

Crossed Immuno-electrophoresis and Crossed Radioimmuno-electrophoresis Analysis of "Yellow Jacket - Common Wasp" (*Vespula* spp.)

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Crossed immuno-electrophoresis (CIE) pattern of Yellow jacket vespid venom showing 25 immunoprecipitates was used in crossed radioimmuno-electrophoresis (CRIE) analysis with sera from 35 subjects allergic to vespid venom. Phospholipase A and B, hyaluronidase, acid and alkaline phosphatases and a lytic active component were demonstrated in immunoprecipitates using zymography and direct haemolysis of sheep erythrocytes. CRIE analysis proved the allergenicity of these proteins. In addition, another antigen (Ag 17) with no detectable enzymatic or lytic activity was identified as an important allergen. This allergen is most likely identical to the previously described Antigen 5 in vespid venoms. By using CIE/CRIE the presence and complexity of multiple forms of the allergenic protein components were well demonstrated.

Key words: CIE/CRIE; vespid venom.

Accepted for publication 20 October 1984

Hymenoptera venoms are complex mixtures of pharmacologically and biochemically active substances including proteins (enzymes), peptides and biogenic amines. The antigenic/allergenic composition of honey bee venom has been extensively studied and many of the individual bee venom components have been characterized biochemically (5, 10). Furthermore, the allergenic composition with respect to major, intermediate and minor allergens has recently been described using a combination of crossed immuno-electrophoresis (CIE)/crossed radioimmuno-electrophoresis (CRIE) (1).

The antigenic/allergenic composition of vespid venoms has been subjected to relatively little study, mainly because of limited availability of vespid venom raw materials. The composition of the various vespids' venoms is rather similar (7).

Vespid venom has been shown to contain many different components such as phospholipase A, phospholipase B, hyaluronidase, acid

phosphatase, proteases, kinin, histamine, serotonin, etc. (7, 12). The allergenic fraction has been found to consist of acid phosphatase, hyaluronidase and phospholipase (9). King et al. (11) have also postulated that a protein, Antigen 5, is a major allergen in vespid venom.

In this paper we will report on CIE/CRIE analysis of Yellow jacket vespid venom raw material (6). The individual antigens in the CIE pattern of unfractionated Yellow jacket venom were identified by examination of enzyme activity and haemolytic activity. The role of the antigens acting as allergens in a selected group of patients sensitive to vespid venoms was determined by CRIE.

MATERIAL AND METHODS

Yellow jacket venom

Vespid venoms were obtained from Vespa Laboratories, Spring Mills, PA, as a mixture for

commercial use in diagnosis and therapy. The studied vespid venom mixture (Lot No. 11048035) was prepared from *Vespula squamosa*, *V. vulgaris* and *V. rufa* groups by extraction of venom sacs with beta-alanine-acetic acid buffer. The protein content in the lyophilised venom material was determined by Lowry protein assay.

Antisera

Antisera to Yellow jacket vespid venom proteins were raised in rabbits (New Zealand White) by multiple bi-weekly intracutaneous immunizations with 300 µg vespid venom in Freund's complete adjuvant essentially as described by Harboe & Ingild (8).

Yellow jacket venom components

Isolated vespid venom components were prepared by combination of various biochemical techniques. Gelfiltration on Sephadex G-75, high speed aqueous gel filtration on Waters I-250 protein column, chromatofocusing and fast protein liquid chromatography (FPLC) on ion exchanger (Mono-S) (4). The isolated vespid venom components were identified by assaying the enzyme activity and direct haemolytic activity. The homogeneity of the isolated fractions was checked by rocket immunoelectrophoresis, isoelectric focusing (Pharmalyte®), SDS-polyacrylamide gradient gel electrophoresis and isoelectrophoresis.

