

Allergens in Hymenoptera venom. XXII. Comparison of venoms from two species of imported fire ants, *Solenopsis invicta* and *richteri*

Donald R. Hoffman, PhD, A. Mason Smith, PhD,* Margit Schmidt, PhD,
John E. Moffitt, MD,** and Miles Guralnick, MS***
Greenville, N.C., Jackson, Miss., and Spring Mills, Pa.

Venoms were collected by electrical stimulation from the two major species of imported fire ants found in the United States, Solenopsis invicta (Sol i) and S. richteri (Sol r). Antigens similar to three of the four known Sol i venom proteins (I, II, III, and IV) were isolated from Sol r. The N-terminal amino acid sequences for the antigens III were identical; but those for the antigens II demonstrated only nine of 20 residues to be identical. Two monoclonal antibodies raised against Sol i II did not react to Sol r. No protein IV could be detected in Sol r by molecular weight, charge, or immunologically with either polyclonal mouse antibodies or five monoclonal antibodies. Both venoms were compared with a panel of 60 sera from Sol i-allergic individuals; mean bindings were similar with an $r = 0.94$ for linear regression. RAST inhibition was performed with 17 individual sera representing a variety of Sol i allergen specificities. Four sera were tested from patients resident in the Sol r endemic area and five sera from patients who experienced reactions to S. xyloni stings. All sera reacted comparably to both imported fire ant venoms. The two venoms appear to be allergenically similar, although antigen IV is absent from Sol r and the antigens II have significant sequence variation. Sol i venom appears to be sufficient for diagnostic purposes. (J ALLERGY CLIN IMMUNOL 1990;85:988-96.)

In 1918 the imported fire ant *Sol r* Forel was introduced into Mobile, Ala., from Argentina or Uruguay. It spread northward but is now restricted to an area along the Mississippi-Alabama border from Pontotoc to Meridian. A second South American species, native to Mato Grosso, Brazil, *Sol i* Buren, was first discovered around Mobile in 1939. *Sol i* is very aggressive and has rapidly spread throughout the southeast-

Abbreviations used

HPLC:	High-performance liquid chromatography
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
<i>Sol i</i> :	<i>Solenopsis invicta</i>
<i>Sol r</i> :	<i>Solenopsis richteri</i>
BSA:	Bovine serum albumin

From the Departments of Pathology and *Microbiology and Immunology, East Carolina University School of Medicine, Greenville, N.C.; **Department of Pediatrics, University of Mississippi Medical Center, Jackson, Miss., and ***Vespa Laboratories, Inc., Spring Mills, Pa.

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Reprint requests: Donald R. Hoffman, PhD, Department of Pathology, East Carolina University School of Medicine, Greenville, NC 27858-4354.

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ern United States, displacing native species and *Sol r*. *Sol i* is presently found north to Beaufort County, N.C., and west to central Texas, with isolated colonies reported from Arizona and New Mexico.¹ In 1985 it was found that the two species, *Sol i* and *Sol r*, can hybridize; the hybrid appears to be more cold resistant than either species.² At present the hybrids can only be recognized by analysis of venom alkaloids or cuticular hydrocarbons.³

Imported fire ants have become the leading

cause of allergic-sting reactions in the southeastern United States,⁴ and their stings have caused many verified allergic deaths.⁵ An experimental *Sol i* venom preparation is undergoing clinical trials. This study compares the venom protein allergens from the two most important species of imported fire ant, *Sol i* and *Sol r*.

MATERIAL AND METHODS

Venoms

Venoms from both *Sol i* and *Sol r* were obtained by electrical stimulation with the proprietary commercial process. *Sol i* venom collected in this manner has been demonstrated to be comparable to hand-collected venom by our laboratory.⁶ Venom was also similarly collected from hybrid colonies. The collected venoms were verified as *Sol i*, *Sol r*, or hybrid by gas chromatographic analyses of the venom alkaloids, kindly provided by Dr. Robert K. Vander Meer of the United States Department of Agriculture, Gainesville, Fla.³

Patients and sera

All patients in the *Sol i*-reactive group had experienced systemic reactions from imported fire ant stings within 6 months, and all patients lived in the *Sol i* endemic area. Four sera were obtained from imported fire ant-allergic patients resident in the *Sol r* endemic area in northern Mississippi. These four sera were tested against the panel of purified allergens from both fire ant species, and only one serum demonstrated any *Sol r* specificity. This patient was tenfold more sensitive to *Sol r* by skin testing. All the patients in both groups had typical fire ant stings, including pustule formation at the sting sites within 24 hours. Five sera were obtained from subjects who suffered reactions from fire ant stings in California. In one of these cases, the culprit ants were captured and identified as *S. xyloni*. All five of these patients reported being stung by small reddish ants and did not experience pustule formation. This group is described in detail in a recent publication.⁷ Sera were allowed to clot at room temperature, separated after centrifugation, and stored at -20°C until use. Control sera used in these studies have been described in detail previously.^{6, 8}

SDS-PAGE

SDS-PAGE was performed in 15% gels with 3.3% cross-linking, as was previously described.⁶ Gels were stained with ICN Rapid-Ag silver stain (ICN Radiochemical Division, Irvine, Calif.)

