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Species-specific allergens from the salivary glands of Triatominae (Heteroptera: Reduviidae)

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We investigated allergenic cross-reactivity among species of the blood-feeding insects of the subfamily Triatominae. By skin testing, patients allergic to either Triatoma protracta or T. rubida gave positive responses only to the respective salivary antigen. RAST-inhibition experiments demonstrated that binding of IgE antibodies to T. protracta antigen was not inhibited by salivary extracts from T. rubida, T. cavernicola, T. rubrofasciata, or Rhodnius prolixus. The same level of species specificity was found for IgE antibodies to T. rubida. By direct RAST, no T. rubida positive serum bound T. protracta antigen, and 29 of 30 T. protracta positive sera failed to bind T. rubida. One serum from a T. protracta-allergic patient contained IgE antibodies to both T. protracta and T. rubida. RAST-inhibition experiments demonstrated that these antibodies did not cross-react and that this person had separate species-specific antibodies to T. protracta and T. rubida antigens. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of salivary extracts demonstrated that the lower molecular weight bands that contain the antigens responsible for human allergic reactions differed in number and size in all species tested. These studies demonstrate species specificity for the allergic response to Triatoma and stress the importance of accurate insect identification and the need for species-specific antigens for diagnosis and immunotherapy. (J ALLERGY CLIN IMMUNOL 78:430-5, 1986.)

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Blood-feeding insects in the subfamily Triatominae (Heteroptera: Reduviidae) have been documented to cause serious immediate hypersensitivity reactions in humans caused by sensitization to the salivary proteins that are injected during a blood meal.¹ Salivary extracts have been reported for use as diagnostic reagents as well as effective immunotherapeutic materials for *T. protracta*.² There are nine important species in the genus *Triatoma* in the continental United States that generally overlap in distribution. The two most prominent species in the southwest United States are *T. protracta* and *T. rubida*; they account for all the *Triatoma* allergy that we have observed. Recently, in

Abbreviations used

BSA:	Bovine serum albumin
M _r :	Relative molecular weight
OA:	Ovalbumin
PAGE:	Polyacrylamide gel electrophoresis
PBS:	Phosphate-buffered saline
RIA:	Radioimmunoassay
SDS:	Sodium dodecyl sulfate

a study in an area of southern California where this insect is endemic, we found a 6.7% prevalence of serum IgE antibodies to *T. protracta*.³

As in Hymenoptera allergy, the question of potential cross-reactivity of the various species needs to be addressed since both *T. protracta* and *T. rubida* cause a similar range of IgE-mediated allergic reactions. Definition of potential cross-reactivity is necessary to permit accurate diagnosis by way of identifying the offending species and to design appropriate immunotherapy regimens. Furthermore, knowledge of cross allergenicity will be important to counsel patients regarding bites from other, potential allergy-inducing species of *Triatoma*, especially since there are clear regional distribution differences in the United States among the species.²

We therefore investigated, in *Triatoma*-allergic persons, cross-reactivity between the salivary extracts from *T. protracta* and *T. rubida*. The studies were done by skin test reactivity, RAST inhibition, direct binding of allergic sera to the salivary extracts, and SDS-PAGE analysis. We failed to find substantial evidence for cross-reactivity between these two species. Additional studies were undertaken with salivary extracts from *T. cavernicola*, *T. rubrofasciata*, and *R. prolixus*, and these also failed to demonstrate allergic cross-reactivity between the saliva from these species and that from *T. protracta* and *T. rubida*.

MATERIAL AND METHODS

Antigen preparation

Colonies of *Triatominae* were maintained at University of California-Los Angeles, and all species were from those colonies. Salivary gland dissections were performed, and antigen from each species was prepared from dissected salivary glands as described.^{1,2} *Triatominae* used as antigen sources were *T. protracta*, Riverside Co., Winchester, Calif.; *T. rubida*, Riverside Co., Indio, Calif.; *T. cavernicola*, Kuala Lumpur, Malaysia; *T. rubrofasciata*, Honolulu Co., Waianae, Hawaii, and *R. prolixus*, Venezuela.