Crossed immunoelectrophoresis (CIE)

CIE was carried out on 8 × 8 cm glass plates, essentially in accordance with the previously described procedure (5), using 0.023 M Tris/Tricine buffer (Bio-Rad), pH 8.6, in 1% agarose (Agarose A®, Pharmacia Fine Chemicals, Uppsala, Sweden). Samples of 5 µl Yellow jacket venom (15 g/l) were run in the first dimension electrophoresis at 10 V/cm for 40 min. The second dimension electrophoresis was performed for 18 h with 2 V/cm at 15°C, using 25 µl antiserum/cm² gel.

Identification of individual components in the

CIE-pattern was performed utilizing tandem CIE and crossed line immunoelectrophoresis (CLIE) in accordance with procedures described by Axelsen et al. (2).

Crossed radioimmuno-electrophoresis

The washed CIE-plates were incubated with 0.74 ml of the patient's serum diluted to 13 ml with the CRIE incubation buffer (0.05 mol/l phosphate buffer, pH 7.4, containing 0.9% sodium chloride, 3.2% tween and 0.3% dextran 10) for 18 h at 22°C in suitable plastic boxes. The plates were washed as previously described (13), and incubated with approximately 500,000 cpm ¹²⁵I-anti-IgE (Phadebas RAST® Isotope Reagent) in 13 ml total volume for another 18 h. The plates were washed, dried and placed on X-ray photographic film for autoradiographic exposure at -70°C in cassette with intensifying screen. All plates were exposed several times, using time periods varying from 1 h to 27 h (13), so that each exposure corresponded to one particular CRIE class, using a CRIE reference system.

IgE

Specific and total IgE in the patient sera were determined using Phadebas RAST and Phadebas IgE PRIST® kits (Pharmacia Diagnostics AB), respectively, according to the manufacturer's instructions.

Patient sera

Sera were obtained from 35 individuals with clinically documented systemic reactions to Yellow jacket stings, a positive skin prick test and a positive RAST with Yellow jacket vespid venom (RAST class 2-4) and a total IgE in the range 10-2000 U/ml. The patients had not been subjected to immunotherapy.

Enzyme assays

Acid phosphatase, phospholipase A and hyaluronidase enzyme activities were assayed as previously described (5). Phospholipase B en-

zyme activity was assayed using agarose with beta-lyso phosphatidylcholine as substrate. Proteolytic activity was detected by measuring

the hydrolysis of bovine serum albumin adsorbed to a polyester and subsequently incorporated in agarose (13).

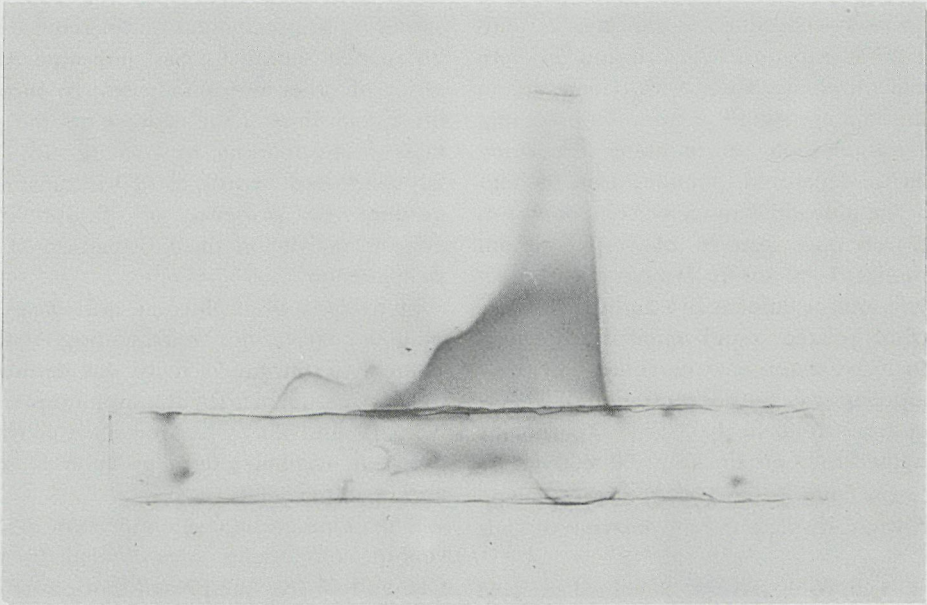


Fig. 1a. Crossed immunoelectrophoresis of Yellow jacket vespid venom against hyperimmune rabbit antisera. 5 μ l of Yellow jacket venom source material (15 g/l) analysed in agarose electrophoresis (pH 8.6) with 10 V/cm, 15 °C for 60 min followed by electrophoresis perpendicular to the first dimension with 2 V/cm, 15 °C for 18 h in agarose containing rabbit antiserum (25 μ l/cm²). Anode to the right in the first dimension and at top in the second dimension.

