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Antiallergic Action of Magnolia Officinalis on Immediate Hypersensitivity Reaction

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We studied the effect of aqueous extract of *Magnolia officinalis* bark (Magnoliaceae) (MOAE) on the immediate hypersensitivity reaction. MOAE (0.01 to 1 g/kg) dose-dependently inhibited compound 48/80 induced systemic anaphylaxis in rats. MOAE (0.1 and 1 g/kg) also significantly inhibited local immunoglobulin E (IgE)-mediated passive cutaneous anaphylactic reaction. When MOAE was pretreated at concentrations ranging from 0.01 to 1 g/kg, the levels of plasma histamine were reduced in a dose-dependent manner. MOAE (0.001 to 1 mg/ml) dose-dependently inhibited the histamine release from rat peritoneal mast cells (RPMC) activated by compound 48/80 or anti-dinitrophenyl (DNP) IgE. The level of cyclic AMP (cAMP) in RPMC, when MOAE was added, significantly increased compared with that of the normal control. Moreover, MOAE (0.01 to 1 mg/ml) had a significant inhibitory effect on anti-DNP IgE-induced tumor necrosis factor-α production from RPMC. These results indicate that MOAE inhibits immediate hypersensitivity reaction *in vivo* and *in vitro*.

Key words: *Magnolia officinalis*, Immediate hypersensitivity reaction, Compound 48/80, anti-DNP IgE, Tumor necrosis factor-α, Cyclic AMP

INTRODUCTION

The dried bark of stem, root or branch of *Magnolia officinalis* Rehd. et Wils. or *Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils. (Magnoliacae), well known as "Hu-bak" in Korea, has been used for centuries as traditional medicine. This crude drug has the pharmacological functions of antiallergic effect (type IV allergic reaction) (Taniguchi *et al.*, 2000), myocardial protective effect (Tsai *et al.*, 1996), anti-inflammatory effect (Wang *et al.*, 1995), antioxidative effect (Zhou and Xu, 1992), central depressant effect (Watanabe *et al.*, 1983), anti-ulcerative effect and laxative effect (Chang and But, 1987). It is, therefore, used in the treatment of distention and pain of the abdomen, dyspepsia, and asthmatic cough (Chang and But, 1987). It has been reported that Magnoliae Flos (flower-bud of *Magnolia sp.*) showed anti-allergic effect(type I allergic reaction) (Kim *et al.*, 1999; Tsuruga *et al.*, 1991). However, the bark of *Magnolia sp.* has not been studied. Hypersensitivity may be classified into four types. One of these, type I hypersensitivity (immediate hypersensitivity reaction), popularly known as allergy, is a major clinical problem in human. It has been found that the histamine release from mast cells is an essential step in the pathological process of a type I hypersensitivity (Ishizaka *et al.*, 1977). Compound 48/80 and polymers of basic amino acids, such as substance P, are some of the most potent secretagogues of mast cells (Ennis *et al.*, 1980). Compared with the natural process, a high concentration of compound 48/80 induces almost a 90% release of histamine from mast cells. Thus, an appropriate amount of compound 48/80 has been used as a direct and convenient reagent to study the mechanism of anaphylaxis (Allansmith *et al.*, 1989). The secretory response of mast cells can also be induced by aggregation of their cell surface-specific receptors for immunoglobulin E (IgE) by the corresponding antigen (Segal *et al.*, 1977; Metzger *et al.*, 1986; Alber *et al.*, 1991). It has been established that the anti-IgE antibody induces passive cutaneous anaphylaxis (PCA) reactions as a typical model for the immediate hypersensitivity (Saito

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and Nomura, 1989). Although mast cells store small amounts of cytokines in their granules (Gordon and Galli, 1990), these cells dramatically increase their production of tumor necrosis factor-α (TNF-α), IL-6, and other cytokines within 30 min after their surface FcεRI are cross-linked with specific antigen (Plut *et al.*, 1989; Wodnar-Filipowicz *et al.*, 1989; Burd *et al.*, 1989; Gurish *et al.*, 1991; Galli *et al.*, 1991). Therefore, modulation of TNF-α production by mast cells should provide us with a useful therapeutic strategy for allergic disease.