Skin testing

Salivary antigens from *T. protracta* and *T. rubida* were simultaneously used to skin test persons previously diag-

nosed as having immediate hypersensitivity reactions to *Triatoma*. Eleven *T. protracta*- and three *T. rubida*-sensitive patients were tested with tenfold increasing concentrations of 0.02 to 0.03 ml of each antigen injected intradermally, beginning with a 0.00001 U/ml solution. Testing was stopped when a positive reaction was produced (wheal ≥ 5 mm) or a concentration of 100 U/ml was reached. The lowest concentration producing a positive reaction was recorded. The amount of antigen required to block 50% binding of 10 μ l of a reference serum as previously described² was taken as 0.4 units.

Determination of total IgE

Sera were assayed for total IgE by use of a solid-phase RIA as described previously.² Polyvinyl chloride microtiter plates were coated with a mixture of two monoclonal antihuman IgE Fc-specific antibodies, CIA/E-7.12 and CIA/E-4.15. Diluted serum samples, 100 μ l per well, were added, and the plates were incubated for 2 hours and then washed five times with 0.05% Tween 20 in PBS, pH 7.0. The plates were incubated with goat affinity-purified antihuman IgE (Kirkgarrrd and Perry Laboratories, Gaithersburg, Md.) labeled with ¹²⁵I. After final washing, the plates were dried, and individual wells were cut out and counted in a gamma counter.

The assay was quantitated by use of twofold dilutions (1/100 to 1/51,200) of a standard serum to form a control curve that was run in parallel with the test sera. The standard serum was standardized against the World Health Organization International IgE Standard (National Institute of Biological Standards and Controls, London, U. K.) and contained 20,000 IU of IgE per milliliter. Sera were assayed at dilutions of 1/5, 1/25, or higher where it was appropriate. The diluent for all samples was 1% BSA in PBS-Tween.

Triatoma-specific IgE radioimmunoassay

The radioimmunoassay for *Triatoma*-specific IgE has been previously reported.² Briefly, microtiter plates were coated with 100 μ l of a 100 U/ml solution of *T. protracta* or *T. rubida* antigen. After overnight incubation, the plates were washed with PBS-Tween and blocked with 1% OA in PBS. Sera were diluted 1/10, and 80 μ l was added per well. After overnight incubation and three washes, 100 μ l of ¹²⁵I-labeled anti-IgE was added, and the plates were incubated for 4 hours. The plates were then washed with PBS-Tween, washed with water, and dried, and the wells were cut out and counted in a gamma counter. Sera tested were from 30 *T. protracta*-allergic patients, two *T. rubida*-allergic patients, and four nonallergic control subjects. Assays were performed separately with salivary gland extracts from *T. rubrofasciata*, *T. cavernicola*, and *R. prolixus* as antigen. The sera used in these latter assays included two anti-*T. rubida*, three anti-*T. protracta*, and two negative control sera. All samples were assayed in duplicate. Units of *Triatoma*-specific IgE were extrapolated from a control curve constructed with a serum that was arbitrarily defined as containing 160 U/ml anti-*T. protracta* IgE. This serum elicited significant binding (twofold to threefold above background) at a dilution of 1/160 in the *T. protracta* RAST.

TABLE I. Comparison of the *T. protracta* skin tests, specific IgE antibody, and total serum IgE in patients and control subjects

Subjects	<i>T. protracta</i> skin test positive*	IgE antibody to <i>T. protracta</i> (U/ml)	Total IgE (IU/ml)
<i>T. protracta</i> allergic (N = 30)	27/27	116 (5 to 600)†	188 (7 to 1515)
Atopic control subjects (N = 14)	nd	<2	377 (18 to 1208)
Nonatopic control subjects (N = 17)	nd	<2	50 (5 to 215)

nd = Not done.

*Positive tests were defined as wheal >5 mm with surrounding erythema to testing with 10 U/ml or less of antigen.

†Numbers in parenthesis represent range.

RIA inhibition

Microtiter plates were coated with *T. protracta* or *T. rubida* antigen at 100 U/ml, incubated overnight, and washed; then any remaining binding sites were blocked by a 1-hour incubation with 1% OA. Serum or serum preincubated with various amounts of *Triatoma* antigen were then added. Two *T. protracta* positive, two *T. rubida* positive, and one negative control sera were tested. Before plate application, each serum was divided into 30 aliquots. To one aliquot each was added one of five concentrations of the five triatomine antigens (above). This was done in fourfold decreasing antigen concentrations (50, 12.5, 3.2, 0.8, and 0.2 units and an OA control). All antigen dilutions were made with 1% OA, and the final serum dilutions were 1/10. After the sera and antigen were incubated for 1 hour, the samples were added to the wells, and the assay was completed in the same fashion as the direct binding radioimmunoassay discussed above.

