak cartridge, vacuum evaporation, redisolution, and passage through HPLC.

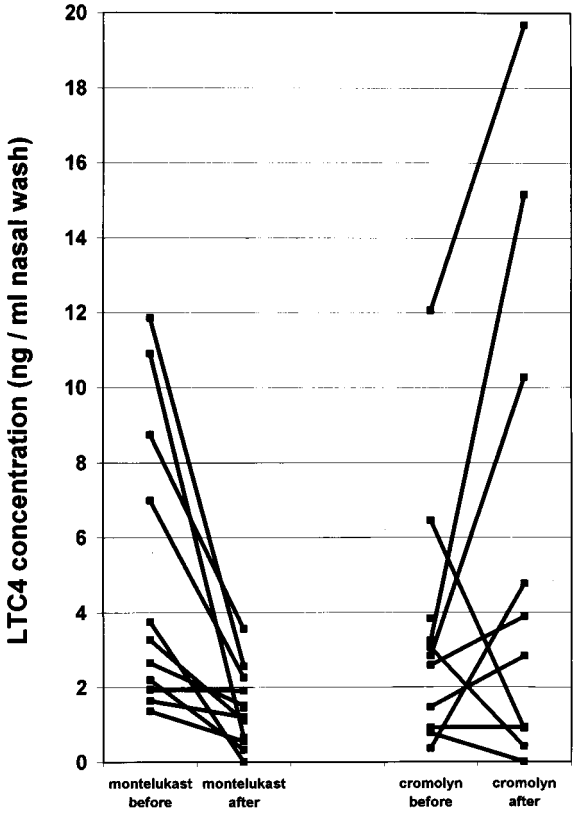

FIG 2. LTC_4 concentration (in nanograms per milliliter) in nasal washes of children before and after first month of treatment with montelukast or cromolyn.

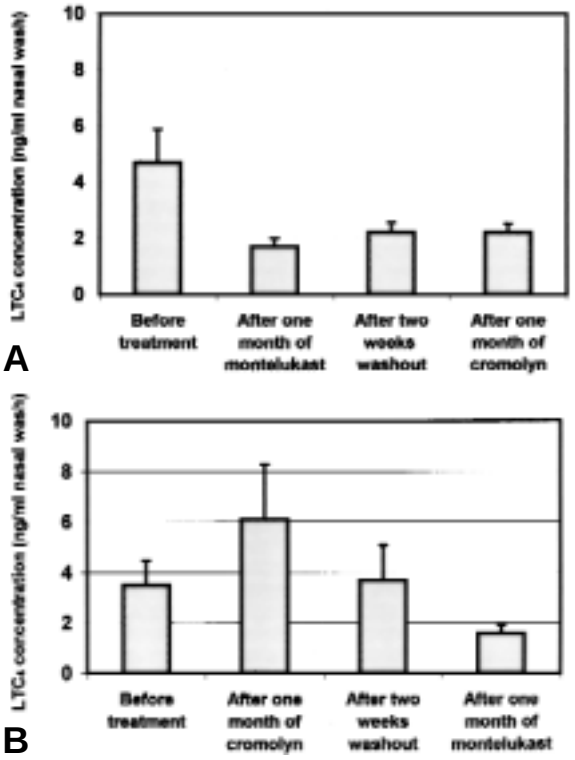

FIG. 3. LTC_4 concentration in nasal washes of the children in all 4 periods of study. **A,** Children starting treatment with montelukast. **B,** Children starting treatment with cromolyn.

Measurement of ECP

ECP was measured by double-antibody RIA with the Pharmacia ECP RIA kit (Phamacia and Upjohn Diagnostics, Uppsala, Sweden). The assay uses a specific antibody against human ECP raised in rabbit with iodine 125–labeled ECP as tracer. The assay was conducted in 50 μL of nasal supernatant. The sensitivity of the assay was 2 μL/L, and the within-assay variation was 11%.