In this study, we showed that MOAE inhibited compound 48/80-induced systemic anaphylaxis, anti-dinitrophenyl (DNP) IgE antibody-induced PCA, and histamine and TNF-α production from rat peritoneal mast cells (RPMC). We also investigated intracellular cAMP content to clarify the mechanism by which the MOAE inhibited histamine release from RPMC.

MATERIALS AND METHODS

Reagents

Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA), α-minimal essential medium (α-MEM), o-phthaldialdehyde and metrizamide were purchased from Sigma Chemical Co. (St Louis, MO). Cyclic AMP was purchased from Amersham Pharmacia Biotec (UK), and Murine TNF-α was obtained from R & D Systems Inc. (USA).

Animals

The original stock of male Sprague-Dawley rats (200~250 g) were purchased from Dae-Han Experimental Animal Center (Taejeon, Korea), and the animals were maintained in the College of Pharmacy, Woosuk University. The animals were housed five per cage in a laminar air flow room maintained under a temperature of 22 ± 2°C and relative humidity of 55 ± 5% throughout the study.

Preparation of MOAE

The dried bark of *Magnolia officinalis* was purchased from the oriental drug store, Bohwa Dang (Chonju, Korea) and identified by J.P.Lim, College of Pharmacy, Woosuk University. A voucher specimen (number WSP-96-10) was deposited at the Herbarium of the College of Pharmacy, Woosuk University. The plant sample was extracted with distilled water at 70°C for 5 h (two times). The extract was filtered through a 0.45 μm filter, and the filtrate was lyophilized, and kept at -4°C. The yield of dried extract from starting crude materials was about 13.2%. The dried extract was dissolved in saline or Tyrode buffer A (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, 0.1% bovine serum albumin) before use.

Compound 48/80-induced systemic anaphylaxis

Compound 48/80-induced systemic anaphylactic reaction was examined as previously described (Shin *et al.*, 1999). Rats were given an intraperitoneal injection of 0.008 g/kg body weigh (BW)of the mast cell degranulator, compound 48/80. MOAE was dissolved in saline and administered by intraperitoneal injection from 0.005 to 1 g/kg BW 1 h before the injection of compound 48/80 (n =10/group). In time dependent experiment, MOAE (1 g/kg BW) was injected intraperitoneally at 5 and 10 min after compound 48/80 injection (n=10/group). Mortality was monitored for 1 h after induction of anaphylactic shock. After the mortality test, blood was obtained from the heart of each rat.

Induction of PCA

An IgE-dependent cutaneous reaction was generated by sensitizing the skin with an intradermal injection of anti-DNP IgE followed 48 h later with an injection of DNP-HSA into the rat's tail vein. The anti-DNP IgE and DNP-HSA were diluted in PBS. The rats were injected intradermally with 0.5 μg of anti-DNP IgE into each of four dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. Each rat, 48 h later, received an injection of 1 mg of DNP-HSA in PBS containing 4% Evans blue (1:4) via the tail vein. MOAE (0.001 to 1 g/kg BW) was orally administered 1 h before the challenge. Then 30 min after the challenge, the rats were sacrificed and the dorsal skin was removed for measurement of pigment area. The amount of dye was then determined colorimetrically after extraction with 1 ml of 1 M KOH and 9 ml of mixture of acetone and phosphoric acid (5:13) based on the method of Katayama *et al.* (1978). The absorbent intensity of the extraction was measured at 620 nm in a spectrophotometer (Shimadzu, UV-1201, Japan).

Preparation of plasma and histamine determination

The blood was centrifuged at 400 × g for 10 min. The plasma was withdrawn and histamine content was measured by the o-phthaldialdehyde spectrofluorometric procedure of Shore *et al.* (1959). The fluorescent intensity was measured at 438 nm (excitation at 353 nm) in a spectrofluorometer (Shimadzu, RF-5301 PC, Japan).

