

IgE sensitivity and cross-reactivity to crude and purified mite allergens (Der f 1, Der f 2, Der p 1, Der p 2) in atopic dogs sensitive to *Dermatophagoides* mite allergens

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Abstract

The present study investigates IgE-reactivity to crude and purified mite allergens by intradermal skin test (IDST), Immunodot method, and ELISA in atopic dogs sensitive to mite allergens, as well as the allergenic cross-reactivity between *Dermatophagoides* (D) *farinae* (DF) and *D. pteronyssinus* (DP) in dogs by IgE-ELISA inhibition. IDST and Immunodot method for crude mite allergens were performed for atopic dogs and 16 atopic dogs showed sensitivity to mite allergens. Of the 16 dogs, all dogs had anti-DF IgE and 11 had anti-DP IgE. We measured specific IgE to purified major allergens (Der f 1, Der f 2, Der p 1, Der p 2). Of the 16 atopic dogs, six had anti-Der f 1 IgE and seven had anti-Der f 2 IgE. Similarly, of the 16 dogs, six had anti-Der p 1 IgE and seven had anti-Der p 2 IgE. However, eight dogs had no specific IgE to these mite allergens. These dogs may be sensitive to other major mite allergens except Der 1 and Der 2. In the dogs that had both anti-DF and DP IgE, IgE binding to DF was greatly inhibited by DP, and reciprocal inhibition was observed. Based on these data, it appears that there is a strong cross-reactivity between DF and DP in dogs.

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Similarly, a cross-reactivity between DF and DP in purified allergens was also observed. IDST and Immunodot method are useful methods for the diagnosis of atopic diseases in dogs, and ELISA is a useful method for further investigation of IgE-reactivity for the allergens. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Canine atopic dermatitis is a common disease in veterinary dermatology (Halliwell and Schwartzmann, 1971; Nesbitt et al., 1984; Willemse, 1986). Various clinical signs have been reported, including pruritus, otitis externa, and recurrent and chronic dermatitis, especially in the axillary, inguinal and flexor and extensor regions of the limbs (Willemse, 1986). Intradermal skin tests (IDST) and in vitro allergen-specific IgE assays have shown positive reactions to specific allergens in atopic dogs, indicating that type I hypersensitivity can be caused by these allergens. It has been determined that the allergens of canine atopic dermatitis reflect environmental factors (Nesbitt et al., 1984; Sture et al., 1995). Our epidemiological study demonstrated that more than 50% of atopic dogs are IgE antibody-positive to mite allergens (Hasegawa et al., 1996). In dogs, house dust mites play an important role in the pathogenesis of atopic dermatitis.

In humans, allergy to house dust allergens is common among patients with allergic diseases (Platts-Mills and Solomon, 1993). Two species of *Dermatophagoides* mite, *D. farinae* (DF) and *D. pteronyssinus* (DP), are important sources of house dust allergens (Platts-Mills et al., 1997). Many mite allergens have been purified from each *Dermatophagoides* species, and Der 1 and Der 2 groups as major allergens have been analyzed in human patients with mite allergies (Thomas and Smith, 1998).

In dogs, skin test reactions are much more common to DF than to DP (Sture et al., 1995; Noli et al., 1996). ELISA and western blots have confirmed the presence of specific IgGd against Der 1 and Der 2 (Noli et al., 1996). However, there has been no report of specific IgE for Der 1 and Der 2 in atopic dogs sensitive to mite allergens. Furthermore, there has been no report of cross-reactivity to Der 1 and Der 2 with use of IgE in sera of dogs sensitive to mite allergens.

The present study investigates IgE-reactivity to crude and purified mite allergens by IDST, Immunodot method, and ELISA in atopic dogs sensitive to mite allergens, as well as the allergenic cross-reactivity between DF and DP by IgE-ELISA inhibition.

2. Materials and methods

2.1. Mite antigen

Mite crude extract from *D. farinae* (DF) and *D. pteronyssinus* (DP) was prepared as described previously (Yasueda et al., 1994). The major mite allergens, Der f 1, Der f 2, Der p 1, and Der p 2 were purified from whole culture of DF and DP as previously described (Yasueda et al., 1986, 1989a).

