Producing Monoclonal Antibodies To Phospholipase A2

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PRODUCING MONOCLONAL ANTIBODIES TO PHOSPHOLIPASE A2

Submitted in Partial Fulfillment of the Requirements for Graduation with Honors to the Department of Biology at Carroll College, Helena, Montana

Marie Anne Kernie
April 1, 1985
This thesis for honors recognition has been approved for the Department of Biology by:

Dr. John A. Christenson, Advisor

Dr. James J. Manion

Rev. William Greytak

April 1, 1985
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I would also like to thank Dr. Walt Waterbury for allowing me to use the facilities at Hollister-Stier Laboratories in Spokane, Washington.

Many thanks are extended to Dr. Greg Plunkett for his helpful advice and enthusiasm while I was working on the project.

Finally, a very special thank you goes to Polly Lally. Her patience, helpfulness, enthusiasm, and friendship while I was at Hollister-Stier Laboratories are much appreciated.
Monoclonal antibodies were produced to phospholipase A2, a protein allergen of honey bee venom. The hybridoma technique, which involves fusing antibody-producing spleen cells to myeloma cells was used. The antibodies were of the IgG1 subclass and they did not cross-react with phospholipase components of other bee venoms.
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INTRODUCTION

When a foreign substance enters an animal, one of the ways the animal responds is by the production of antibodies which react with the foreign substance (the antigen). B-lymphocytes recognize a certain site (determinant) on the antigen, some of them are then converted to plasma cells which secrete serum proteins called antibodies that react with this determinant in such a way that causes the antigen to either be neutralized or destroyed (1).

Because of their specificity, antibodies can be used to identify, purify, and quantify a variety of substances (2) including separating and identifying the various protein allergens (antigens that cause an allergic reaction) that compose honey bee venom. Currently, the allergens considered to be the most important in honey bee venom are hyaluronidase, acid phosphatase, melittin, and phospholipase A₂ (3). Obtaining antibodies specific for each of these components would be useful. The objective of my research was to produce monoclonal antibodies to the phospholipase A₂ component.

The most common means of obtaining antibodies is to inject an animal (i.e., a rabbit) with the desired antigen and then to collect the antiserum the animal produc-
es. The most difficult problem with this method is that instead of consisting of only one kind of antibody, the antiserum is a mixture of many kinds of antibodies including different antibodies that recognize different determinants and also any antibodies to any impurities that were injected with the antigen. It is difficult to separate and purify these many antibodies from each other (4).

The fact that this supply of purified antiserum will eventually run out is another problem. A new animal has to be injected with the desired antigen. There is no guarantee that this new animal will respond to the antigen like the other animal did and if it does, the whole purification process has to be done again. A reliable, alternative method of obtaining pure samples of antibodies would be desirable.

Producing monoclonal antibodies using the hybridoma technique first done by Köhler and Milstein in 1975 (5) is an alternative. It involves fusing antibody-producing spleen cells with myeloma cells. Spleen cells alone will not grow in culture, but when fused with myeloma cells (which grow indefinitely in culture), they acquire the immortality of the myeloma cells. The hybridomas (the fused myeloma spleen cells) can be screened for the ones producing the desired antibodies and then be cloned (6, 7). The result is a pure, never-ending supply of identical antibodies. These identical antibodies are called monoclonal antibodies because they are all derived from the same
Because phospholipase A₂ is one of the more important allergens in honey bee venom it would be beneficial to have a reagent that could identify, purify, and quantify it. Monoclonal antibodies would be ideal for this because they are specific, homogeneous, and the supply of them never runs out (8). The objective of my research was to produce monoclonal antibodies to phospholipase A₂ using the hybridoma technique.
LITERATURE REVIEW

Many monoclonal antibodies to a variety of substances have been reported. Among these are monoclonal antibodies to melanoma cells (9, 10), methanobacteria and other microorganisms (11, 12), tobacco mosaic virus (13), influenza virus (14), various parts of chromosomes (15), and others (2, 4, 16, 17). Monoclonal antibodies are being used in all aspects of research including diagnosis of disease, purification assays, and cancer research (1, 2, 4, 18).

To produce monoclonal antibodies one must first obtain cells that are producing the desired antibody. This is done by immunizing an animal with the desired antigen and then harvesting the antibody-producing B-lymphocytes. B-lymphocytes are often obtained from the spleen of the immunized animal because they are at a high concentration in this organ (6, 7).

Spleen cells alone cannot grow in culture. They are "immortalized" by fusing them with myeloma cells which proliferate indefinitely. Myeloma cells are tumorous antibody-producing cells. The myeloma cells often used in monoclonal antibody production, however, are ones that have lost their ability to produce antibodies yet still grow indefinitely in culture. One such line is called
x63-Ag8.653 (19).