Preparation of RPMC

RPMC were isolated as previously described (Kanemoto *et al.*, 1993). In brief, rats were anesthetized by ether and injected with 20 ml of Tyrode buffer B (137 mM NaCl, 5.6 mM glucose, 12 mM $NaHCO_3$, 2.7 mM KCl, 0.3 mM NaH_2PO_4 and 0.1% gelatin) into the peritoneal cavity and the abdomen was gently massaged for about 90 seconds. The peritoneal cavity was carefully opened and the fluid

containing peritoneal cells was aspirated by a Pasteur pipette. After that, the peritoneal cells were sedimented at 150 × g for 10 min at room temperature and resuspended in Tyrode buffer B. Mast cells were separated from the major components of rat peritoneal cells, i.e. macrophages and small lymphocytes, according to the method described by Yurt *et al.* (1977). In brief, peritoneal cells suspended in 1 ml of Tyrode buffer B were layered on 2 ml of metrizamide (22.5 W/V%) and centrifuged at room temperature for 15 min at 400 × g. The cells remaining at the buffer-metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1 ml Tyrode buffer A.

Inhibition of histamine release

Purified RPMC were resuspended in Tyrode buffer A for the treatment of compound 48/80. RPMC suspensions (2×10^5 cells/ml) were preincubated for 10 min at 37 °C before the addition of compound 48/80 (5 μg/ml). The cells were preincubated with the MOAE (0.001 to 1 mg/ml), and then incubated (10 min) with the compound 48/80. RPMC suspensions (2×10^5 cells/ml) were also sensitized with anti-DNP IgE (10 μg/ml) for 6 h. The cells were preincubated with the MOAE at 37°C for 10 min prior to the challenge with DNP-HAS (1 μg/ml). The cells were separated from the released histamine by centrifugation at 400 × g for 5 min at 4°C. Residual histamine in cells was released by disrupting the cells with perchloric acid and centrifugation at 400 × g for 5 min at 4°C.

Assay of histamine release

The inhibition percentage of histamine release was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{A-B}{A} \times 100$$

A: Histamine release without MOAE
B: Histamine release with MOAE

Assay of TNF-α production

TNF-α production was measured with the quantitative sandwich enzyme immunoassay technique, using a commercial kit (R & D Systems, U.S.A.). RPMC (3×10^5 cells/ml) were sensitized with anti-DNP IgE (1 μg/ml) and incubated for 18 h in the absence or presence of MOAE (0.001 to 1 mg/ml) before the challenge DNP-HAS (0.1 μg/ml). TNF-α production was measured by ELISA. The ELISA was performed by coating 4-well plates with murine polyclonal antibody with specificity for murine TNF-α Sandard, controls, and samples are pipetted into the wells and any mouse TNF-α present is bound by the immunobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse TNF-α is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution (100 μl) is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop solution (100 μl) is added. The intensity of the color measured is in proportion to the amount of mouse TNF-α bound in the initial step. Optical density readings were made on a Titertek Multiscan (Flow Laboratories) with a 405 nm filter. The sample values are then read off the standard curves.

Measurement of cAMP level

The cAMP level was measured according to the method of Peachell *et al.* (1988). In brief, purified mast cells were resuspended in prewarmed (37°C) Tyrode buffer A. Typically, an aliquot of cells (2×10^5 cells) were added to an equivalent volume (50 μl) of prewarmed buffer containing the drug in an Eppendorf tube. The reaction was allowed to proceed for discrete time intervals, terminated by the addition of ice-cold acidified ethanol (0.9 ml of 86% ethanol/1 M HCl, 99:1) with brief vigorous vortexing and then snap frozen in liquid nitrogen. The MOAE was later thawed and vortexed, then the debris was sedimented in a centrifuge (400 × g at 4°C, for 5 min), and an aliquot (0.9 ml) of the supernatant was removed and evaporated to dryness under reduced pressure. The dried sample was reconstituted in assay buffer (150-200 μl) and stored frozen. The cAMP level was determined by enzyme immunoassay, using a commercial kit (Amersham Pharmacia Biotech).

Statistical analysis

The results obtained were expressed as mean ± SEM. The Student's t-test was used to make a statistical comparison between the groups. Results with $p<0.05$ were considered statistically significant.