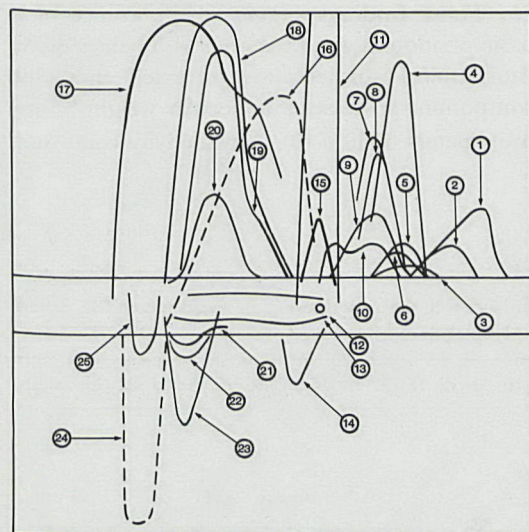


Fig. 1b. Schematic drawing of the immunoprecipitates (Fig. 1a) arbitrarily numbered 1–25. —: protein stained precipitates; ----: visible after radiostaining.

Lytic activity

The presence of lytic components in the CIE-pattern of Yellow jacket was measured by direct haemolytic activity, using washed sheep erythrocytes in phosphate-buffered saline, pH 7.4 (5).

RESULTS

The results of CIE analysis of vespid venom raw material are demonstrated in Fig. 1a (Coomassie Brilliant Blue stained immunoprecipitates), and Fig. 1b depicts a schematic drawing of the CIE reference pattern with arbitrarily numbered immunoprecipitates (1–25). The precipitates are numbered from anode to cathode. As can be seen in Fig. 1 many antigens migrated towards the anode or the cathode, and some antigens migrated towards both. This demonstrates that