Gel filtration

Venoms were separated on a 1.5 by 1600 cm column of Sephadex G-75 superfine with 0.1 mol/L of ammonium formate buffer, as we have previously described.⁹ The column was calibrated with molecular-weight-marker proteins and was periodically recalibrated with insect venom proteins.

Cation-exchange chromatography

Fractions from gel filtration were further purified by ion exchange on a Pharmacia (Piscataway, N.J.) Mono S high-performance, cation-exchange column. Elution was by a sodium chloride gradient in 0.05 mol/L of sodium acetate, pH 5.2, as previously described.¹⁰

Amino acid sequences

Amino acid sequences were determined by coupling the proteins to Immobilon membrane (Millipore, Bedford, Mass.) and automated Edman degradation in a pulsed liquid microsequencer (model 477A, Applied Biosystems, Foster City, Calif.). Identification was performed by an online phenylthiohydantoin amino acid analyzer, model 120A, Applied Biosystems. When peaks did not match closely, no assignment was made, as indicated by "X" in the sequences. *N*-terminal sequences of *Sol i* proteins were determined with specimens from at least two different isolations. Sequencing was performed by the Bio-Technologies Unit of Louisiana State University in New Orleans.

ELISA

ELISA was performed by binding antigen to Immulon 2 removable wells (Dynatech Laboratories, Chantilly, Va.) in carbonate buffer.¹¹ Mouse antibodies were detected with biotinylated antimouse IgGAM purchased from Sigma Chemical Co. (St. Louis, Mo.) at 1:1000, followed by streptavidin-horse radish peroxidase (Bethesda Research Laboratories, Gaithersburg, Md.) at 1:1000. The substrate solution consisted of 1 mg/ml of 2,2'-azinobis(3-ethylbenzthiazoline sulfonic acid) diammonium salt (ABTS) and 0.03% hydrogen peroxide in 0.1 mol/L of sodium-citrate buffer, pH 4.2. Dilutions of antibodies and streptavidin peroxidase were made in 1 mg/ml of BSA in 0.05 mol/L or Tris HCl, pH 7.4, 0.9% NaCl.

Inhibition assays were performed by biotinylating mouse monoclonal antibodies, purified with immobilized protein G, with *N*-hydroxysuccinimidyl, long chain-biotin purchased from Pierce Chemical Co., Rockford, Ill. The biotinylated antibody was dialyzed, and the optimal titer was determined for a lot of antigen-coated plates. Inhibition testing was performed by adding dilutions of ascites fluids containing monoclonal antibodies to the optimal amount of biotinylated antibody. Detection was by streptavidin peroxidase.

Blots

Dot blots were prepared by spotting antigens onto WCN nitrocellulose membranes (Whatman Ltd., Maidstone, England). After blocking with 5% BSA, membranes were incubated with diluted monoclonal antibodies, washed, incubated with biotinylated antimouse IgGAM, washed, incubated with streptavidin-peroxidase, again washed, and developed.¹²

RAST

RAST was performed as previously described.^{6, 13} Many of the sera and disks used were the same ones that we have

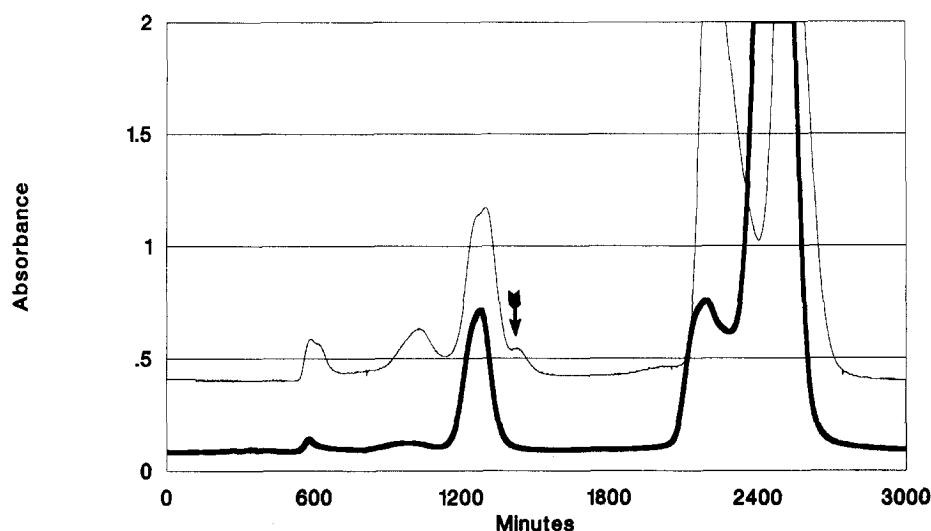


FIG. 1. Gel-filtration profiles of venoms from *Sol r* (heavy line) and *Sol i* (light line) on a 1.5 by 1600 cm column of Sephadex G-75 superfine. The position of *Sol i* IV is indicated by the arrow. The two final peaks and most of the exclusion peak consist of alkaloids. *Sol r* II and *Sol r* III are only partially resolved in the asymmetric peak at 1300 minutes.

used in previous studies⁶⁻⁸ of fire ant allergy. RAST inhibition was performed as we have previously described.^{8, 14} Sera from individual patients and inhibiting antigens were mixed 1 hour before the antigen-coupled disks were added. RAST inhibition was performed with mouse monoclonal antibodies with ascites fluid in place of inhibiting antigen. A pool of 14 sera from *Sol i*-allergic patients was used for the mouse antibody-inhibition experiments.