Statistical analysis

The statistical analysis was performed with use of BMDP statistical software.[24] Means and SEMs were computed for each of the continuous variables, and differences were compared with 1-way ANOVA and ANOVA with repeated measures. The significance for categorical variables was computed with use of Pearson's chi-square test as well as Fisher's exact test. A P value <.05 was considered significant.

RESULTS

The results of 3 of the original 26 children who refused to continue with the collection of nasal washes were excluded from the analysis. Nasal samples were obtained before and after the first treatment period in the remaining 23 children. The cross-over analysis was performed only in the 17 children (9 given montelukast and 8 cromolyn) from whom nasal wash samples were obtained at all 4 stages of the study. Twelve children participated in the extension trial (6 were treated with montelukast and 6 with beclomethasone).

Twelve children started treatment with montelukast and 11 with cromolyn. The 2 groups were comparable for baseline data (Table I). Most of the children (74%) had been treated continuously with inhaled corticosteroids before the study. The mean prestudy peak expiratory flow rate of the whole cohort was 71% to 78% of predicted. Eighty-three percent of the children had a family history of asthma. During treatment with montelukast 75% of the children used fewer than 2 puffs of β_2-agonists (Ventolin) per week compared with 27% during treatment with cromolyn ($P < .04$).

During the entire follow-up period with montelukast, the children did not have an asthma exacerbation and they did not use any antiasthma drug except for β_2 agonists and these only "as needed." However, 2 of the children treated with cromolyn withdrew from the study because of asthma exacerbation. When asked at the end of the study, 82% of the parents and 76% of the children preferred the treatment with montelukast, and only 2% of the parents and 27% of the children preferred cromolyn (the others had no preference). No adverse effects were noted in any of the children with either treatment.

All children treated first with montelukast showed a decrease in the LTC_4 concentration after 1 month of treatment, from a mean (± SE) of 5.03 ± 1.17 ng/mL of nasal wash to 1.42 ± 0.33 ng/mL ($P < .005$). By contrast,

the LTC_4 concentration in the children receiving cromolyn rose although not significantly, after the first month from 3.37 ± 1.11 ng/mL before treatment to 5.88 ± 2.17 ng/mL after (P = .17) (Fig 2).

In the children who started treatment with montelukast, the LTC_4 concentration did not return to the pretreatment level at the end of the washout period (Fig 3, *A*). In the children who started with cromolyn, the LTC_4 concentration returned to the pretreatment level at the end of the washout period and then further decreased when montelukast was administered (Fig 3, *B*). The LTC_4 concentration of all the children (from both arms of the cross-over study) was significantly lower during the month in which they were treated with montelukast than in the month they received cromolyn (1.58 ± 0.23 vs 4.09 ± 1.21 ng/mL, P = .02) (Fig 4).

The recovery rate of standard LTC_4 including all the separation steps was 40%, whereas the rates for LTD_4, LTB_4, and LTE_4 were 15%, 10%, and 0%, respectively.

LTD_4 was detected only in 9 of 23 of the children before the study, in 5 of 23 after 1 month of treatment with montelukast, and in 8 of 23 after 1 month of treatment with cromolyn. The small number of patients with detected LTD_4 did not allow for statistical evaluation.

During the extension trial 6 children received montelukast and 6 beclomethsone. The mean concentration of LTC_4 in the children treated with montelukast remained low and was lower than the concentration found in the children treated with beclomethasone after 3 months (0.8 ± 0.7 vs 1.5 ± 0.6 ng/mL, P = .12) and after 6 months (1.0 ± 0.3 vs 1.5 ± 1.0 ng/mL, P = .26). The difference was not statistically significant, possibly because of the small number of patients.

The concentration of ECP decreased after the first month of treatment in most nasal washes of the children given montelukast (9/12 children), from a mean (±SE) of 284 ± 15 ng/mL of nasal wash to 241.3 ± 23 ng/mL (P = .12). By contrast, a small change in the opposite direction was noted in the children receiving cromolyn: 246 ± 20 ng/mL before and 260 ± 28 ng/mL after treatment. There was a borderline significant interaction between treatment group and effect of treatment (P = .08).

