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## HYMENOPTERA ALLERGY: PRECIPITIN AND PASSIVE CUTANEOUS ANAPHYLAXIS TESTS IN HUMAN AND RABBIT SERA

By

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Compared to the frequency of asthma, and/or allergic rhinitis, of about 25 per cent (5), Hymenoptera allergy represents a small but complex group of patients whose prevalence may be as low as 0.4 per cent (12). These latter patients make up for their lack in numbers by frequently presenting themselves in life threatening situations. Atypical reactions to "bee sting" have been reported both in the treated and untreated Hymenoptera allergy cases. Although most bee sting allergy patients are successfully hyposensitized, a few individuals who have undergone a full hyposensitization program with Hymenoptera extract were unimproved and again have experienced anaphylaxis with a subsequent sting (6). Other individuals have experienced peculiar reactions, some with an onset of anaphylaxis occurring an hour or more after the sting. Barnard (2) has reported fatal bee sting reactions occurring as long as several days later.

In this study, immunological procedures were undertaken as a possible aid in understanding some of these unusual reactions to Hymenoptera allergy.

## MATERIAL AND METHODS

A total of 24 sera were obtained from four groups of human subjects. The first group was called "non-allergic recent,, (NR) and included six individuals who were stung by a bee within two years of this study, resulting only in a local reaction. The second group was called "non-allergic distant,, (ND) and included six individuals who were last stung more than ten years prior to this study, again resulting in only a local reaction. The third group included six patients who were stung within two years of this study, resulting in generalized urticaria, wheezing, and/or shock. In addition these individuals had not undergone any hyposensitization treatment and were called allergic untreated (AU). The fourth category was called allergic treated (AT) and included six individuals who developed generalized urticaria, wheezing, and/or shock with their last bee sting which occurred within six years prior to this study. In addition, all the patients in this last category (AT) were undergoing hyposensitization treatment at least at the level of 0.1 ml. of 1/100 (wt./vol.) mixed whole body Hymenoptera extract (wasp, yellow jacket, hornet, honey bee) at that time. The average age of the NR, ND, AU, and AT groups were 21, 20, 16, and 47 years of age respectively. All subjects were males except for one female in the AU group and three females in the AT group.

Ten male, young adult, albino rabbits were immunized with a 1/1 mixture of 1/100 (wt./vol.) whole body Hymenoptera extract and Freund's complete adjuvant.\*\*\*) Five separate aqueous commercial whole body extracts\*) of each of the following were used: wasp, yellow jacket, hornet, honey bee, and mixed. The mixed extract is designated commercially as an equipart mixture of the other four. The ten rabbits were divided into five groups of two rabbits each. Each pair received one of the five antigen-adjuvant emulsion: one of the rabbits in each group was immunized by footpad injections in the forepaws, the other was immunized by subcutaneous injections in the dorsal skin.

A baseline bleeding was performed at week 0 before immunization and trial bleedings were done at week 8 and week 9. The blood samples, 7-10 ml., were taken from a peripheral ear vein. The samples were allowed to stand for 48 hours at 5°C, centrifuged at 2,000 rpm for 30 minutes, and the serum was collected and stored at 5°C.

The injection schedule for the immunization of the rabbits is similar to one used by *Langlois, Shulman & Arbesman* (7). One ml. mixture of equal parts of one of the whole body Hymenoptera extract (1/100 wt./vol.) and Freund's complete adjuvant was given on the 1st, 2nd, 4th, and 6th week to each rabbit.

Precipitin-in-gel test were performed by the Ouchterlony technique and plates were frequently observed for a period of 10 days. Undiluted human and rabbit sera were tested for precipitin, to each of the Hymenoptera extracts, which were at various dilutions (1/10, 1/100, 1/1000 wt./vol.).

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\*) Center Laboratory, Port Washington, New York.

\*\*) Hyland Laboratories, Los Angeles, California.