Separation of the natural fragments of *Sol i* II

Sol i II was reduced with dithiothreitol and alkylated with iodoacetamide.¹⁵ The reduced and alkylated peptides were then separated by HPLC on a Vydac C₁₈ column (The Separations Group, Hesperia, Calif.) with a gradient of 0.1% trifluoroacetic acid to 75% acetonitrile in 0.1% trifluoroacetic acid during 50 minutes.

Mouse monoclonal antibodies

BALB/C mice were immunized with purified *Sol i* allergens with a multiple immunization schedule slightly modified from the method of Cianfriglia et al.¹⁶ The injections on days -15 and -8 were administered in Ribi adjuvant (Ribi Inc., Hamilton Mont.), and each mouse received a total of 100 µg of antigen. Some of the antibodies against *Sol i* III were produced from mice hyperimmunized with Freund's complete adjuvant and boosted with incomplete adjuvant. Spleen cells from mice with positive serum antibody titers were fused with the Sp2/0 or P3-X63-ag8.653 myeloma lines, as described by Lane et al.¹⁷ After growth, culture supernatants were tested by solid-phase ELISA. Positive master wells were cloned by limiting dilution, and those chosen for study were subcloned at least twice. Monoclonal antibodies were prepared either from tissue-culture supernatants or ascites produced after injections of selected

clones into pristane-primed BALB/C mice. Antibodies were typed by ELISA and immunodiffusion (The Binding Site, Inc., San Diego, Calif.). All the antibodies against *Sol i* II and IV were of the IgG1 subclass. Antibodies against *Sol i* III were IgG1, IgG2a, and IgM. All monoclonal antibodies were tested for nonspecific binding against BSA used for blocking and the other fire ant allergens.

The care and use of animals followed "Principles of Laboratory Animal Care" and "Guide for the Care and Use of Laboratory Animals." The protocol was approved by the Animal Care Committee of East Carolina University School of Medicine.

RESULTS

Venoms were collected from colonies of *Sol i*, *Sol r*, and hybrid fire ants. The species identification was verified by gas chromatographic analysis of the venom alkaloids.³ Each preparation was analyzed by gel filtration on a 1600 cm column of Sephadex G-75 superfine. The profiles of *Sol i* and *Sol r* venoms are compared in Fig. 1. The *Sol i* venom pattern was similar to the pattern previously reported,⁶ whereas the *Sol r* venom did not demonstrate a peak corresponding to *Sol i* IV (Fig. 1, arrow). Hybrid venom elicited a pattern similar to the pattern of *Sol i*, but with a small *Sol i* IV peak. The *Sol r* G-75 fractions were pooled as described for *Sol i*⁶ and further characterized and purified by HPLC cation exchange on a Mono S column. The asymmetric peak at about 1300 minutes in the Sephadex G-75 separation was divided at the dip into two fractions, the first containing mainly *Sol r* II and the second *Sol r* III. The results are illustrated in Fig. 2. Proteins *Sol r* I and *Sol r* II