The spleen cells and the myeloma cells are induced to fuse by using any one of several agents including the Sendai virus, lysolecithin, and polyethylene glycol. The latter is most commonly used (6).

Once the cells are fused, there has to be a way to separate the hybridomas from the spleen cells and the myeloma cells. This has most often been accomplished by growing the cells in the selective medium, HAT medium (6), which includes hypoxanthine, aminopterin, and thymidine. Aminopterin blocks the main biosynthetic pathway for nucleic acid synthesis. Cells can grow using an alternative pathway if they are provided with hypoxanthine and thymidine. The myeloma cells, however, lack the enzyme hypoxanthine quanine phosphoritosyl transferase (HGPRT) and this prevents them from incorporating the hypoxanthine so they perish. The hybridomas have obtained HGPRT from which their spleen cell parents and live. The spleen cells die naturally.

An assay has to be developed to screen for the hybridomas that are producing the desired antibodies. Different screening procedures have been used including immunofluorescence assays, rosetting assays, radioimmunoassays and Enzyme Linked Immunosorbent Assays (6).

There are many types of ELISA assays, however, the one used in this project was the indirect method for detection and measurement of antibodies (20). It involves
attaching the antigen to a solid phase. The supernatants from the hybridomas are then added. If antibodies to the desired antigen are present they will bind to the antigen on the plate. The extra antibodies that do not bind are washed off. An anti-species-globulin that is labeled with an enzyme is then added and this binds to the antibodies that have bound to the antigen. The last thing that is added is an enzyme substrate and a color change is observed. The more antibodies that have bound to the antigen, the more intense the color change. If there are no antibodies present in the supernatant the anti-species-globulin cannot bind and thus no color change is observed when the substrate is added.

After the screened hybridomas have been growing a while they need to be cloned. One of the most common methods of cloning is the Limiting Dilution Technique. It involves diluting the cells out to a stage where there is, statistically, only one viable cell per well in a microtiter plate (6).

Finally, feeder cells can be utilized in both fusing the cells and cloning the hybridomas if desired. They serve two functions. One is to give a high enough concentration of cells so that the isolated hybridoma will grow. The second is to provide a growth stimulating chemical. Under ideal conditions these are not necessary, and many labs have had successful fusions without them (6).

Producing monoclonal antibodies using the hybridoma
technique can be tedious. Once made, however, the supply of antibodies specific to one antigen is limitless (8).
METHODS AND MATERIALS

Fusion Protocol

1. Preparation

1.1 Immunization of Mice

Solutions

Complete Freunds Adjuvant

Phospholipase A₂ (PLA) - Hollister-Stier Laboratories had obtained the PLA by running honey bee venom through a Bio-Gel P-30 column (BIO-RAD Laboratories Cat. #1501340) and collecting the PLA peak (approximately 18,000 MW). The column was 76 cm in length, 4.5 cm in diameter and the buffer used was 50 mM acetate, pH=4.5

Phosphate Buffered Saline (PBS) - 1.8mM sodium phosphate monobasic monohydrate, 8.2 mM sodium phosphate dibasic anhydrous, 0.12 M NaCl, pH adjusted to 7.3.

Injection Schedule

Hollister-Stier Laboratories immunized four BALB/C mice to phospholipase A₂ (PLA) as follows:

PLA in Complete Freunds Adjuvant (25 ug PLA/0.5 ml) was injected at five sites: 0.1 ml into each of the two axillary lymph node regions, 0.1 ml into each of the two inguinal lymph node regions
and 0.1 ml was injected intraperitoneally.

Fifty-five days later the mice were boosted intraperitoneally with 0.5 ml of PLA in Complete Freund's Adjuvant (25 µg PLA/0.5 ml).

Three days prior to the fusion (5 days after the second booster) the mice were given a third booster, 0.5 ml PLA in Phosphate Buffered Saline (PBS) (12 µg PLA/0.5 ml), intravenously.

1.2 Testing for Antibody Production in the Imunized Mice using an Immunodiffusion Assay

Preparation of Agarose Gel Square

An agarose gel square was prepared using Agarose Immunodiffusion Tablets (BIO-RAD Laboratories Cat #170-3002) according to the manufacturer's instructions. The support medium for the agarose gel was Gelbond Film.

Immunodiffusion Assay

Antiserum from each of the four mice immunized to PLA was put into four different wells on the agarose gel square. Surrounding each of these four wells were six wells containing different dilutions of PLA in PBS. The most concentrated dilution was 1.62 µg PLA/ml and the least concentrated was 0.00052 µg PLA/ml.