For the passive cutaneous anaphylaxis (PCA) tests, the technique of Ovary (11) was followed using male, albino guinea pigs weighing between 250 to 320 grams. Intracutaneous injections of 0.1 ml. of the undiluted serum of the human subjects and of the immunized rabbit were made on the shaved abdomens: Fifteen guinea pigs were used on each of two trial runs. Each animal had a positive control site of 0.275 mg. histamine diphosphate and a negative control site of normal saline. The sera test sites were administered five hours before challenge while the positive and negative controls were administered just prior to the challenge. The intravenous challenge dose consisted of 1.5 ml. mixture of 1.0 ml. of the insect whole body extract (1/10) and 0.5 ml. of 0.5 per cent Evans blue dye.

A test site was considered positive only if the resulting blue stain was significantly larger than that produced by the normal saline control. No site was considered positive if the histamine control site was negative or if the saline control site was positive. The results were read 45 minutes after the challenge. Each human serum was run twice and tested with each of the five insect extracts. Sera from the rabbits immunized with the mixed whole body extract also were tested with each of the five extracts, using the PCA procedure.

## RESULTS

Rabbit anti-wasp, anti-yellow jacket, anti-honey bee, and anti-hornet and anti-mixed Hymenoptera sera when tested with various Hymenoptera whole body extract by the Ouchterlony plate technique demonstrated varied amounts of precipitin bands, the results of which are summarized in Table 1. Each rabbit anti-sera generally contains the maximum number of precipitin bands when tested with mixed Hymenoptera extract as well as with its specific corresponding extract. In decreasing order, the best antibody response, resulting in the greatest number of precipitin bands, was produced by mixed Hymenoptera extract followed by wasp, hornet, yellow jacket, and honey bee extract. Honey bee extract produced a relatively poor antibody response. Dilution of the rabbit anti-sera or the Hymenoptera extract concentration greatly diminished the number of precipitin bands. Fused precipitin lines of identity were noted in the varied patterns of Ouchterlony plates. The overall results of these plates showed that wasp, yellow jacket, and hornet extracts contained at least two common antigens as noted by the fusion of precipitin lines. Honey bee extract, however, did

not produce any fusion of precipitin lines with other Hymenoptera extracts. However, rabbit anti-honey bee serum did produce precipitins with each of the other Hymenoptera extracts (Table 1).

TABLE 1  
*Maximum number of Precipitin Bands using Various Rabbit Antisera.*

| Insect<br>Extract (1/10)<br>(wt./vol.) | Anti-Mixed<br>Insect Serum | Anti-Wasp<br>Serum | Anti-Yellow<br>Jacket<br>Serum | Anti-<br>Hornet<br>Serum | Anti-<br>Honey Bee<br>Serum |
|----------------------------------------|----------------------------|--------------------|--------------------------------|--------------------------|-----------------------------|
| Mixed Insects                          | 5                          | 5                  | 3                              | 4                        | 2                           |
| Wasp                                   | 5                          | 4                  | 2                              | 3                        | 1                           |
| Yellow Jacket                          | 3                          | 3                  | 2                              | 3                        | 1                           |
| Hornet                                 | 3                          | 2                  | 3                              | 3                        | 2                           |
| Honey Bee                              | 1                          | 0                  | 1                              | 2                        | 1                           |

As a rule the footpad immunization site resulted in a higher production of antibodies than a subcutaneous immunization site. Generally, there was no increase in antibody titer from the eighth to ninth week of the immunization period.

The guinea pig passive cutaneous anaphylaxis test was performed using rabbit anti-mixed Hymenoptera sera in the intracutaneous sites. Positive results were obtained on repeated occasions on intravenous challenge using the following extracts: mixed Hymenoptera, yellow jacket, wasp, and hornet. Negative results were obtained on repeated trials when honey bee extract was used as the intravenous antigen.