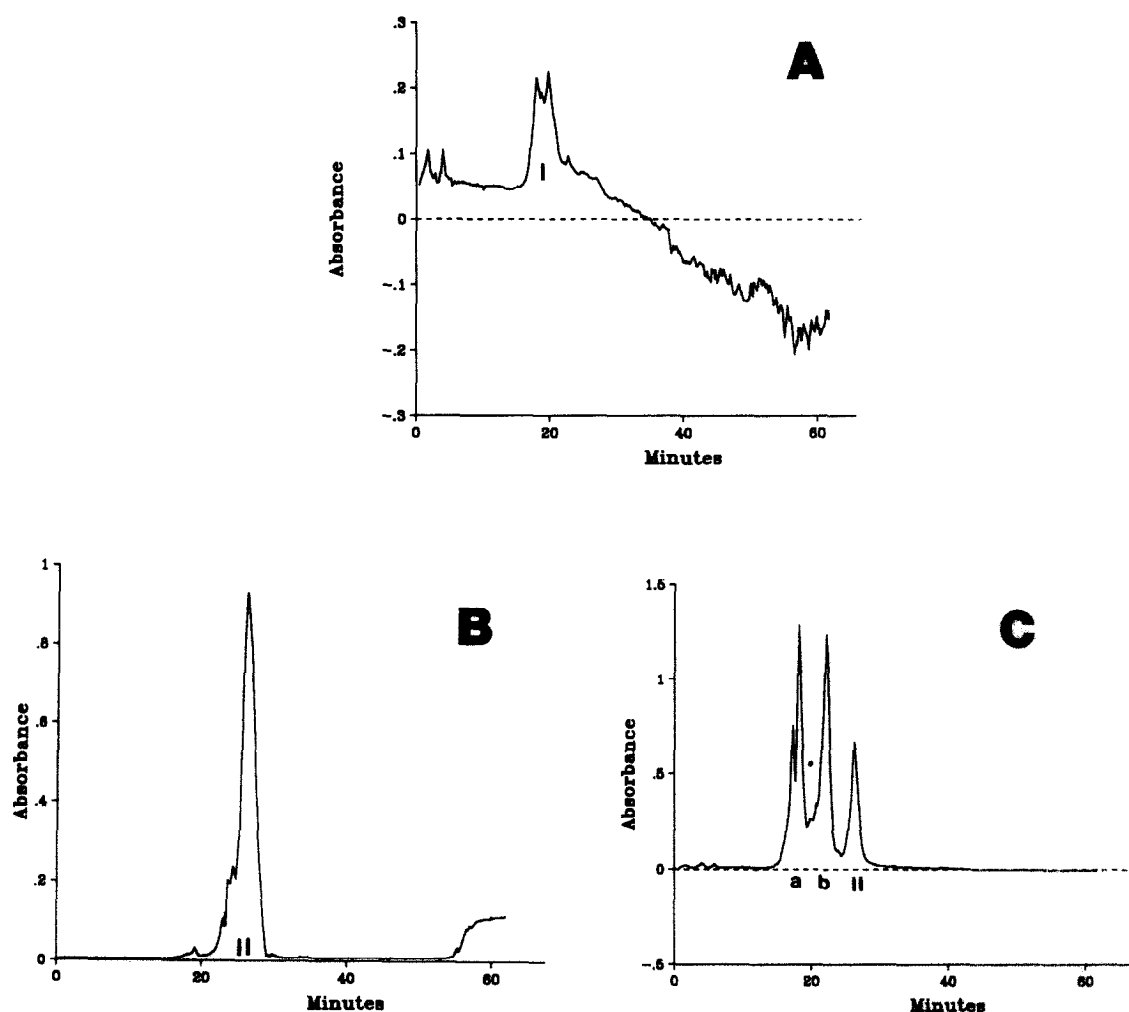


FIG. 2. Cation-exchange chromatography of *Sol r* venom fractions from the gel filtration illustrated in Fig. 1. A, The small amount of *Sol r* I. B, *Sol r* II eluting as a single major peak. C, *Sol r* III eluting as two groups of peaks, the first IIIa and the second IIIb. The G-75 fraction with *Sol r* III also contains some *Sol r* II.

corresponding to *Sol i* I and *Sol i* II were isolated. Two forms of *Sol r* III of differing charge were isolated. No protein was found corresponding to either the size or highly basic charge of *Sol i* IV.

The purified proteins from *Sol r* were further studied by SDS-PAGE under reducing conditions (Fig. 3). Two differences are observed between *Sol r* and *Sol i* venoms. The intact II band is almost absent in *Sol r* venom, and the leading part of the large band at 15,000, corresponding to *Sol i* IV, is absent. This leading fraction is present in hybrid. The purified protein *Sol r* I appears similar to *Sol i* I.⁶ Phospholipase, *Sol r* II, is almost all naturally cleaved, as is true for most *Sol i* II. Both charge forms of *Sol r* III have the same mobility in SDS-PAGE.

N-terminal amino acid sequences were obtained for the purified II and III proteins from both species and

for *Sol i* IV. Only small amounts of the protein I were present; these proteins contain at least two peptide chains. The sequences are compared in Fig. 4. The N-terminal sequences of *Sol i* II and *Sol r* II demonstrated some similarity, but only nine of 20 residues were identical. The N-terminal sequence of the internal natural fragment of *Sol i* II was also determined. The first 20 residues of *Sol i* III and both charge forms of *Sol r* III were identical. The sequence of *Sol i* IV was unlike any of the others.

The proteins from *Sol i* and *Sol r* were compared immunologically with monoclonal antibodies raised against *Sol i* II, *Sol i* III, and *Sol i* IV. Two monoclonal antibodies raised against *Sol i* II, C6 and A4, reacted with *Sol i* venom, but not with *Sol r* venom. The antibodies demonstrated about 10% as much reactivity with venom from hybrid ants as with *Sol i*. Both of

