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The Effects of *Apis Mellifica* on Type I Hypersensitivity in Mice

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ABSTRACT

The philosophy of homeopathic medications, like *Apis mellifica,* is that by introducing infinitesimal doses of a possible allergen, the symptoms of stress and allergic reactions can be reduced. The purpose of this study is to determine if *Apis mellifica* affects the allergic reaction by reducing the amount of immunoglobulin type E (IgE) antibodies in the blood. IgE is the antibody that produces a type I hypersensitivity, the body's immediate reaction to allergens. A treatment and control group of 6 mice each were used in the study. Gel electrophoresis was performed to separate proteins in the blood samples taken from the mice at four different times throughout the course of the study. Immunoprobing and visualization techniques identified possible broken-down IgE peptides but no difference in the levels of IgE was found between the treatment and control groups. These smaller molecules were visualized most heavily in the third blood sample following the final oral treatment of *Apis mellifica* and placebo. Immunoreactive peptides indicate that IgE was present in the blood. Earlier blood sampling may have yielded intact IgE antibodies that could be separated, visualized, and quantitatively compared between the treatment and control groups.

Keywords: Immunoglobulin type E (IgE) antibody, type I hypersensitivity, allergen, homeopathic medicine, *Apis mellifica,* gel electrophoresis, Western blotting, immunoprobing.

INTRODUCTION

It is estimated that more than 50 million people suffer from allergies in the United States. These allergies include hay fever, rhinitis, dermatitis, chronic sinusitis, drug reactions, and food intolerance. Insect stings are known to cause severe anaphylaxis in 3.3 percent of the U.S. population (Gergen, et. al., 1987). The environment is filled with probable allergens yet not everyone is affected. The immune system is responsible for fighting these probable allergens as well as other diseases and foreign material. Each person's immune system responds differently when exposed to this foreign material. When the immune system becomes over-active, an allergic reaction takes place.

Allergens are the chemical substances responsible for these allergic reactions and are found on the surface of many types of pollen, fungal spores, animal dander, feathers, plant seeds, and house dust. When these allergens, also known as antigens, enter the body they are recognized by the antibodies the immune system has produced to protect the body from disease. When the immune system has produced an excess amount of these antibodies the immediate reaction to the antigen is a type I hypersensitivity. A type I hypersensitivity occurs when an antigen is recognized by an immunoglobulin type E (IgE) antibody. These antibodies are found on the surface of mast cells. When an antigen is introduced into the system, it binds to these IgE molecules which in turn link together. This linking causes mast cells to release chemicals such as histamine into the surrounding tissues (Coleman, et. al., 1989). This leakage of histamine and other immune chemicals affect the blood vessels by causing fluid to collect in the tissues, which then stimulates the local pain receptors. These results, termed an allergic reaction, usually are exhibited by an inflammatory response associated with swelling, redness, and sensitivity in the tissue (Coleman, et. al., 1987).

Homeopathy is an alternative form of medicine from the western pharmaceutical practices. The philosophy behind homeopathic medicine is that an infinitesimal amount of a substance can reduce symptoms of stress and allergic response. In theory, homeopathic drugs work by affecting the regulation of complex systems instead of treating only the symptoms. *Apis mellifica* contains melittin, the active ingredient in honeybee venom. At a concentrated dosage melittin has many allergic reactions including edema (swelling), erythema (redness), and itching. As a homeopathic medicine *Apis mellifica* is used to cure similar symptoms caused by antigens (Bellavite and Signorini, 1995).

The purpose of this project is to determine whether *Apis mellifica* has an affect on type I allergic reactions by way of the IgE antibody. Mice were used in this study because it is a research system with the same immune system response as humans. If the mice undergoing the treatment of *Apis Mellifica* display a significantly lower IgE level it would show a probable process for how this homeopathic medicine affects the immune system. Thus, new avenues for allergy treatments, such as needed in anaphylaxis, could be developed using homeopathic medicines. If IgE levels do not change significantly there are other possibilities of how the homeopathic treatment is affecting the system. *Apis Mellifica* might affect the binding affinity of the IgE being made so that it no longer recognizes the antigen. In this case IgE levels would not be affected but the allergic reaction would still be reduced.

MATERIALS AND METHODS

Twelve CD-1 male mice between 16-18 grams were

purchased from Charles River Laboratories, Inc (251 Ballardvale Street, Wilmington, MA 01887. 1-800-522-7287). Mice were divided into two groups: 6 mice for the control group and 6 mice for the treatment group. Mice were kept in four cages with three mice per cage, to alleviate stress caused by overcrowding. Mice were given an eight-day period of acclimation to alleviate skewed results due to stress from handling. Throughout the study both groups were fed nutrient pellets appropriate for rodents and unlimited water.