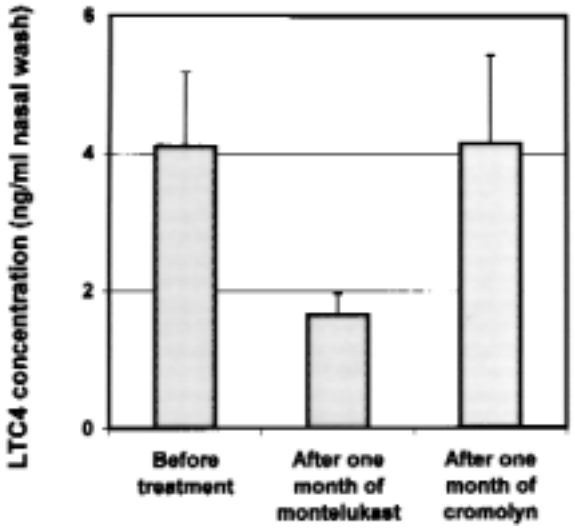

FIG 4. LTC_4 concentration in all the children (both arms of cross-over study) before and after treatment with montelukast or cromolyn.

DISCUSSION

This study demonstrates that treatment with montelukast, a leukotriene receptor antagonist, is associated with a significant suppression of LTC_4 release in the respiratory tract of children and with concomitant clinical improvement in their asthma status. These pathophysiologic and clinical effects were not observed when the same children received cromolyn therapy.

The ideal model for the investigation of mediator metabolism in asthma is the respiratory mucosa of the lung by either bronchial biopsy specimen study or by segmental BAL.[19] However, because these procedures are invasive, several groups have used the nose to study the underlying mechanisms of allergic and nonallergic reactions in the respiratory tract.[25,26] The nose allows for the repetitive collection of nasal secretions after different interventions. In a trial with tracheotomized children,[18] we demonstrated a positive correlation between the concentration of leukotrienes in the bronchial tree (trachea) and in the nose.

LTC_4 was detected in all the samples at baseline and in most of the samples during the other treatment periods of the study, whereas LTD_4 and LTB_4 were detected in fewer samples. Studies have shown that LTC_4 is the principal leukotriene secreted by activated eosinophils.[27] Wenzel et al[14] reported that LTC_4 was the predominant sulfidopeptide leukotriene found in the BAL fluid of patients with atopic asthma undergoing allergen challenge, followed by LTD_4 and LTE_4. In previous studies we demonstrated the presence of LTC_4 in the nasal washes of children with acute viral infection,[15] with bronchiolitis caused by respiratory syncytial virus infections,[16] and after exposure to ragweed antigen.[17] We have detected LTC_4 in tracheal secretions,[18] ear fluid,[21] and segmental BAL fluid.[19] The results of the current study indicate that LTC_4 is present in the respiratory mucosa of children with persistent asthma and that an active leukotriene receptor antagonist such as montelukast can suppress these elevated levels.

The exact mechanisms underlying the suppression of LTC_4 in the nasal washes of the children treated with montelukast are unclear. We offer 2 possible explanations. (1) Both LTC_4 and LTD_4 are known to share the same cysteinyl leukotriene T_1 receptor ($CysLT_1$).[28] Cysteinyl leukotrienes promote eosinophil recruitment in the airways,[29] and eosinophils in turn, release leukotrienes.[28] Montelukast, which is a $CysLT_1$ receptor antagonist,[28] indirectly leads to a decrease in leukotriene concentration in the airways by reducing the influx of eosinophils. During the active asthma state (end of the run-in period), when the first baseline samples were obtained, the con-

centrations of leukotrienes in the respiratory mucosa were elevated. Immediately after treatment with montelukast, there may still have been a transient accumulation of leukotrienes, but they are unstable in the respiratory mucosa and undergo rapid metabolism and degradation.[29] Once they were absent from the airways, all the clinical effects of the leukotrienes,[30] including the attraction of eosinophils into the lung,[31] were prevented, ultimately resulting in a reduction in LTC_4 production. This theory is supported by the relative decrease in ECP (produced by eosinophils) observed in our study after treatment with montelukast. (2) The reduction in leukotrienes in the mucosa during treatment with montelukast may also be associated with another mechanism. LTD_4 increases nasal mucosal blood flow.[32] A montelukast-induced reduction in the concentration of leukotrienes may result in a reduction in blood flow into the respiratory mucosa, leading to a lower number of eosinophils arriving from the blood to the nose and thereby a reduced production of LTC_4.