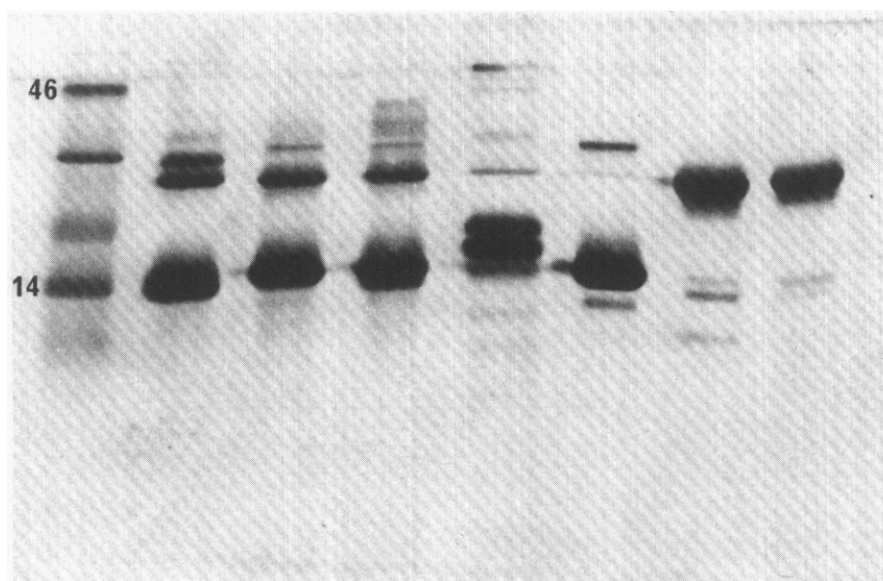


FIG. 3. SDS-PAGE analysis of imported fire ant venoms and purified allergens from *Sol r* venom. The first lane contains molecular-weight markers at 46,000, 30,000, 21,500, 14,300, and 6500 (faint); lane 2, *Sol i* venom; lane 3, *Sol r* venom; lane 4, hybrid fire ant venom. The purified *Sol r* allergens are in lanes 5 to 8, *Sol r* I, *Sol r* II, *Sol r* IIIa, and *Sol r* IIIb, respectively. The gel was run under reducing conditions and stained with silver. Note that only a trace of intact *Sol r* II is present and that there is no protein in *Sol r* venom that corresponds to *Sol i* IV, the unresolved leading part of the 15,000 molecular-weight band. The purified *Sol r* allergens were purposely overloaded in order to assess cross-contamination.

	1	5	10	15	20	25	30
<i>Sol i</i> II	AspAsnLysGluLeuLysIleIle-X-LysAspValAlaGluGlnLeuLeuThrLeuProLys-X-GlyAsnGlnProProAspAspLeu						
<i>Sol r</i> II	AspIleGluAlaGln-X-ValLeu-X-LysAspIleAlaGlu-X-Ala-X-ThrLeuPro						
<i>Sol i</i> II fragment	ProAlaValIleGlnAsnGlnAlaLysValAlaIleThrLysIleIle-X-Lys						
<i>Sol i</i> III	ThrAsnTyr-X-AsnLeuGlnSer-X-Lys-X-AsnAsnAlaIle-X-ThrMet-X-GlnTyrThrSer						
<i>Sol r</i> III a	ThrAsnTyr-X-AsnLeuGlnSer-X-Lys-X-AsnAsnAlaIle-X-ThrMet-X-Gln						
<i>Sol r</i> III b	ThrAsnTyr-X-AsnLeuGlnSer-X-Lys-X-AsnAsnAlaIle-X-ThrMet-X-Gln						
<i>Sol i</i> IV	LeuAspIleLysGluIleSerIleMetAsn-X-IleLeuGluLys-X-Ile-X-ThrValProLys-X-GluAsnAspProIleAsnProLeu						

FIG. 4. N-terminal amino acid sequences of imported fire ant venom allergens determined by automated Edman degradation. X is used to mark all residues that did not correspond to the standard reference positions in reversed-phase HPLC analysis. *Sol i* II fragment is the naturally occurring internal fragment of *Sol i* II.

these antibodies demonstrated a species specificity for *Sol i* II. Neither C6 nor A4 could significantly inhibit IgE binding of a pool of sera from fire ant venom-allergic patients to *Sol i* II.

Five monoclonal antibodies were raised against *Sol i* IV. In dot blots with whole venoms, all five antibodies were reactive with *Sol i*, only weakly reactive with hybrid venom, but not reactive with *Sol r*. The five monoclonal antibodies were pooled and used in

an inhibition ELISA with *Sol i* IV bound to the plastic plates. At 0.5 mg/ml, *Sol i* venom elicited 63% inhibition, hybrid venom elicited 31% inhibition, and *Sol r* venom elicited no inhibition. In a RAST-inhibition system, each of the five monoclonal antibodies could inhibit human IgE binding to *Sol i* IV from 38% to 56%.