2.2. Dogs

Based on Willemse's criteria (Willemse, 1986), 42 dogs were diagnosed as having atopic dermatitis at the Veterinary Medical Center, The University of Tokyo, Japan. After screening using both a skin test and an Immunodot method (de Weck et al., 1998), 21 dogs showed sensitivity to crude mite allergens in both methods. Of 21 dogs, 16 dogs were available in this study (Table 1). IDST and serum sampling for Immunodot and ELISA were performed at the same time. Various clinical signs have been recognized in the dogs (Table 1). Chronic dermatitis was recognized in dogs of No. 3, 9, 11, 15, resulting in lichenification of skin, while simple pruritus without obvious skin lesions, acute dermatitis with or without superficial pyoderma, and self-induced dermatitis causing hair loss were seen in the other dogs. Dogs have been treated with corticosteroid, anti-histaminics, and anti-bacterial agents if skin lesions became severe. Before intradermal skin test and antigen-specific IgE test, the treatment was discontinued for at least one week.

Dermatomycosis and ectoparasitosis caused by *Demodex canis*, fleas, and scabies were excluded. Prior to the study, the dogs were treated with anti-bacterial therapy such as anti-bacterial shampoo and /or antibiotics in order to exclude the possibility of simple bacterial infection.

Twenty dogs (12 female and 8 male) (mean age $\pm$ SD, 3 years $\pm$ 2 years 8 months) (range from 1 to 6 years) that had never shown signs of atopic dermatitis and had demonstrated no specific IgE to mite allergens by IDST and Immunodot method were used as a negative control.

2.3. Intradermal skin test (IDST)

IDST was performed according to Greer's instructions. Briefly, the mite allergens that we prepared (Yasueda et al., 1994) were diluted to a concentration of 1 : 20 or 1 : 50 with diluent containing 0.9% sodium chloride and 0.4% phenol. The diluent was also used as a negative control. A histamine solution (0.0275 mg/ml) was used as a positive control. These solutions of the mite allergens and the controls were injected intradermally into clipped skin of the ventro-lateral thorax at a volume of 0.05 ml, using a test syringe with a 26-gauge needle. The diameter of wheals was measured 15–20 min after the injections. The sizes of the wheals were classified into four grades. The size equal to or greater than that of positive control was ++++. If the size was equal to or smaller than that of negative controls, it was determined as negative (–). The mean size between the positive and the negative controls was used to divide ++ and +; if the size was equal to or greater than the mean size, it was ++. The size smaller than the mean size was +.

2.4. Immunodot

Specific IgE against mite allergens was measured by Immunodot method (CMG Laboratories, Switzerland) (de Weck, 1995; de Weck et al., 1998). Briefly, dog serum samples were incubated on a nitrocellulose strip spotted with mite allergens. After

Table 1
Clinical signs in dogs sensitized with mite allergens

No.	Breeds	Age	Onset ^a	Sex	Clinical signs						
					Itching	Papules	Pustules	Pigmentation	Lichenification	Hair loss	Otitis externa
1	Shiba Inu	11	9	F	+	+	+	–	–	–	–
2	Golden Retriever	4	2	F	+	+	+	–	–	+	–
3	Mongrel	9	5	F	+	–	–	–	+	–	–
4	Shiba Inu	1	1	M	+	+	+	–	–	–	+
5	Great Pyreneese	2	1	F	+	+	–	–	–	–	–
6	Mongrel	9	5	F	+	+	–	–	–	–	–
7	Old English Sheepdog	4	2	M	+	+	+	–	–	+	–
8	Great Pyreneese	1	1	M	+	–	–	–	–	+	–
9	Pug	5	2	F	+	+	+	+	+	+	+
10	Pug	9	5	M	+	+	+	–	–	+	+
11	Akita	9	2	F	+	+	+	+	+	–	+
12	Kai	2	1	F	+	+	+	–	–	–	–
13	Mongrel	4	3	M	+	+	+	–	–	–	+
14	Golden Retriever	2	1	M	+	+	–	–	–	–	–
15	Shetland Sheepdog	4	2	F	+	+	+	+	+	+	+
16	Miniature Dachshund	4	1	M	+	+	–	–	–	–	–

^a Onset: first onset of clinical signs (years).

incubation, peroxidase-conjugated monoclonal anti-canine IgE was applied for detection. The color intensity was measured with a densitometer. If the O.D. value in the densitometer was two or more by the Immunodot method, the reaction was considered to be positive.