Human sera of both recently and distantly stung non-allergic subjects and the sera of both treated and untreated allergic subjects were examined for the presence of Hymenoptera antibody using the Ouchterlony and passive cutaneous anaphylaxis technique. Separate and mixed Hymenoptera extracts were used as antigens. The only subject eliciting a positive Ouchterlony test was a non-allergic but recently stung individual whose sera revealed precipitin bands to wasp, yellow jacket, and hornet extracts.

The only human subject manifesting a positive PCA test

was an allergic but treated individual. The positive PCA was to both yellow jacket and wasp extracts. The subject positive on the Ouchterlony test was negative on the PCA test.

#### DISCUSSION

Each Hymenoptera insect contains species specific antigens as well as antigens shared in common with other Hymenoptera species. Arbesman's group (1) demonstrated by chromatography, and gel-diffusion 12 to 13 antigens for wasps, 9 for bees, and 9-10 for yellow jacket. In addition they reported that wasp and yellow jacket have 2 antigens in common; while honey bee shares one antigen in common with yellow jacket and wasp. Those antigens shared in common were found in the whole-body of the insects and not in the venom sacs which, they stated, contains only species specific antigens.

*Foubert & Stier* (4) working on whole-body extracts of wasp, hornet, yellow jacket, and bumble bee with the agar double diffusion technique found that each species contained 4 to 6 antigenic proteins, two of which were common antigens for each species.

*Loveless & Fackler* (9) using intracutaneous and conjunctival tests as well as a serum passive transfer inhibition test reported that "yellow jacket, hornet, wasp, honey bee, and bumble bee possess a common allergenic specificity while each also contains in its venom a component which is peculiar to it.,, *Loveless* (8) later reported that immunization with yellow jacket will protect sensitive individuals from subsequent stings from yellow jackets and wasp. The relationship between venom as found in a venom sac and venom that is obtained by stinging was explained by *Shulman et al.* (13) who stated that the former contains one prominent species specific component while the latter contains, in addition, trace amounts of body antigens.

Our study was in agreement with these past investigations in that at least 2 common antigens were found in whole-body

extracts of wasp, yellow jacket, and hornet while honey bee shared only one common antigen with wasp, and yellow jacket, but 2 common antigens with hornet. The presence of species specific antigens as well as antigens common to different species may explain some of the atypical clinical findings where one "bee sting,, produces an anaphylactic reaction while a second "bee sting,, some time later may not produce anaphylactic reaction in the same, untreated, individual. For example, an individual allergic to a species specific antigen from a yellow jacket may not manifest anaphylactic symptoms when stung by a hornet, since the latter does not contain this particular antigen. Conversely, an individual allergic to an antigen common to several species may manifest anaphylactic symptoms if stung by either a yellow jacket, hornet, wasp, or honey bee.

Mixed Hymenoptera extract when tested with anti-wasp serum and anti-hornet serum produced a greater number of precipitin bands than did wasp and hornet extract when these were tested with their anti-serum counterparts. One explanation for this phenomenon is that mixed Hymenoptera extract may contain significant amounts of a particular antigen held in common by two or more species. This antigen may not be quantitatively large enough to produce a precipitin band when one single, species extract is used. Similarly, the presence of a small amount of one antigen may also explain why honey bee extract had only one precipitin band with anti-honey bee serum while hornet had two precipitin bands. Honey bee extract may have had trace amounts of this second antigen which was enough for stimulating the rabbit into producing an antibody against, it but not quantitatively enough to produce a precipitin band by Ouchterlony plate analysis. Hornet, by contrast, might have had a larger amount of this second, held-in-common, antigen which was quantitatively enough to produce a second precipitin band. Of the individual species extracts, wasp contained the strongest antigenic stimulant for rabbits with 4 precipitin bands, while honey bee was significantly weaker antigenically with one precipitin band. If a significant

beneficial mechanism mechanism of "hyposensitization,, in Hymenoptera allergy is the production of non-reaginic antibody and if the immunological system of humans is similar to rabbits, then the honey bee allergy may be the most difficult to treat since this extract may result in relatively poor antibody production. Hyposensitization failures in honey bee allergy may be due to this phenomenon.