PAGE

Salivary gland extracts from *T. protracta*, *T. rubida*, *T. rubrofasciata*, and *R. prolixus* were separated by SDS with a 5% stacking gel and a 15% running gel.⁴ Samples containing 20 µg of protein were boiled 2 minutes in 5% SDS and 0.2 mol/L of dithiothreitol and then were electrophoresed for 4 hours at 30 mA in 0.025 mol/L Tris-0.192 mol/L glycine buffer, pH 8.3. Protein bands were visualized in the gels by Coomassie blue staining.

RESULTS

IgE antibodies in *T. protracta*-allergic patients

Skin tests to *T. protracta*, IgE anti-*T. protracta* antibody, and total serum IgE levels of 30 patients with a history of immediate hypersensitivity to *T. protracta* bites were compared with those of 14 allergic and 17 nonallergic control subjects (Table I). Twenty-seven of the *T. protracta*-allergic patients were skin tested, and all gave positive immediate reactions (5

mm or greater wheal to 10 U/ml or less) to *T. protracta* salivary gland extract at a concentration of 10 U/ml or less. All 30 of these patients had detectable IgE anti-*T. protracta* antibody in their serum, whereas, in keeping with previous results,² control sera did not. Most *T. protracta*-allergic patients (24/30) had total IgE levels within the normal range (<200 IU/ml), and the geometric mean levels were twofold lower than those of the allergic control subjects (Table I).

Assessments of allergenic cross-reactivity between *T. protracta* and *T. rubida*

Quantitative intradermal skin tests. Allergenic cross-reactivity between salivary gland extracts of *T. protracta* and *T. rubida* was assessed by quantitative intradermal skin testing, RAST inhibition, and direct RAST. Intradermal skin tests with salivary gland extracts from both species were compared in 11 *T. protracta*- and three *T. rubida*-allergic patients. The results (Fig. 1) demonstrated that *T. protracta*-allergic patients were highly sensitive to *T. protracta* allergen (eliciting positive responses at 0.001 to 1.0 U/ml) but either failed to react or were questionably reactive (responding at 100 U/ml) to *T. rubida* allergen. In previous studies,² approximately 5% of persons with no history of *Triatoma* sensitivity gave positive skin tests to the 100 U/ml of allergen concentration; therefore, reactions at this dose are likely to be nonspecific. Similarly, the *T. rubida*-allergic patients gave good responses to *T. rubida* allergen but demonstrated little or no sensitivity to *T. protracta* allergen.

RAST inhibition and direct RAST. Salivary gland extracts from *T. protracta*, *T. rubida*, *T. rubrofasciata*, and *R. prolixus* were tested for cross-reactivity by RAST inhibition with sera from either *T. protracta*- or *T. rubida*-allergic patients. The *T. protracta* extract

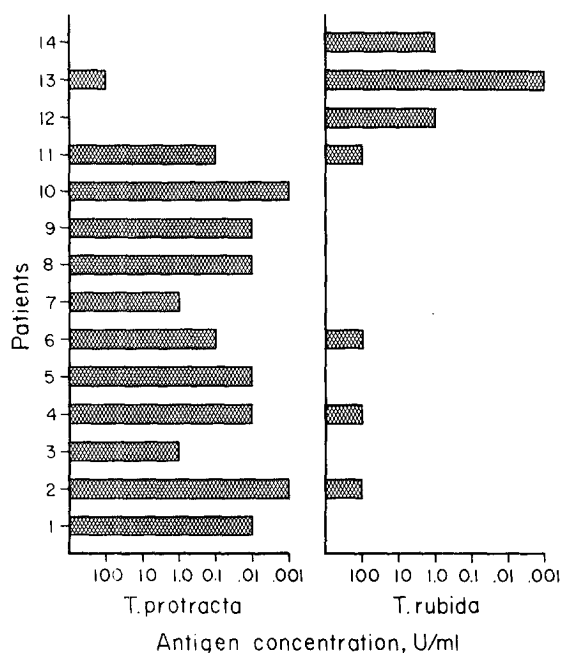


FIG. 1. Results of quantitative skin tests used to investigate cross-reactivity between *T. protracta* and *T. rubida* in allergic patients. Patients 1 to 11 were historically and clinically allergic to *T. protracta* as were patients 12 to 14 allergic to *T. rubida*. Positive tests were defined as wheal >5 mm with surrounding erythema when patients were tested with 10 U/ml or less.