RESULTS

In vivo effect of MOAE on compound 48/80-induced systemic anaphylaxis

Initially, to determine the effect of MOAE in systemic anaphylaxis, we used compound 48/80 (0.008 g/kg BW) as a systemic fatal anaphylaxis inducer. After the intraperitoneal injection of compound 48/80, the rats were monitored for 1 h, after which the mortality rate was determined. As shown in Table I, an intraperitoneal injection of 200 μl saline as a control induced a fatal shock in 100% of rats. When rats were pretreated with MOAE at concentrations ranging from 0.005 to 1 g/kg BW for 1 h, the mortality with compound 48/80 was reduced dose-dependently. The mortality of rats injected intraperitoneally with MOAE (1 g/kg) 5 min after compound 48/80

injection was 0%. However, the mortality of rats injected with 10 min after compound 48/80 injection was 20% (n=10/group) (Table II).

Effect of MOAE on compound 48/80-induced plasma histamine release

The ability of MOAE to influence compound 48/80-induced plasma histamine release was investigated. MOAE was given from 0.005 to 1 g/kg BW 1 h before (*n* =10/group) compound 48/80 injection. The correlation results with those of the mortality test were shown when their plasma histamine contents were measured (Fig. 1). The inhibition rate of histamine by MOAE was significant at doses of 0.1 to 1 g/kg.

Effect of MOAE on anti-DNP IgE-induced PCA

PCA is one of the most important *in vivo* models of anaphylaxis in local allergic reaction(Wershil *et al.*, 1987). As described in this experimental procedures, local extravasation was induced by a local injection of anti-DNP IgE followed by an antigenic challenge. Oral administration of MOAE (0.1 and 1 g/kg) showed a marked inhibition rate in PCA reaction (Fig. 2).

Table I. Effect of MOAE on compound 48/80-induced systemic anaphylaxis

MOAE treatment (g/kg BW)	Compound 48/80 (0.008 g/kg BW)	Mortality (%)
None (saline)	+	100
0.005	+	100
0.01	+	90
0.05	+	60
0.1	+	10
0.5	+	0
1	+	0
1	–	0

Groups of rats (*n*=10/group) were intraperitoneally pretreated with 200 μl saline or MOAE. MOAE was given at various doses 1 h before the compound 48/80 injection. The compound 48/80 solution was intraperitoneally given to the group of rats. Mortality (%) within 1 h following compound 48/80 injection was represented as the number of dead rats × 100/total number of experimental rats.

Table II. Time-dependent effect of MOAE on compound 48/80-induced systemic anaphylaxis.

MOAE treatment (g/kg BW)	Compound 48/80 (0.008 g/kg BW)	Mortality (%)	
		5 min after	10 min after
None(saline)	+	100	100
1	+	0	20
1	–	0	0

Groups of rats (*n*=10/group) were intraperitoneally pretreated with 200 μl saline or MOAE. MOAE was given at 5 min or 10 min after the compound 48/80 injection. The compound 48/80 solution was intraperitoneally given to the group of rats. Mortality (%) within 1 h following compound 48/80 injection was represented as the number of dead rats × 100/total number of experimental rats.

In vitro effect of MOAE on compound 48/80-induced or anti-DNP IgE-mediated histamine release from RPMC

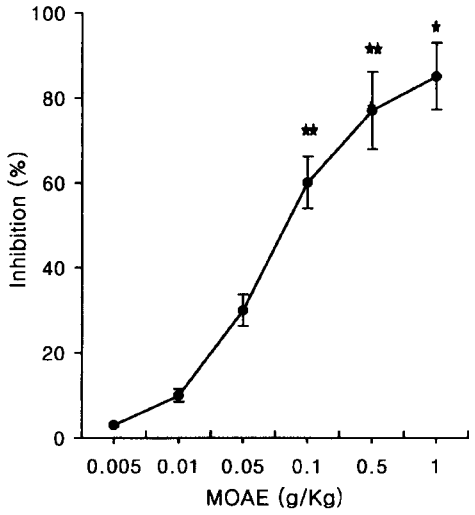

Fig. 1. In vivo effect of MOAE on compound 48/80-induced plasma histamine release. Groups of rats were intraperitoneally pretreated with 200 μl saline or MOAE. MOAE was given with various doses 1 h before the compound 48/80 injection. All values are expressed as a percentage of the control; 100%=2.14 ± 0.08 ng/ml. Each value is the mean ± SEM of three independent experiments. *p<0.05, **p< 0.01; significantly different from the saline value.