Another possible clinical association of this experiment is to confirm the rationale of using mixed Hymenoptera extracts in hyposensitization. A patient allergic to a species specific antigen either in the whole-body or in the venom, and may not be allergic to an antigen that is shared in common between the species. Therefore, a faulty hyposensitization with the wrong species may not be protective against another species of Hymenoptera.

*McCormick* (10) reported the presence of precipitin antibodies as determined by gel diffusion technique to whole-body wasp extract and to a lesser degree to honey bee whole-body extract in the serum of a patient who died from 2 wasp stings. In our study, the one individual who had serum precipitin antibodies to wasp, yellow jacket, and hornet extract was a non-allergic healthy individual who was stung a few months prior to this experiment. This particular individual was stung again a few years after this experiment, reportedly, by a yellow jacket, resulting in only a small local reaction, which subsided one hour after the sting.

The prevalence of human serum precipitin antibodies to Hymenoptera extract is not firmly established as yet. Only one out of 24 human sera that we examined had precipitin antibodies while *Terr & McLean* (14) found no precipitin antibodies on examining the sera of 26 untreated bee sting allergy patients and 16 control subjects. Some of their cases, two of which were beekeepers, had a past history of numerous stings. However, in 1964, *Hala Brown* (3) reported that 17 untreated Hymenoptera allergy patients had serum precipitins to Hymenoptera extract, and that in most cases these precipitins were no longer detectable by gel-diffusion techniques after

a series of hyposensitization injections. These data has not been adequately confirmed by other investigators.

*Terr & McLean* also reported that 13 sera out of 26, untreated bee sting allergy patients had a positive PCA test in guinea pigs when honey bee antigen was used intravenously and 3 out of the 26 were also positive when yellow jacket antigen was used intravenously. No PCA reactions were elicited with wasp or hornet antigens. Our results differ from these authors in that only one out of 12 allergic patients had a positive PCA. This patient had undergone hyposensitization treatment and the positive PCA reaction occurred twice, once on using yellow jacket extract and the other on using wasp extract.

Ecologically, honey bee differs significantly from hornet, yellow jacket, and wasp by the following: it is not a predacious insect but a strict nectar feeder, and it has a barbed stinger which prevents removal of the stinger from the victim's skin, causing self evisceration and death after one sting. This difference perhaps extends to the immunologic level where honey bee extract was found to be a relatively poor antibody stimulator, and appears to have less antigens in common with other extracts in the Hymenoptera group.

#### SUMMARY

A total of 24 human sera from 12 subjects with no Hymenoptera allergy and 12 patients with definite Hymenoptera allergy were studied for precipitin antibodies and passive cutaneous anaphylaxis (PCA) reactions in guinea pigs. The non Hymenoptera allergy group was further subdivided into 6 recently stung subjects and 6 distantly stung subjects. The Hymenoptera allergy group was subdivided into 6 untreated and 6 hyposensitized patients. Only one serum demonstrated a positive precipitin test and that belonged to a non allergic but recently stung individual. One serum demonstrated a positive guinea pig passive cutaneous anaphylaxis (PCA) test, and

this belonged to a Hymenoptera allergy patient who was fully hyposensitized.

Immunized rabbit serum revealed that at least 2 common antigens are present in whole body extracts of wasp, yellow jackets, and hornet, when tested by Ouchterlony technique. Honey bee was found to have only one common antigen with wasp and yellow jacket but two common antigens with hornet. In rabbits, antibody response to honey bee extract appears relatively weaker than to other Hymenoptera extracts. Honey bee extract was the only Hymenoptera extract that failed to demonstrate a positive PCA test when rabbit anti-mixed Hymenoptera sera was used in the intracutaneous sites. Immunologically, honey bee appears to be significantly different from other Hymenoptera insects.

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