demonstrated the expected dose-dependent inhibition of the binding of human IgE antibody to plates coated with *T. protracta* extract (Fig. 2). In contrast, salivary gland extracts from the other four species did not significantly inhibit the *T. protracta* RAST when extracts were assayed at similar allergen concentrations (0.2 to 50 U/ml). At much higher concentrations (1300 U/ml) the *T. rubida* extract did inhibit the *T. protracta* RAST, but the quantity of extract required to elicit 50% inhibition was a thousandfold greater than that of the homologous extract (data not presented). In the reverse experiment, the *T. rubida* RAST could be inhibited by *T. rubida* extract but not by extracts of the other four species (Fig. 3). These results strongly suggested that the allergens in the saliva of by *T. protracta* and *T. rubida* were species specific. To confirm and extend these observations, we compared 30 sera from *T. protracta*-allergic patients and two sera from *T. rubida*-allergic patients by direct RAST with plates coated with either allergen. Only 1/30 of the sera that contained IgE antibody to *T. protracta* (Table I) demonstrated detectable IgE binding to *T. rubida*. The increase in IgE anti-*T. protracta* binding of this serum relative to four negative controls was 3.3 fold. This compares to sera from *T. rubida*-allergic persons that bound 4.9 times the

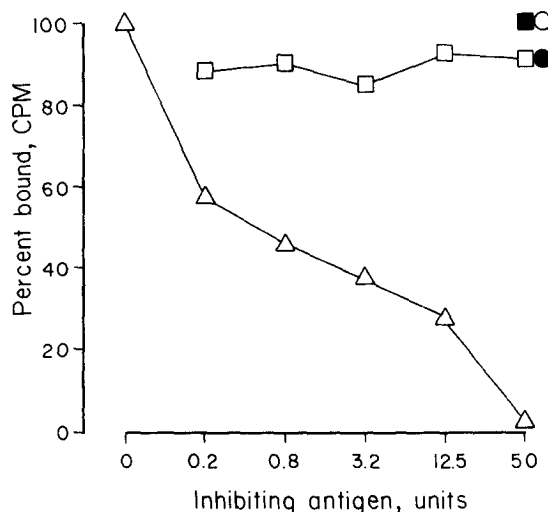


FIG. 2. RAST-inhibition experiment with *T. protracta*. Results demonstrate binding inhibition of anti-*T. protracta* IgE in serum by various triatomine antigens. *T. protracta* (Δ), *T. rubida* (□), *T. cavernicola* (○), *T. rubrofasciata* (■), and *R. prolixus* (●). Activity is expressed in percent bound ^{125} I-labeled anti-IgE. The antigen concentrations are presented on a fourfold geometric scale.

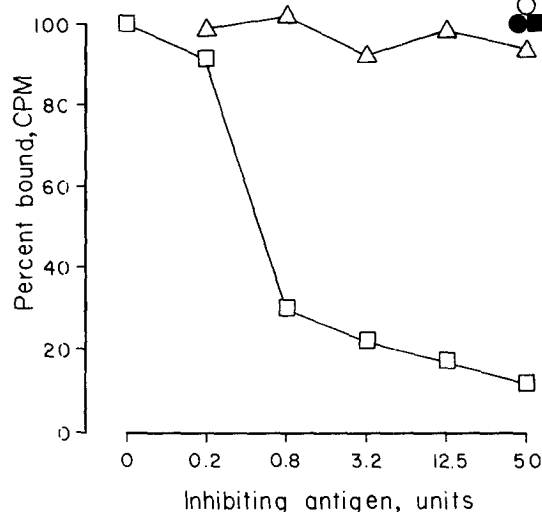


FIG. 3. RAST-inhibition experiment with *T. rubida*. Results demonstrate binding inhibition of anti-*T. rubida* IgE in serum by various triatomine antigens. *T. protracta* (Δ), *T. rubida* (□), *T. cavernicola* (○), *T. rubrofasciata* (■), and *R. prolixus* (●). Activity is expressed in percent bound ^{125} I-labeled anti-IgE. The antigen concentrations are presented on a fourfold geometric scale.