Blood Samples

Over the duration of the study four 20-microliter blood samples were taken from the tail of each mouse and added to 5-microliters of 5x SDS loading buffer in pre-labeled aliquots (Ausubel, et. al., 1988). The samples were frozen for electrophoresis and western blotting (Henry, 2000).

The first sample was taken on day 9, one day following the acclimation period. The samples on day 13 and 28 were taken one hour following casein injections. The sample on day 25 was taken one hour after the final *Apis mellifica* and placebo treatment

Milk Casein and *Apis mellifica* treatments

On day 11, 13, and 28 each mouse was given 100 micrograms milk casein in 0.2-ml intraperitoneal (ip) injection (Hasegawa, et. al., 1999).

Apis mellifica and placebo treatment lasted 12 days starting on day 14 of the study. Mice were given a 20-mg/kg per day dosage of *Apis mellifica* (Wyczolkowska, 1993). *Apis mellifica* bought at a health food store at a dosage of 30x was diluted at 1-mg *Apis mellifica* to 2-microliters de-ionized water (Bellavite and Signorini, 1995). Mice in the treatment group were given a daily dosage of 2-microliters *Apis mellifica* orally by using a micropipette. Mice in the control group were given 2-microliters dH_2O placebo dosage by the same method.

Electrophoresis

Laemmli SDS-PAGE gel electrophoresis was used to separate proteins in blood samples (Ausubel, et. al., 1988). An 8% acrylamide separating gel was used based on the molecular weight of IgE (Sambrook, et. al., 1989).

The blood samples were prepared for electrophoresis by boiling samples at 100ºC for 8 minutes and then centrifuging for 2 minutes immediately following to reduce the amount of precipitate in the samples. Purified mouse IgE standard and molecular weight markers were boiled 8 minutes before being loaded into gel. Gels were loaded using 10-microliter Hamilton syringes. Electrophoresis lanes were loaded with 5-microliters of each blood sample and mouse IgE standard and 10-15-microliters of the stained molecular weight markers as per manufacturer instructions. Gels were run at constant current, 15 mA for a single gel and 30 mA for a double gel assembly (Ausubel, et. al., 1988).

Western Blotting and Immunoprobing

The separated proteins were then transferred to a membrane by western blotting. Transfer was completed using protocol of a semi-dry electroblotter system (Owl, 2000) with a polyvinylidene fluoride (PVDF) transfer membrane (Millipore, 1995). Transfer blotting system was assembled as follows:

+ Anode, 3 sheets filter paper soaked in transfer buffer, PVDF membrane, Gel, 3 sheets filter paper soaked in transfer buffer, -Cathode.

Semi-dry system was attached to a constant current of 400 mA with a low voltage between 10-15 mV for 1-½ hours. The gel was then removed and stained with Coomassie Blue to check for protein transfer. The PVDF membrane was used in immunoprobing with an avidin-biotin coupling to the secondary antibody (Ausubel, et. al., 1988). The membrane was incubated in TTBS blocking buffer for 1 hour. Rat anti-mouse IgE was used as the primary antibody. The Vectastain rat IgG system was used with alkaline phosphatase as the chromogenic reagent (Vector, 2000).

The distances traveled by the stained molecular weight markers were then used to determine the molecular weights of the bands visualized through immunoprobing.

RESULTS

Immunoprobing and visualization techniques identified bands of proteins in 5 out of 6 mice in the control group and 4 out of 6 mice in the treatment group. The third blood sample in both groups showed the most bands (Fig. 2).

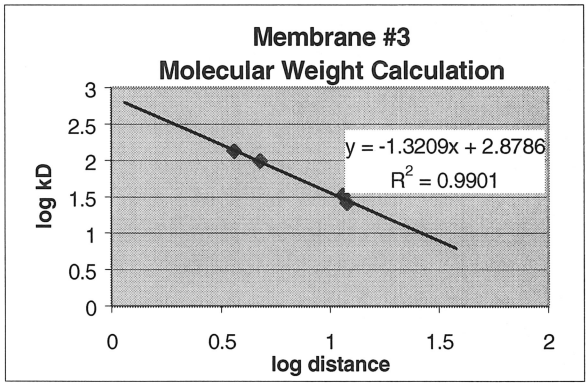

Figure 1. Plot of logarithmic functions of molecular weight (kD) versus distance traveled in gel to determine molecular weights of the bands visualized through immunoprobing.

The majority of bands, however, were not at the level of the mouse IgE standard. By using the stained molecular weight markers it was possible to calculate the molecular weights by distance traveled in the acrylamide gel (Fig. 1).