2.5. Fluorometric ELISA for specific IgE

Specific IgE antibodies to crude (DF and DP) and purified (Der f 1, Der f 2, Der p 1, Der p 2) mite allergens were assayed by a fluorometric ELISA with a slight modification (Sakaguchi et al., 1997). Measurement of these specific IgE is not available in the Immunodot method, so these IgE were measured with this fluorometric ELISA. A microplate (Immulon 2, Dynatech, Chantilly, VA, USA) was coated with crude mite antigens (10 µg/ml) (Yasueda et al., 1994) or purified mite allergens (1 µg/ml) overnight at 4°C. After washing, several concentrations of serum were added to the wells and the plate was incubated for 3 h at room temperature. The plate was washed, and a mouse monoclonal anti-dog IgE antibody (0.5 µg/ml) (De Boer et al., 1993), different from the monoclonal antibody used in the Immunodot method, was then added. The plate was incubated overnight at 4°C. After washing, biotinylated rat monoclonal anti-mouse IgG₁ (Zymed Laboratories, San Francisco, CA, USA) was added. The plate was incubated for 1 h at room temperature. After washing, β-D-galactosidase conjugated streptavidin (Zymed) was added. The plate was incubated for 1 h at room temperature. After final washing, 0.1 mM 4-methylumbelliferyl-β-D-galactoside (Sigma, MO, USA) was added to each well. The plate was incubated for 2 h at 37°C. The enzyme reaction was stopped with 0.1 M glycine-NaOH (pH 10.2), and the fluorescence intensity was read as fluorescence units (FU) on a microplate fluorescence reader (Fluoroskan, Flow Laboratories, McLean, VA, USA). The concentration of mite-specific IgE in each serum was calculated from standard titration curves of reference standard (a pooled serum) and was expressed in arbitrary units (U/ml).

2.6. Inhibition of specific IgE to mite allergens

The antigenic cross-reactivity between DF and DP was analyzed by fluorometric ELISA inhibition. Crude DF and DP (5 µg/ml) and purified mite allergens (0.5 µg/ml) were adsorbed to microplate wells. After overnight incubation at 4°C, the serum diluted 1:10 was mixed with mite allergens (at concentrations that produced maximal inhibition: 100 µg/ml as a crude allergen, 10 µg/ml as a purified allergen) as an inhibitor and added to the wells. After 3 h of incubation at room temperature, monoclonal anti-dog IgE antibody (0.5 µg/ml) was added. The following procedures were the same as those in the above-mentioned fluorometric ELISA. The percentage of inhibition was calculated as follows:

$$\left(1 - \frac{\text{FU in presence of allergen}}{\text{FU in absence of allergen}}\right) \times 100 = \text{percentage of inhibition}$$

3. Results

3.1. Reactivity to DF and DP in atopic dogs sensitive to mite allergens

In 16 atopic dogs that had specific IgE to crude mite allergens (mixed DF + DP) as determined by the Immunodot method, IDST for crude DF and DP were carried out (Table 2). Of the 16 dogs, all dogs had an IDST reaction to DF and 10 had an IDST reaction to DP.

We measured specific IgE to crude DF and DP in the sera of these 16 atopic dogs by fluorometric ELISA (Table 2). Of the 16 dogs, all dogs had anti-DF IgE and 11 had anti-DP IgE. As a negative control, 20 dogs that had no signs of allergy and no specific IgE to mite allergens by the Immunodot method showed no specific IgE to DF and DP as determined by this ELISA (data not shown).

Comparing IDST and ELISA results, of the 16 dogs with positive IDST for DF, all dogs had anti-DF IgE (Table 2). Of the 10 dogs with positive IDST for DP, eight had anti-DP IgE. Of the six dogs with negative IDST for DP, three had anti-DP IgE and three had no anti-DP IgE.

3.2. Reactivity to mite major allergens in atopic dogs sensitive to mite allergens

In six atopic dogs, IDST was performed using purified mite allergens (Der f 1, Der f 2, Der p 1, Der p 2) (Table 3). Of the four dogs that had positive IDST for purified mite allergens, three had specific IgE to the allergens. The two dogs that had negative IDST for purified mite allergens, also had no specific IgE for the allergens.