control counts per minute in that assay. The sera from *T. rubida*-sensitive patients did not demonstrate significant IgE binding to *T. protracta* (mean 1.3 fold above background).

We further investigated the nature of the binding

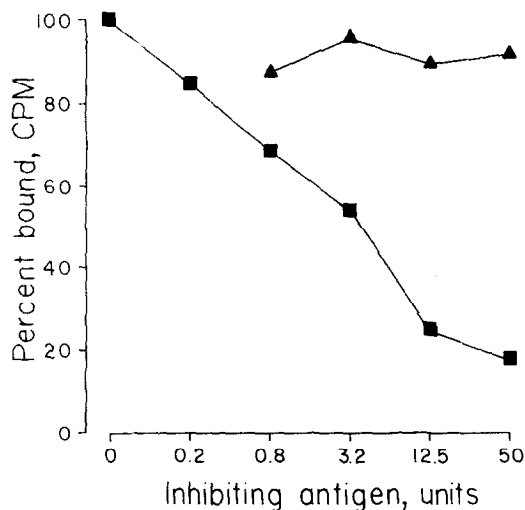


FIG. 4. RAST inhibition with *T. rubida* as the competing antigen. *T. rubida* was used to inhibit binding of the patient's serum that reacted with both *T. rubida* antigen (100% binding equal 13,000 CPM) and *T. protracta* antigen (100% binding equal 31,000 CPM). *T. rubida* blocked binding to *T. rubida* (■) by 90% at 50 units but failed to block binding to *T. protracta* (▲) (4% at 50 units). The antigen concentrations are presented as a fourfold geometric scale.

of the single apparent cross-reacting serum. RAST-inhibition experiments were undertaken with this serum. *T. protracta* or *T. rubida* antigens were bound to individual microtiter wells, and the ability of each antigen to inhibit binding of the serum was assessed. Sera that had not demonstrated cross-reactivity in the direct RAST were run in parallel as controls. The binding of the putative cross-reacting serum to *T. protracta* or *T. rubida* in the solid phase could only be inhibited by the homologous antigen and not the other (Fig. 4). Thus, this serum actually contained two sets of IgE antibodies to *Triatoma*, one directed against *T. protracta* and another that reacted with *T. rubida* salivary antigens. The anti-*Triatoma* antibodies in this serum did not cross-react.

Finally, the two most reactive sera from each patient group were tested by direct RAST for IgE cross-reacting with *T. rubrofasciata*, *T. cavernicola*, and *R. prolixus* salivary extracts. No binding above background to these preparations was detected.

SDS-PAGE analysis of salivary gland extracts

Salivary extracts from *T. protracta*, *T. rubida*, *T. rubrofasciata*, and *R. prolixus* demonstrated distinct profiles on SDS-PAGE (Fig. 5). The *T. protracta* extract demonstrated a major band, M_r 70 K, and six bands, M_r 17 to 25 K. Extracts of the other species

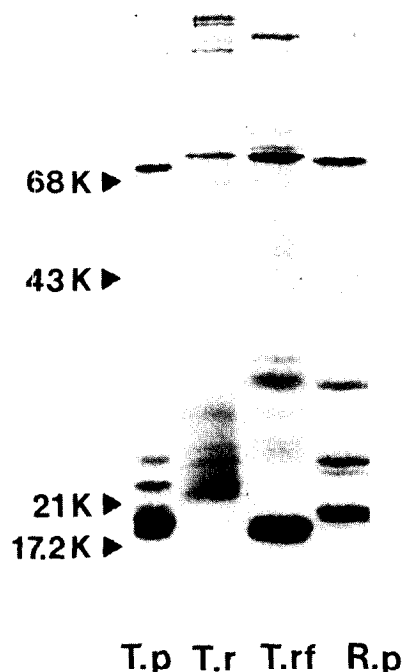


FIG. 5. SDS-PAGE analysis of triatomine salivary gland extracts. Extracts run were *T. protracta* (T.p), *T. rubida* (T.r), *T. rubrofasciata* (T.rf), and *R. prolixus*. Control markers (not illustrated) were BSA (molecular weight 68,000), OA (molecular weight 43,000), soybean trypsin inhibitor (molecular weight 21,000) and myoglobin (molecular weight 17,200).