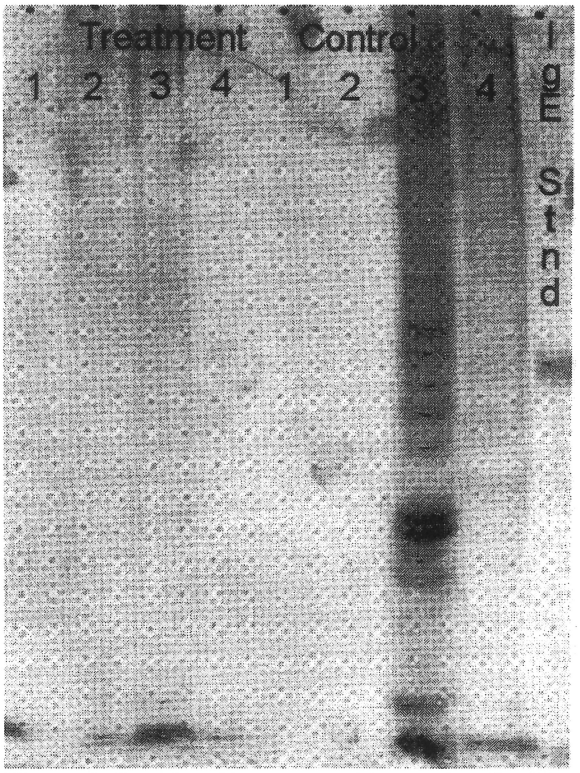

Figure 2. Results of immunoprobing to visualize bands of proteins in blood samples.

The calculated molecular weight of the mouse IgE standard was 66 kD. The two lowest bands have average molecular weights of 30.2 kD and 28.61 kD (Fig. 2). The sum of these two bands is slightly less than the molecular weight calculated for the band of IgE standard.

DISCUSSION

Although no immunoreactive bands were found at the level of the mouse IgE standard, there were two prominent smaller molecular weight bands that were visualized. The sum of these two bands was approximately equal to the intact IgE standard band. This would indicate that breakdown products of IgE molecules were being identified. All blood samples were taken I hour after oral treatment or ip injections so it is possible that the IgE had already began hydrolization. This would account for the bands of smaller molecular weights rather than intact IgE molecules. Even smaller fragments would have been lost from the lanes due to electrophoresis running them off the gel.

It is also possible that in the preparation of the samples for loading in gel electrophoresis, IgE could have began hydrolization during boiling or centrifugation. However, the IgE standard was also boiled. Immunoprobing revealed no indications that hydrolization had occurred in the IgE standard samples.

By using a polyclonal primary antibody it is possible that the immunoprobing system could be reacting with another immunoglobulin such as IgG. However, if this cross-reaction were taking place it would be expected to visualize bands in the second, third and fourth samples of all the mice. IgG is the most common immunoglobulin and is found in the blood after any exposure to an allergen (Benjamini and Leskowitz, 1991).

Although there is not evidence to support that *Apis mellifica* reduced the levels of IgE, the results do indicate that IgE is present in a broken-down state. This makes it difficult to quantify the amounts of IgE in the blood, which would have been produced when exposed to the allergen. Further testing in which blood samples are taken within a shorter time of the casein injection may produce bands of intact IgE antibodies. These bands could then be compared quantitatively between the treatment and control groups.

The practice of homeopathic medicines like *Apis mellifica* is still being questioned due to the extreme dilution process. A 30x dosage signifies that the homeopathic solute has been diluted at a concentration of 1:100. This dilution process is then repeated 30 times. At this theoretical dilution of $1x10^{-60}$M it is questionable whether any of the original solute is still present. If solute were present there would probably be variability in the percentage of active ingredient found in each individual dosage (Wynn, 1998).

This variability in dosage could affect the level of IgE in the blood. Previous studies suggest that there is an optimal dosage of homeopathic drug in treating an allergy. It is possible that a different dilution of *Apis mellifica* could make a profound difference in the level of IgE antibodies found in the blood.

Studies have not yet determined how homeopathic medicines affect the immune system. There is the possibility that *Apis mellifica* prevents the IgE that is being made from binding to the antigen. If the antigen is unable to bind, then histamine cannot be released and no allergic reaction will take place (Benjamini and Leskowitz, 1991). This study would not be able to distinguish such a result from there being no effect in the allergic reaction.

In identifying proteins by immunoprobing, a primary antibody was used. This antibody has a specific allotype. It is not known what type of histamine, compatibility complex CD-1 mice represent. If there is a discrepancy in the allotype this could cause a lack of visualization of the protein bands. However, bands were detected in immunoprobing. This would indicate that either the allotypes were compatible or that it did not matter whether or not the antibodies matched exactly.

It is also likely that the CD-1 mice would show some variation due to the fact that they are outbred. These mice come from a mixed population and would show some genetic heterozygous tendencies, such as susceptibility to allergens. The difference between the treatment and control groups in the visualization of bands could be due to these genetic tendencies.

The reason the third blood samples showed more traces of IgE is unknown. Perhaps the mice were reacting to another allergen at the time of the blood sample. However, of the techniques used, the treatment of *Apis mellifica* and placebo was least stressful. There were no changes in buffers used or the timing of the blood samples that would provide an explanation for these discrepancies in the data.

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