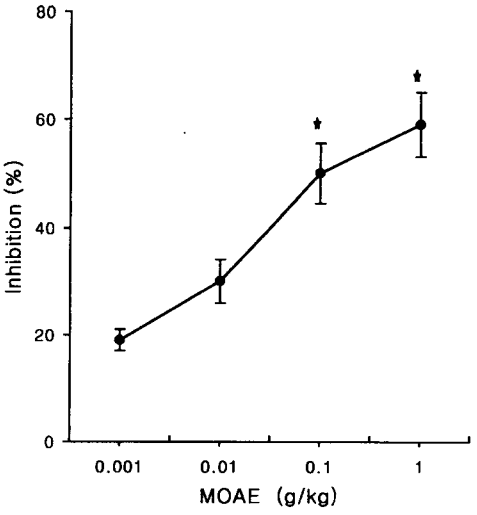

Fig. 2. Effect of MOAE on 48 h PCA. MOAE was administered orally 1 h prior to the challenge with antigen. All values are expressed as a percentage of the control; 0%=29.15 μg (amount of dye/site); 100%=0 μg(amount of dye/site). Each value is the mean ± SEM of three independent experiments. *p<0.05; significantly different from the saline value.

The inhibitory effect of MOAE on compound 48/80-induced or anti-DNP IgE-mediated histamine release from RPMC are shown in Fig. 3. MOAE dose-dependently inhibited compound 48/80-induced or anti-DNP IgE-mediated histamine release at concentrations of 0.001 to 1 mg/ml. Especially, MOAE significantly inhibited the compound 48/80-induced or IgE-mediated histamine release at the concentrations of 0.1 and 1 mg/ml.

Effect of MOAE on anti-DNP IgE-induced TNF-α production from RPMC

We next examined whether MOAE could also regulate TNF-α production by RPMC. MOAE significantly inhibited TNF-α production at concentrations of 0.01 to 1 mg/ml (Table III). No significant cytotoxicity of MOAE on the culture was observed in the concentrations used in the experiments, as assessed by trypan blue uptake.

Effect of MOAE on cAMP level of RPMC

Finally, we examined the cAMP content to clarify the mechanism by which MOAE inhibits histamine release from RPMC. RPMC were preincubated with or without MOAE at 37°C for 10 min prior to incubation with or without compound 48/80. When RPMC were incubated with MOAE, the cAMP content significantly increased, compared with that of control cells (Table IV). The increase in cAMP content by MOAE was little affected by the addition of compound 48/80.

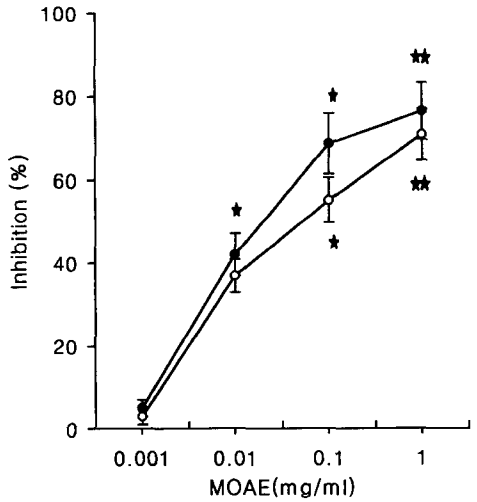

Fig. 3. In vitro effect of MOAE on compound 48/80-induced (●) or IgE-mediated (○) histamine release from RPMC. The cells (2×10^5 cells/ml) were preincubated with MOAE at 37°C for 10 min prior to incubation with compound 48/80 or challenge with DNP-HAS. All values are expressed as a percentage of the control; 100%=4.78 ± 0.93 μg/ml. Each value is the mean ± SEM of three independent experiments. *p<0.05, **p<0.01; significantly different from the saline value.