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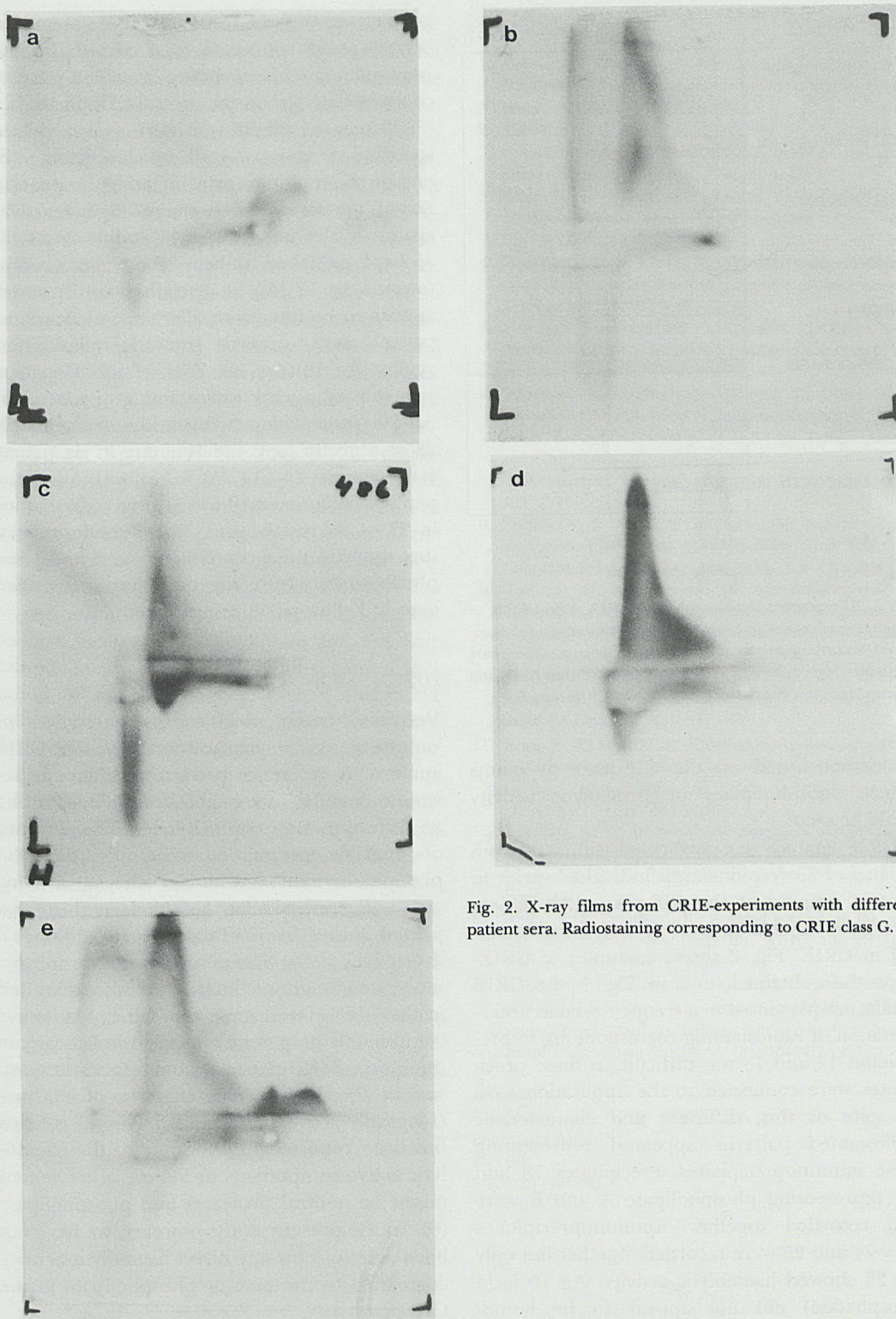


Fig. 2. X-ray films from CRIE-experiments with different patient sera. Radiostaining corresponding to CRIE class G.

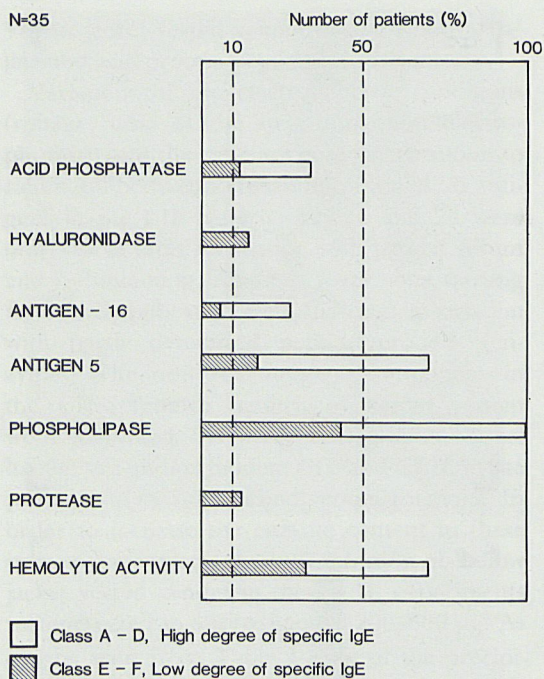


Fig. 3. Allergogram showing average CRIE radiostaining of important allergens in vespid venom. Precipitates are numbered according to the CIE reference pattern. Open bars symbolize high degree of radiostaining (CRIE class A-D) and hatched bars low degree of radiostaining (CRIE class E-F).

be demonstrated on the CIE-plate at points where phospholipases or proteolytic activity could be seen.