The monoclonal antibodies raised against *Sol i* III by immunization in Ribi adjuvant had about 10%

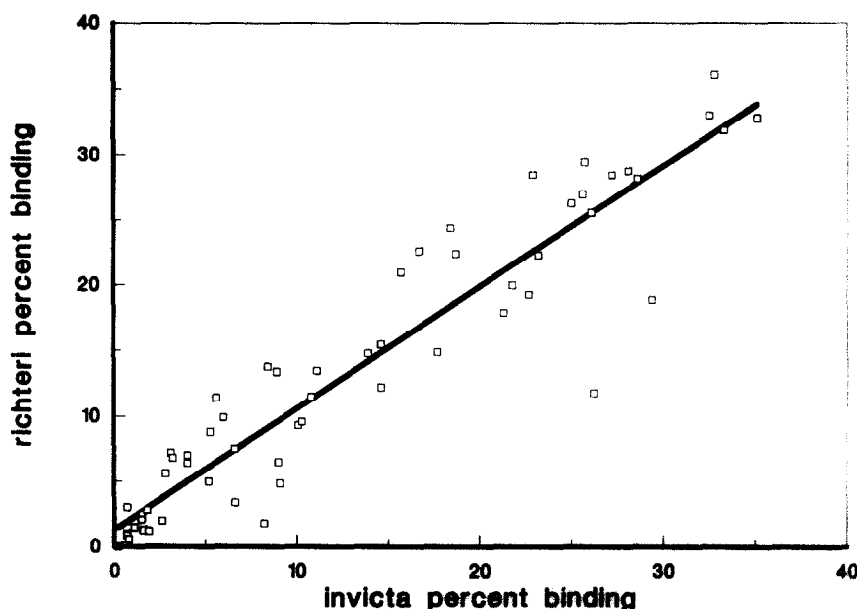


FIG. 5. RAST to *Sol i* and *Sol r* venoms for 60 sera from individuals allergic to *Sol i* venom. The plotted line is the best-fit linear regression with a slope of 0.99 and $r = 0.94$.

cross-reactivity with *Sol r* III and were only weak inhibitors of human IgE binding to *Sol i* III eliciting maximum inhibitions of 11% to 20%. The antibodies raised by immunization with Freund's adjuvant were totally cross-reactive with *Sol r* III. These mouse monoclonal antibodies inhibited human IgE binding to *Sol i* III from 23% to 45%. The initial pooled mouse polyclonal antiserum raised against *Sol i* III elicited 41% inhibition of human IgE binding.

No protein corresponding to *Sol i* IV could be found in *Sol r* venom by size, charge, SDS-PAGE, or immunologic reactivity. Proteins similar to *Sol i* I, *Sol i* II, and *Sol i* III were readily isolated from *Sol r* venom.

The allergenic relationships between *Sol i* and *Sol r* venoms were studied by direct RAST and RAST-inhibition experiments. Direct RAST to both venoms was performed with 60 individual sera from *Sol i* venom-allergic patients. The results are demonstrated in Fig. 5. The mean binding after subtraction of negative control was 12.9% with a 10.7 SD for *Sol i* venom and 13.3 ± 10.5 SD for *Sol r* venom. The coefficient of linear regression was $r = 0.94$ with a slope of 0.99. This finding suggests that the venoms are very closely related. RAST-inhibition experiments were performed with 17 individual sera selected from the 60. The results are summarized in Table I, and the curves for serum 15368 are illustrated in Fig. 6. The sera were chosen to have different specificities for *Sol i* antigens by RAST with purified *Sol i* proteins, although none was *Sol i* IV specific. Four of the sera

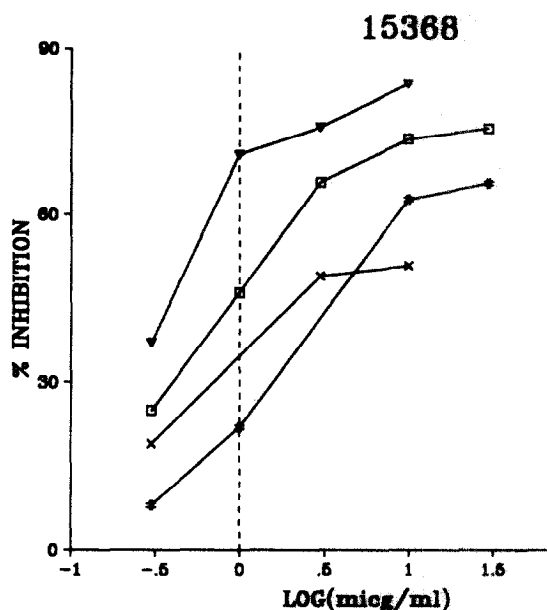


FIG. 6. RAST inhibition of IgE binding of serum 15368 to *Sol i* and *Sol r* venoms. Inhibition of *Sol i* by *Sol i* (■), inhibition of *Sol i* by *Sol r* (×), inhibition of *Sol r* by *Sol i* (▲), and inhibition of *Sol r* by *Sol r* (●). The relative inhibition constant for binding to *Sol i* is 8.3, and for binding to *Sol r*, 3.1. This serum exhibits some *Sol i* venom specificity.

demonstrated evidence of significant *Sol i* specificity, whereas most sera did not distinguish strongly between *Sol i* and *Sol r*. All five of the sera from patients who had experienced reactions from *S. xyloni* stings