also demonstrated a densely staining band at M_r 70 K, but the lower molecular weight bands in each of these extracts differed in number and in their electrophoretic mobilities. Although there is a cluster of bands near M_r 25 K, they actually have slightly different molecular weights. These differences in the lower molecular weight range are of particular relevance as we have reported that the lower molecular weight proteins comprise the relevant antigens in human allergic responses to *T. protracta*.⁵

DISCUSSION

We have demonstrated through quantitative skin tests, direct binding RIAs, and RAST-inhibition experiments that there is no substantial cross-reactivity among the salivary allergens from various species of the genus *Triatoma*. These results extend preliminary findings reported by Lindberg et al.⁶ that suggested species specificity among the *Triatoma*. The major reason for our findings became apparent from the SDS-PAGE experiments on the salivary material where we demonstrated unique SDS-PAGE patterns for the lower molecular weight proteins. In the accompanying paper in this JOURNAL, we demonstrate

that these low-molecular-weight protein bands comprise the major allergen(s) of *T. protracta*.⁵ Experiments with RAST inhibition indicated that the single individual whose serum did demonstrate binding to both *T. protracta* and *T. rubida* had, in fact, IgE directed to species-specific components of *T. protracta* and other IgE antibodies to *T. rubida* salivary products. Thus species-specific antibodies to *Triatoma* did not cross-react, even when two such antibodies occurred in a single individual. This latter person must have therefore encountered and been sensitized to *T. rubida* as well as *T. protracta*. It was confirmed that the individual, residing in a *T. protracta* area, had also travelled extensively in areas occupied by *T. rubida*.

Our finding represent a major exception to a generally occurring pattern of cross-reactivity of allergic reactions to related insects, particularly at the level of species within the same genera. The major Hymenopteran superfamilies, Apoidea and Vespoidea, demonstrate limited cross-reactivity. Within the family Vespoidea, cross-reactivity has clearly been demonstrated between various genera such as *Polistes* (paper wasps), *Vespula* (hornets), *Dolichovespula* (yellow jackets), and *Vespa* (giant hornets).^{7,8} Within any one genus of Hymenoptera, such as *Apis* or *Vespula*, there is extensive cross-reactivity. Inhalant allergy-producing insects also commonly cross-react at the genus and species level. Cross-reactivity has been noted between the American cockroach, *Periplaneta americana* and the German cockroach *Blattella germanica*⁹ and is also common among the different species of gnats (Chironomidae).¹⁰ Thus, the exquisite species specificity observed with allergic antibody to the *Triatoma* we have studied is without precedent among insect allergies. As more patients are studied and more species of *Triatoma* are investigated, this lack of species cross-reactivity may not turn out to be absolute, and minor cross-reacting antigens/allergens may be identified. We recently found that most *Triatoma* salivary extracts contain a 70,000 MW antigen that cross-reacts with rabbit antisera and also reacts with some human sera.⁵

In the continental United States, allergic reactions to *T. protracta*, *T. rubida*, *T. sanguisuga*, and *T. gerstaeckeri* have been reported¹¹ (reference 2 has range map). The distribution of *T. protracta* overlaps with that of *T. rubida* as do the distributions of *T. sanguisuga* and *T. gerstaeckeri*. It will be important there-

fore that antigens from the appropriate species be available for the diagnosis and treatment of allergy to *Triatoma*. Furthermore, it is necessary to be familiar with the various species of *Triatoma* in the area where a patient resides in order to rationally approach management of this problem. Finally, because of the species specificity of *Triatoma* allergy, the distribution of *Triatoma* species becomes important in terms of insect avoidance. Allergic individuals who elect not to undergo immunotherapy must consider areas of residence and recreation free of the *Triatoma* species to which they are sensitive.

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