The agarose gel square was allowed to incubate at room temperature overnight. The next day the square was stained with Coomassie Blue in 50%
methanol, 10% acetic acid and then destained with 10% acetic acid. The presence of precipitin bands indicated the presence of antibody to PLA in the mouse antiserum.

1.3 Preparation of Media and Solutions

**Fusion RPMI** - RPMI 1640 (powder by Gibco Laboratories Cat #430-1800) prepared according to manufacturer's instructions. Other chemicals added were 94.4 μM penicillin (Sigma Chemical Co.), 68.6 μM streptomycin (Sigma Chemical Co.), 2 mM glutamine (Gibco Laboratories, 1 mM Na pyruvate (Gibco Laboratories), and 0.5 μM 2-mercaptoethanol (Sigma Chemical Co.).

**Full RPMI** - 15% Fetal Bovine Serum (Hyclone) in Fusion RPMI.

**HT RPMI** - Full RPMI plus 0.051 μM hypoxanthine and 0.06 μM thymidine.

**HAT RPMI** - HT RPMI plus 0.4 μM Aminopterin.

**50% Polyethylene Glycol (PEG)** - 50% autoclaved PEG 1450 (J.T. Baker Cat #8-6220) in Fusion RPMI. This solution was made on the day of the fusion.

1.4 Thawing of the Myeloma Cells

The myeloma cells used were from the mouse myeloma cell line called X63-Ag8.653. They had been stored in liquid nitrogen and were thawed and grown before they were used in the fusion.

Three days before the fusion a 1-ml cryogenic
vial containing myeloma cells was thawed in a 56°C water bath for 1.5 min. The contents were transferred to a 50-ml conical centrifuge tube. Twenty ml of Full RPMI were added over a 10 min period. The cells were left alone for 15 min. Then an additional 30 ml of Full RPMI were added and the suspension was centrifuged at 200g for 10-15 min. The supernatant was poured off. The pellet was resuspended in 5 ml of Full RPMI. The cells were then counted and diluted to about $5 \times 10^5$ cells/ml with Full RPMI and seeded into flasks.

The day before the fusion the myeloma cells were divided so that their concentration was about $8 \times 10^4$ cells/ml. This concentration was lower than the usual concentrations used ($5 \times 10^6$ cells/ml), however, there were still enough myeloma cells to be used in the fusion the next day.

2. Fusion Day

2.1 Preparation of Feeder Cells

Feeder cells were obtained by surgically removing the thymus from a 4 to 6-wk-old BALB/c mouse under sterile conditions. The thymus was then placed on a 150 mesh screen in a sterile petri dish containing a small amount of HAT RPMI. The cells were rubbed through the screen with a rubber policeman. The screen was rinsed with 10 ml of
HAT RPMI and the cells were transferred to a 50-ml centrifuge tube.

The cells were counted using a vital stain and an Improved Neubauer Ultra Plane hemacytometer, then they were diluted to $3 \times 10^6$ cells/ml. One hundred ml at this concentration were prepared.

2.2 Preparation of the Spleen Cells

The spleen, under sterile conditions, was surgically removed from an immunized mouse that had been shown to be producing antibodies to PLA. It was placed on a 100 mesh screen in a sterile petri dish containing Fusion RPMI.

Most of the spleen cells were flushed out of the capsule with Fusion RPMI using a needle and syringe. The spleenic capsule was then teased apart to get the remaining cells. The screen was rinsed with 10 ml of Fusion RPMI and the cells were transferred from the petri dish to a 50-ml centrifuge tube.

After waiting 5 min (to allow debris to collect), the cell suspension was filtered through a 150 mesh screen into another 50-ml centrifuge tube. It was then spun at 400g for 10 min. The pellet was resuspended in less than 1 ml of Fusion RPMI.

Three ml of sterile water (distilled water that had been filtered through a 0.2 mM filter)
were added to the suspension to lyse the red blood cells, and then 30 ml of Fusion RPMI were added. These two steps occurred within 8 sec.

The cells were again centrifuged at 400g for 10 min. The pellet was resuspended in 20 ml of Fusion RPMI and vitally stained cells were counted.

2.3 Preparation of Myeloma Cells

The myeloma cells that had been growing for 3 days (section 1.4) were flushed from their flasks and transferred to 50-ml centrifuge tubes. They were spun at 400g for 10 min. The pellets were resuspended in a small amount of Fusion RPMI and then combined into one centrifuge tube. Thirty ml of Fusion RPMI were added to this tube and the cells were stained and counted.