Table 2
Reactivity to crude DF and DP in dogs sensitized with mite allergens

Dog	IDST		Specific IgE		
	DF	DP	ELISA (U/ml)		Immunodot
			DF	DP	DF + DP
1	+++	+++	238	80	38
2	+++	+++	497	9	36
3	+++	+++	66	177	2
4	+++	+++	114	154	32
5	+++	++	1620	41	30
6	+++	++	184	10	5
7	+++	++	52	3	6
8	+++	++	432	59	4
9	++	+++	48	<1	25
10	+++	++	55	<1	3
11	+	–	52	<1	14
12	++	–	29	<1	14
13	+	–	15	<1	3
14	+++	–	527	5	63
15	++	–	23	2	22
16	++	–	78	24	16

Table 3

Reactivity to DF and DP major allergens in dogs sensitized with mite allergens

Dog	IDST				ELISA (U/ml)			
	DF		DP		DF		DP	
	Der f 1	Der f 2	Der p 1	Der p 2	Der f 1	Der f 2	Der p 1	Der p 2
3	+++	+++	+++	+++	94	1422	23	1860
7	+++	+++	+++	+++	<1	35	<1	33
15	+++	+++	+++	–	23	<1	3	<1
14	–	++	–	–	<1	<1	<1	<1
10	–	–	–	–	<1	<1	<1	<1
12	–	–	–	–	<1	<1	<1	<1

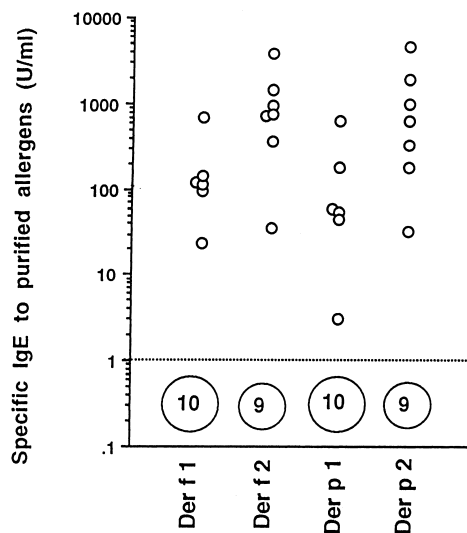


Fig. 1. The levels of specific IgE to purified mite allergens by an ELISA. Numbers in circles are number of sera below 1 U/ml.

Fig. 1 shows specific IgE to purified mite allergens as determined by the fluorometric ELISA. Of the 16 atopic dogs sensitive to mite allergens, six had anti-Der f 1 IgE and seven had anti-Der f 2 IgE. Similarly, of the 16 dogs, six had anti-Der p 1 IgE and seven had anti-Der p 2 IgE. Only five dogs had both specific IgE to Der 1 and Der 2. Eight dogs had no specific IgE to these allergens.

3.3. Allergenic cross-reactivity among mite allergens by ELISA inhibition

Allergenic cross-reactivity between DF and DP was investigated by ELISA inhibition. In the dogs (Nos. 1, 4, and 8) listed in Table 1 that had both anti-DF and DP IgE, incubation of the homologous DF with the serum inhibited binding by more than 90% (Fig. 2(a)). IgE binding to DF was greatly inhibited by DP (Fig. 2(a)). A similar pattern in