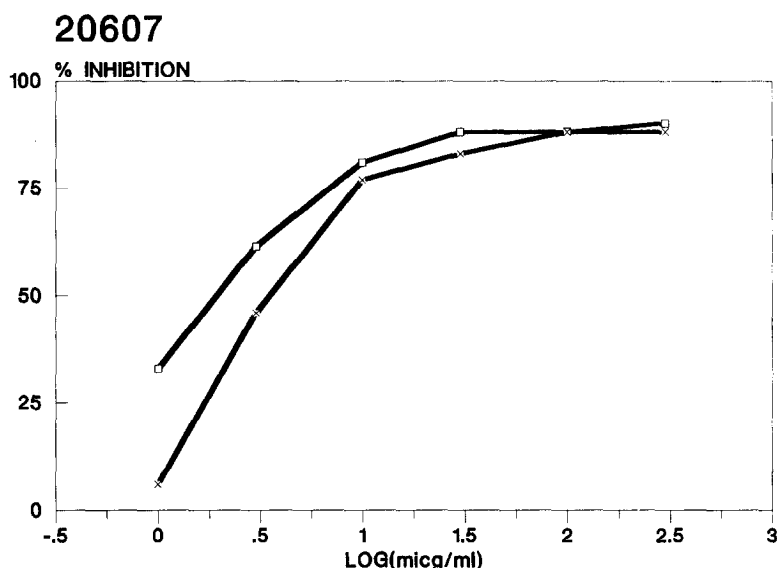


FIG. 7. RAST inhibition of IgE binding of serum 20607 to *Sol r* venom; inhibition by *Sol i* (□) and by *Sol r* (×). This patient was more reactive to *Sol r* by skin test and had IgE antibodies reacting to *Sol r* II, but not to *Sol i* II. *Sol r* elicited more complete inhibition than *Sol i*, adding evidence that this patient was sensitized by *Sol r*.

TABLE I. RAST Inhibition by 100 µg of imported fire ant venoms for *Sol i*-sensitized patients

Inhibition of binding to	<i>Sol i</i>		<i>Sol r</i>	
	By <i>Sol i</i>	By <i>Sol r</i>	By <i>Sol i</i>	By <i>Sol r</i>
Serum				
16427	35	19	49	54
15368	74	49	84	66
16381	84	95	86	74
17096	70	49	76	79
16132*	65	35	45	78
17456*	45	22	63	64
13311*	22	12	72	58
17622	95	95	98	94
17625	88	76	88	86
17670	86	72	79	62
17704	83	17	68	44
18806	93	10	95	90
19898	100	90	83	75
20056	90	98	98	90
20233	78	40	79	81
20424	96	82	91	94
20488	95	18	95	79

Sera 13311, 17704, 18806, and 20488 demonstrate *sol i* specificity by direct RAST and RAST inhibition.

*Inhibition at 30 µg/ml of venom.

in California were reactive with both *Sol i* and *Sol r*. As has been described elsewhere,⁷ one of these patients was probably initially sensitized to *S. xyloni*, whereas the other patients may have been initially sensitized by *Sol i* in the southern United States. The four sera from patients resident in the *Sol r* endemic

area in northern Mississippi bound 7.6%, 16.2%, 22.6%, and 23.6% to *Sol i* venom and 6.5%, 17.4%, 22.5%, and 25.0% to *Sol r* venom, although the first patient was found to be more sensitive to *Sol r* by skin testing, and in RAST with purified allergens, her serum reacted to *Sol r* II but not to *Sol i* II. RAST-

inhibition results for this serum are illustrated in Fig. 7 and provide additional evidence for *Sol r* sensitization. Although there is some species-specific reactivity to *Sol i* venom, it appears that *Sol r* venom is closely related allergenically. *Sol r* venom is deficient in at least one allergenic component corresponding to *Sol i* IV and the phospholipases, *Sol i* and *Sol r* II, appear to have some species-specific sequences and antigenic determinants. There appears to be clinical as well as immunologic cross-reactivity among the *Solenopsis* venoms, as is observed in the four patients probably sensitized by *Sol i* in the South experiencing reactions to *S. xyloni* stings in California. Three of the four sera from patients resident in the *Sol r* endemic area had comparable reactivity to both *Sol i* and *Sol r*. These patients may have been stung by *Sol i* or by the now common hybrid between *Sol i* and *Sol r* found over much of the *Sol r* range.

DISCUSSION

Sol r venom was found to contain three allergens similar to allergens described previously from *S. invicta* venom.⁶ Although *Sol r* III had two different charge forms, both demonstrated the same N-terminal 20 amino acids as *Sol i* III. *Sol r* II, the phospholipase, had nine of the first 20 amino acids identical to *Sol i* II. Mouse monoclonal antibodies against *Sol i* II were species specific but did not inhibit human IgE binding. *Sol r* I appeared to be similar on electrophoresis to *Sol i* I. No protein analogous to *Sol i* IV could be found in *Sol r* venom by gel filtration, ion exchange, or immunologically with a panel of five monoclonal antibodies. Venom from hybrid fire ants, which look like *Sol r*, contain lower amounts of *Sol i* allergens, *Sol i* II and IV. The monoclonal antibodies raised against *Sol i* III demonstrated varying degrees of cross-reactivity with *Sol r* III ranging from 11% to 100%. Some of the highly cross-reactive monoclonals could inhibit human IgE binding.