We used a cross-over design because the study was part of a "preference" international multicenter cross-over trial (not a clinical comparison of the efficacy and safety of the drugs). The main objective of the current study was to verify that montelukast, and not other drugs, induces a reduction in leukotriene concentration in the respiratory mucosa. The results for the group that started with montelukast and continued with cromolyn showed that the mean concentration of LTC_4 at the end of the washout period remained as low as that during active treatment with montelukast and did not reach pretreatment values. This suggests that montelukast had a cross-over effect during the 2-week washout period. We therefore performed both a cross-over analysis and a direct comparison of pretreatment and posttreatment values in the 2 parallel groups. The results proved to be comparable, demonstrating a significant reduction in LTC_4 concentration only during treatment with montelukast and not with cromolyn.

The scarcity of leukotrienes in the human body fluid, their rapid metabolism and degradation once they are formed in the respiratory mucosa,[31] and the cross-reactivity within the group of leukotrienes LTC_4, LTD_4, and LTE_4 in RIAs[23] necessitate the use of refined laboratory techniques, including separation and purification of the samples to detect and measure their concentration with HPLC.

The recovery rate of standard LTC_4 observed here with our separation technique was undesirably low. Measurement of the recovery rate of only 1 step of the procedure might have yielded a result closer to that in our previous study (76%),[15] with the same technique, which was similar to the 77% reported by others for the same separation step.[8] As such, there are 2 main steps in our procedure that might account for the relatively low recovery rate obtained in the current study: (1) Sep-Pak extraction, which precedes HPLC, is associated with a loss of leukotriene concentration and hence a low recovery rate[34] and (2) vacuum extraction and redisolution of the leukotrienes from the dried tube always leaves some leukotrienes on the wall of the assay tube. As in our study, Schwartzberg et al[33] also reported a low recovery rate for LTD_4 that was less than half of that for LTC_4. We did not detect LTE_4 mainly because our HPLC separation technique was optimally adjusted to a good separation of LTC_4 and LTD_4.

The preliminary results of the extension part of the trial indicate that the concentration of leukotrienes in the respiratory mucosa remains low during 3 and 6 months of treatment with montelukast. The LTC_4 concentration was lower in the children treated with montelukast than in those treated with beclomethasone, indicating the specificity of the treatment with the antileukotriene agent.

A recent study had shown that cromolyn only attenuates, without statistical significance, the release of LTC_4 and LTD_4 in the nasal airway of allergic patients undergoing nasal allergen provocation.[34] In our study, treatment with cromolyn did not induce any significant change in the LTC_4 concentration, as opposed to treatment with montelukast. On the contrary, children treated with cromolyn showed an increase (although not statistically significant) in the concentration of leukotrienes in the respiratory mucosa. This may have been the result of deterioration of the clinical and pathophysiologic status of most of these children, who had been previously treated with inhaled corticosteroids.

Treatment with other antileukotriene drugs in humans, such as zileuton, a 5-lipoxygenase inhibitor,[4,10] or MK-0591, a 5-lipoxygenase activating protein inhibitor,[5] has been associated with the inhibition of urinary excretion of LTE_4. Our study is the first to demonstrate a reduction in LTC_4 in the respiratory tract of children in response to a leukotriene receptor antagonist.

The current study demonstrates that montelukast causes a reduction in the concentration of leukotrienes in the respiratory mucosa of children with persistent asthma parallel to reduction in ECP and clinical improvement. This effect was not observed when the same children were treated with cromolyn.

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