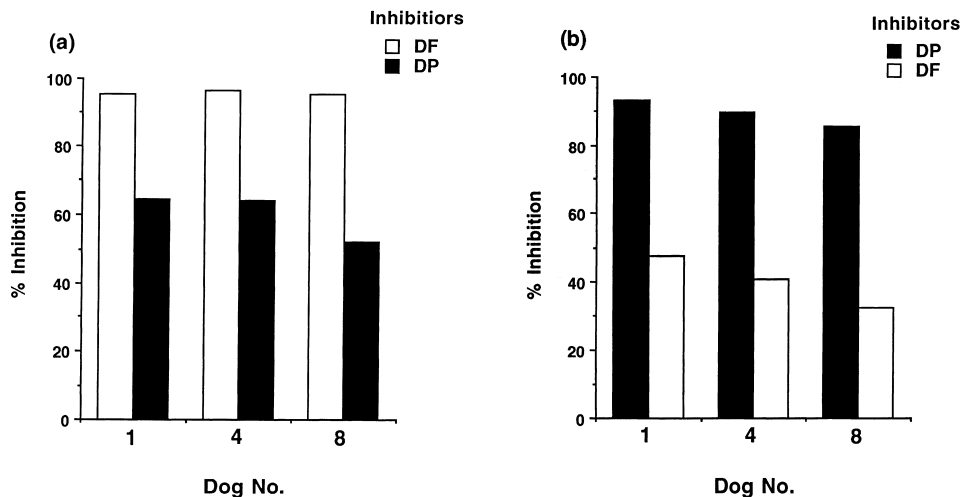


Fig. 2. Cross-reactivity between crude DF and DP by ELISA inhibition analysis using sera of atopic dogs with both anti-DF and -DP IgE. (a) immobilized antigen: DF; (b) immobilized antigen: DP.

the reciprocal inhibition was also observed (Fig. 2(b)). Therefore, we found a cross-reactivity between DF and DP.

Next, the cross-reactivity between purified mite allergens was examined by ELISA inhibition (Figs. 3 and 4). Sera of the dogs (Nos. 1, 3, and 8) that had both anti-Der f 1 and De p 1 IgE (Fig. 3) were used for the analysis (Fig. 3). IgE binding to Der f 1 was inhibited by Der p 1, and reciprocal inhibition was observed (Fig. 3(a)). Sera of the dogs

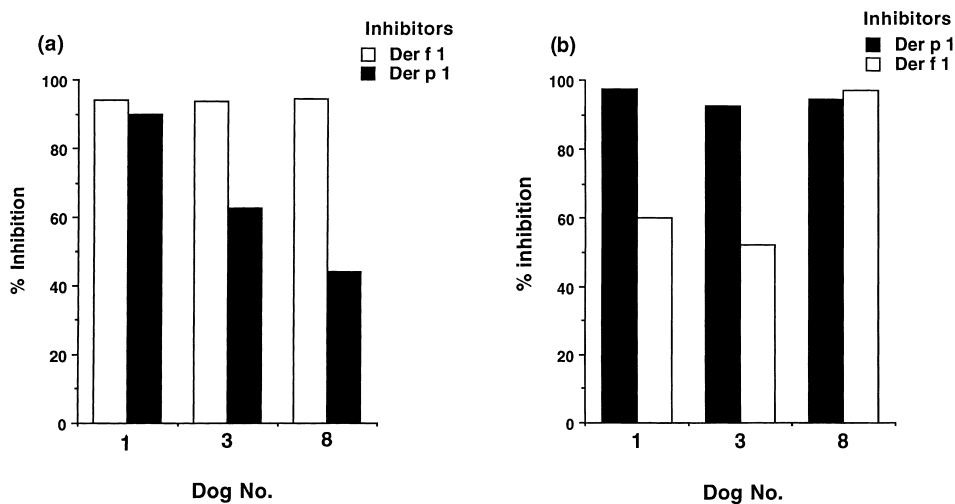


Fig. 3. Cross-reactivity between purified allergens by ELISA inhibition analysis using the sera of atopic dogs with both anti-Der f 1 and anti-Der p 1 IgE. (a) immobilized antigen: Der f 1; (b) immobilized antigen: Der p 1.