Sera from patients allergic to *Sol i* venom demonstrated a high degree of cross-reactivity between the venoms by direct RAST, suggesting that they are recognized as similar by most human IgE antibodies. RAST-inhibition studies with individual sera confirmed that the venoms were allergenically closely related with relative inhibition constants ranging from 0.5 to 10. A few of the sera tested demonstrated *Sol i* specificity, but most sera did not distinguish significantly between the species.

The group of patients who suffered systemic reactions after stings by the native fire ant, *S. xyloni*, demonstrated that immunologic cross-reactivity exists beyond the two sister species that are able to produce fertile hybrids. This cross-reactivity is clinically significant, as was demonstrated by four patients who

probably acquired sensitization to *Sol i* reacting to stings of *S. xyloni*. Of 69 patients studied, only one patient was found with evidence of *Sol r* sensitization. Three other patients resident in the *Sol r* endemic area had totally cross-reactive antibodies. Since most of the *Sol r*-allergic population has hybridized with *Sol i*, patients with pure *Sol r* sensitization are probably very rare. It is not possible to study patients allergic to *S. geminata*, the tropical fire ant, since the range of this ant is inside the *Sol i* endemic area, and *Sol i* is a much more aggressive species.

These studies suggest that RAST and probably skin testing with *Sol i* venom is sufficient for diagnosis of imported fire ant venom allergy and also probably for allergy to native fire ants. *Sol r* is deficient in one allergen, but no proteins dissimilar to proteins found in *Sol i* were found in *Sol r* venom. Species-specific determinants were found on *Sol i* venom allergens by mouse monoclonal antibodies, but they do not appear to be important specificities for human IgE antibodies. The availability of *Sol i* venom for immunotherapy should be sufficient for the relatively small number of *Sol r*-allergic patients and patients sensitized by the hybrid ants. It also appears to detect sensitivity to the native fire ant *S. xyloni*.

The production of monoclonal antibodies that recognize species-specific determinants and antigens in fire ant venoms allows the future development of an immunologic method for distinguishing *Sol i* from other ants and especially for distinguishing hybrid ants from *Sol r*.

We thank Dr. Robert K. Vander Meer for performing gas chromatography on the venom alkaloids to verify the identity of the fire ant species, and Marvin B. Alligood, Jr., for providing technical assistance in some of the assays. The monoclonal antibodies were produced with the technical assistance of Ulla Godwin, Carolyn Jones, and Inga Oleksy. We especially thank Drs. Ellis Moffitt, Robert Holzhauer, Sam Jay Weiss, and Mark Ellis for providing sera and historical information from their patients.

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A long-term follow-up study of hyposensitization with immunoblotting

Erika Jarolim, MD, Lars K. Poulsen, PhD, Beda M. Stadler, PhD,*
Holger Mosbech, MD, Ole Oesterballe, MD,** Dietrich Kraft, MD,*** and
Bent Weeke, MD Copenhagen and Viborg, Denmark, Bern, Switzerland, and
Vienna, Austria

The formation of specific IgE, IgG1, and IgG4 antibodies was investigated by immunoblotting during hyposensitization with timothy grass-pollen extract and 6 years later. Until the end of immunotherapy, specific IgG antibody levels increased. Also simultaneously, the number of allergenic components detected by IgG increased. However, this IgG response was similar in responding and nonresponding patients; thus, it did not correlate with the clinical outcome of the therapy. More allergenic compounds were also detected by IgE on immunoblots, but again without correlation to success of therapy. Six years after immunotherapy, the therapeutic effect was still present, although by now the observed immunoglobulin-binding patterns were similar to patterns observed in the same patients' sera collected before the initiation of hyposensitization. Thus, changes of antibody-binding patterns in immunoblot do not relate to the success or failure of immunotherapy. (J ALLERGY CLIN IMMUNOL 1990;85:996-1004.)

From the Laboratory of Clinical Allergology, Medical Department TTA, State University Hospital, Copenhagen, Denmark; *Institute of Clinical Immunology, Inselspital, Bern, Switzerland; **Pediatric Department, Viborg Sygehus, Viborg, Denmark; and ***Institute of General and Experimental Pathology, University of Vienna, Vienna, Austria.

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Reprint requests: Erika Jarolim, MD, Institute of Clinical Immunology, Inselspital, CH-3010 Bern, Switzerland.

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Hyposensitization has been widely used since it was first recognized in 1911.¹ It has been suggested that "blocking factors" are induced by this kind of immunotherapy that inhibit allergic responses.² These blocking factors have been identified as IgG antibodies.³ Devey et al.⁴ and Van der Giessen et al.⁵ further characterized these antibodies to be mainly of the IgG4 subclass but also questioned that their biologic function was predominantly the trapping of the allergen. Furthermore, they suggested that specific IgG4 inter-