DISCUSSION

The present study showed that MOAE pretreatment greatly affected compound 48/80-induced systemic anaphylaxis and anti-DNP IgE-induced PCA. MOAE inhibited the plasma histamine levels in rats. MOAE also inhibited the compound 48/80 or anti-DNP IgE-mediated histamine release from RPMC. Therefore, we simply speculate that these results indicate that anaphylactic degranulation of mast cells is inhibited by MOAE. There is no doubt that stimulation of mast cells with compound 48/80 or anti-DNP IgE initiates the activation of signal-transduction pathway, which leads to histamine release. Some recent studies have shown that compound 48/80 and other polybasic compounds are able, apparently directly, to activate G-proteins (Mousli *et al.*, 1990a; Mousli *et al.*, 1990b). The evidence indicates that the protein is G inhibitory-like and that the activation is inhibited by benzalkonium chloride (Bueb *et al.*, 1990).

In spite of the increasing evidence of the role of several other mediators (Rafferty and Holgate, 1989; Rimmer and Church, 1990), histamine is still regarded as the principal mediator of antigen-induced skin reactions. In addition, intradermal and intranasal application of chemical mediators and chemical mediator releasers increase vas-

Table III. Effect of MOAE on anti-DNP IgE-induced TNF-α production in RPMC

MOAE treatment (mg/ml)	Anti-DNP IgE plus DNP-HSA	TNF-α production (pg/ml)
None (saline)	−	69.5 ± 5.8
None (saline)	+	209.1 ± 18.7
0.001	+	187.0 ± 18.2
0.01	+	118.2 ± 12.3**
0.1	+	102.6 ± 9.8*
1	+	91.9 ± 9.4**

RPMC (3×10^5 cells/ml) were sensitized with anti-DNP IgE (1 μg/ml) and incubated for 18 h in the absence or presence of MOAE before the challenge with DNP-HAS (0.1 μg/ml). The data represents the mean ± SEM of three independent experiments. *p<0.05, **p<0.01: significantly different from the saline value.

Table IV. Effect of MOAE on compound 48/80-induced cAMP content in RPMC

MOAE treatment (mg/ml)	Compound 48/8 (5 μg/ml)	cAMP (p mol)
None (saline)	−	0.39 ± 0.03
None (saline)	+	0.21 ± 0.03
0.1	−	1.08 ± 0.17*
1	−	2.17 ± 0.31*
1	+	1.81 ± 0.24*

RPMC (2×10^5 cells/ml) were pretreated with MOAE at 37°C. The data represents the mean ± SEM of three independent experiments. *p<0.05: significantly different from the saline value.

cular permeability in a manner similar to that of allergic models (Inagaki *et al.*, 1989, 1990). The MOAE administered rats are protected from IgE-mediated local anaphylaxis. This finding suggests that MOAE might be useful in the treatment of allergic skin reactions. Our data demonstrated that MOAE inhibited anti-DNP IgE-induced TNF-α production from RPMC. The effect of MOAE on mast cell cytokine production *in vivo* and the relative importance of mast cells as source of TNF-α during inflammatory and immune responses are important areas for future studies. According to earlier findings, agents that elevated cAMP levels inhibited mediator release (Hayashi *et al.*, 1976, Sullivan *et al.*, 1975). Xanthine derivatives, prostglandine E and E_2, and so forth, are known to increase intracellular cAMP levels (Sullivan *et al.*, 1975). The possible mechanism of these effects appears to be related to the activation of adenylate cyclase and a subsequent increase in intracellular cAMP level (Makino *et al.*,1987). The intracellular cAMP content of mast cells, when incubated with MOAE, increased more than six fold in comparison with that of basal cells. This result indicates that the inhibitory effect of MOAE on degranulation of mast cells may be mediated through an increase in cAMP level. Our results demonstrated that MOAE inhibited the mast cell-mediated immediate hypersensitivity *in vivo* and *in vitro* in a murine model. Therefore, Further work should address the possibility that MOAE may also be active in the inhibition of human mast cell degranulation.

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