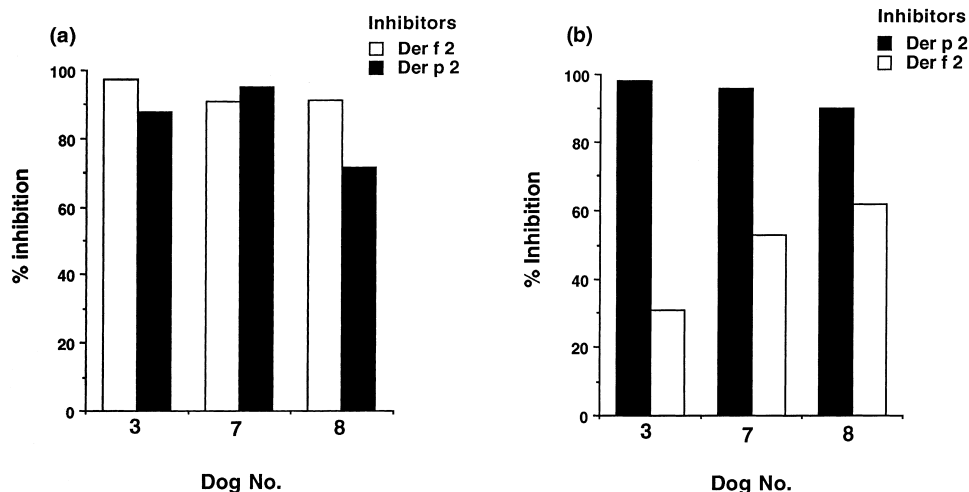


Fig. 4. Cross-reactivity between purified allergens by ELISA inhibition analysis using the sera of atopic dogs with both anti-Der f 2 and anti-Der p 2 IgE. (a) immobilized antigen: Der f 2; (b) immobilized antigen: Der p 2.

(Nos. 3, 7, and 8) that had both anti-Der f 2 and Der p 2 IgE were used for the analysis (Fig. 4). A similar pattern was found in the inhibition between Der f 2 and Der p 2 (Fig. 4(a) and (b)).

4. Discussion

In IDST, all 16 dogs had an IDST reaction to DF, but six had no IDST reaction to DP. In this study, atopic dogs sensitive to mite allergens had more frequent IDST reactions to DF than those to DP. Although DP mite is more predominant in Japan than DF mite (Yasueda et al., 1989b; Sakaguchi et al., 1992), the atopic dogs showed much stronger reactions in IDST to DF than DP. In previous studies, it has been reported that IDST reactions to DF are much more common than those to DP (Sture et al., 1995; Noli et al., 1996). Our IDST and ELISA results in atopic dogs of Japan confirm the results of these previous studies. Recently, however, Lina and Halliwell (1998) reported the same frequency of DF- and DP-specific IgE in atopic dogs.

In this study, of the 16 atopic dogs sensitive to mite allergens, eight had no specific IgE to purified mite allergens (Der 1 and Der 2). It has been reported that atopic dogs have low levels of specific IgGd to these purified allergens (Noli et al., 1996). Willemse et al. (1985a,b) identified a non-IgE anaphylactic antibody, termed IgGd, in the sera of atopic dogs. It has been proposed that IgGd may be important in the pathogenesis of atopic disease. In this study, specific IgGd was not measured, because the assay was not available.

In humans, Der 1 and Der 2 group allergens would be considered major allergens in *Dermatopagoides spp.* mite. The prevalence of specific IgE to groups 1 and 2 allergens is >80%, and immunoabsorption studies have shown that >50% of IgE to mite allergens can

be directed against these allergens (Meyer et al., 1994; Platts-Mills et al., 1997). The dogs with no sensitivity to Der 1 and Der 2 groups may be sensitive to other mite allergens.

To investigate the allergenic cross-reactivity between DF and DP, ELISA inhibition with atopic dogs' sera was performed. In the dogs that had both anti-DF and DP IgE, IgE binding to DF was greatly inhibited by DP, and the reciprocal inhibition was also observed. Based on these data, it appears that there is a strong cross-reactivity between DF and DP in dogs. In this study, the cross-reactivity between purified allergens was also studied by ELISA inhibition. In the dogs that had both anti-Der f 1 and Der p 1 IgE, IgE binding to Der f 1 was inhibited by Der p 1, and the reciprocal inhibition was observed. A similar pattern was found in the inhibition between Der f 2 and Der p 2. There is a cross-reactivity between DF and DP in purified allergens.

In this study, crude extract was prepared from mite bodies. Whole culture extract was chosen as starting material for purification of mite major allergens, since Der f 1 and Der p 1 are mainly included in mite fecal particles (Yasueda et al., 1986). Identification and assessment of purity were achieved by SDS-PAGE (Yasueda et al., 1986).

In conclusion, sensitization to DF might be more predominant than that to DP in dogs. Half of the atopic dogs had sensitivity to Der 1 and Der 2. There is a cross-reactivity between DF and DP in crude and purified mite allergens.

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