CRIE analysis was performed with sera from a panel of 35 vespid allergic individuals prior to protein staining of the CIE-plates. All patient sera gave variable degrees of visible radiostaining in CRIE. Fig. 2 shows examples of CRIE-films thus obtained, and in Fig. 3 the CRIE results are presented in a frequency table. Interpretation of radiostaining corresponding to precipitates 12 and 13 was difficult, as these precipitates were connected to the application well. In spite of this, different and characteristic radiostained patterns appeared representing these immunoprecipitates. Precipitates 18 and 19, representing phospholipase A and B were also recorded together. Immunoprecipitates Nos. 24 and 25 were recorded together but only Ag 25 showed haemolytic activity. Ag 10 (acid phosphatase) did not appear to be homo-

geneous, as in the CIE-pattern there seemed to be two peaks connected by a curved line. No radiostaining corresponding to CRIE class G could be detected on the control CIE-plates.

Thirteen of the 25 sera were shown to have specific IgE against Ag 10, which appears to be one of the multiple forms of acid phosphatase. Six of the sera studied showed IgE reactivity against hyaluronidase (Ag 11), while 28 of the 35 sera exhibited rather strong radiostaining towards Ag 17 (Ag 5). Phospholipase A and B appear to be important allergens, as nearly all patient sera studied showed radiostaining against Ag 18 (Ag 19). Four of the 35 patient sera showed a weak radiostaining to precipitate Ag 20 (proteolytic activity) whilst antigens 21-23 as a group gave radiostaining in six patient sera. Antigen 24 (Ag 25), a haemolytic protein/peptide component, shows strong radiostaining in 27 of 35 patient sera. These results suggest that the lytic active component, Ag 5 and phospholipase constitute the most important allergens in Yellow jacket vespid venom.

DISCUSSION

Vespa venom obtained by extraction of venom sacs was characterized by CIE/CRIE analysis. A reference pattern of Yellow jacket venom in CIE was established demonstrating 25 different immunoprecipitates. The presence of multiple enzymatic activity, i.e. different precipitates exhibited similar enzyme activity, was common. Similar results have been reported earlier by Hoffman (9). In addition, a direct lytic active component, with an approx. molecular weight of 6000 d, was also identified in the CIE reference pattern. Direct lytic active components have been identified in bee venom (melittin, MCD-peptide) but not in Wasp venom (7). However, the existence of pharmacologically active compounds in vespid venoms has been reported (3, 9). Some of the haemolytic active components in Yellow jacket venom might be neutral proteases and phospholipase (6). In the present study, however, we have not been able to show any direct haemolytic activity correlated to protease or phospholipase in the CIE pattern.

King et al. (11) identified three proteins in vespid venom (phospholipase, hyaluronidase and Ag 5) as active allergens in vespid-sensitive individuals. Of these, Ag 5 was classified as a major allergen but individual patients varied in their response. In another study by Hoffman (9) five different allergens were identified of which acid phosphatase, hyaluronidase and Ag 5 were found to be important allergens. In our investigation we were able to identify at least seven different allergens which gave rise to radiostaining in CRIE; acid phosphatase, hyaluronidase, Ag 16, Ag 5, phospholipase A and B, a protease and a haemolytic component. More than 50% of the 35 patients studied showed distinct radiostaining in CRIE for phospholipase, Ag 5 and the haemolytic component, showing their role as important allergens. A complicating factor in the interpretation was the presence of multiple forms of several of the allergens (isoenzymes). This effect has previously been demonstrated in the CRIE studies of bee venom (1). For that reason acid phosphatase, protease, phospholipase and the lytic component should preferably be regarded as a group of similar allergens, even if the rabbit IgG-system sometimes shows better discrimination between molecular variants compared with the human IgE-system.

CIE/CRIE has been used to study the antigenic/allergenic composition of vespid venom without purification of the individual components. It must be stressed that the combination of immunological methods, zymographic methods and haemolytic studies gives more detailed and accurate information regarding the allergens in Yellow jacket venom. The purpose of analysing the components in vespid venoms is to get a better understanding of the venom composition and, consequently, a more rational basis for treatment of sting reactions with a well-characterized preparation.

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