2.4 Cell Fusion

The prepared myeloma cells were mixed with the prepared spleen cells at a ratio of 3:1 and then centrifuged at 400g for 10 min. The pellet was resuspended in less than 1 ml of Fusion RPMI. Again the cells were centrifuged at 400g for 5 min. This time all the supernatent was removed with a Pasteur pipette.

A timer was set for 3 min, then 1 ml of 50% PEG was added and the pellet was gently and gradually dispersed into the 50% PEG with a Pasteur pipette. Within 2 min the pellet was uniformly
dispersed into 0.5-1 μm clumps. During the third minute, a pipette was filled with 10 ml of Fusion RPMI. The Fusion RPMI was added in 0.5 ml aliquots over a 10-min period with gentle mixing. Then 20 ml of Fusion RPMI were added over the next 5 min. Fifteen min later, the mixture was centrifuged at 400g for 10 min. The pellet was gently resuspended in 20 ml HAT RPMI. This mixture was transferred to a flask. The feeder cells were added and then enough HAT RPMI was added to bring the total volume to 200 ml. Using a 10 ml pipette, 3 or 4 drops of the suspension were placed into each well of 10 96-well plates. Polly Lally of Hollister-Stier Laboratories prepared the last five plates. The plates were put in a 10% CO₂, 36°C, humidified incubator.

3. Cell Feeding Schedule

Four days after the fusion the cells were fed for the first time. Using an apparatus called the "Handispense," the old media was suctioned out of the wells of all 10 plates. New HAT RPMI was then added dropwise to all the wells until they were nearly full. Three days later the cells were again fed with HAT RPMI.

On the tenth day after the fusion, the cells were fed with HT RPMI. By this time the myeloma cells
had died so that the selective medium HAT RPMI was no longer needed.

**Screening and Cloning the Hybridomas**

4. **Screening the Hybridomas**

A microtiter plate ELISA (Enzyme Linked Immunosorbent Assay) was used to distinguish the hybridomas that were producing anti-PLA antibody from the ones that were not.

4.1 **Solutions**

- **0.1M citrate buffer** - 0.5M triNaCitrate dihydrate, 0.05M citric acid anhydrous pH 4.3
- **ELISA coating buffer** - 0.1M sodium bicarbonate buffer pH 9.5.
- **ELISA stopping solution** - 2mM sodium azide.
- **0.12M PBS** - same as the PBS (section 1.1).
- **0.5M PBS** - same as 0.12M PBS except it has 0.5M NaCl.
- **ABTS peroxidase substrate** - 0.1M citrate buffer, 20 μg/ml ABTS (2,2'-azino-di-(3-ethylbenzylthiazoline) sulfonic acid), and 30% H₂O₂ in a ratio of 10:0.4:0.01, respectively.

4.2 **Screening Procedure**

Nine hundred sixty microtiter wells of Dynatech Immunol plates were coated with (5 μg/PLA/ml) and incubated overnight at room temperature. The plates are then aspirated and washed three times
in 0.5M PBS, 1.0% Tween 20 (J.T. Baker). To block any remaining adsorption sites, the wells were incubated 1 hr at 37°C with 250 µl of 0.12 M PBS, 0.1% Tween 20, 10% Normal Goat Serum (Colorado Serum Company). The plates were aspirated and washed three times with 0.5M PBS, 0.1% Tween 20.

The plates were then incubated with 50 µl of the supernatant from the wells containing the hybridomas at 37°C for 2 hr. The plates were aspirated and washed three times with 0.5M PBS, 0.1% Tween 20.

Fifty µl of peroxidase labeled Goat Anti-mouse IgG (Tago 6450) diluted 500 times with 0.12M PBS, 0.1% Tween 20, 10% Normal Goat Serum were added to the plates and incubated 2 hr at 37°C. The plates were washed three times with 0.5M PBS 0.1% Tween 20.

One hundred µl of the ABTS peroxidase substrate were then added and color development was allowed to proceed for 25 min before it was stopped with ELISA stopping solution.

The presence of a green color is an indicator of the presence of anti-PLA antibody (see literature review).

5. Expanding and Freezing the Hybridomas

Fifteen hybridomas in the microtiter plates were
positive when screened and were expanded to 24-well plates. These 15 hybridomas were screened again for antibody production and the five that were positive were expanded to six-well plates, then finally to 250 ml flasks. At this point they were frozen.

Freezing Procedure

The hybridoma cells were flushed from their flasks, transferred to 50-ml centrifuge tubes, and centrifuged at 200g for 10 min. The pellet was resuspended in Fetal Bovine Serum (Hyclone), 1 ml FBS/vial that was to be frozen. An equal amount of 24% Dimethylsulfoxide (DMSO) in HT RPMI was added at the rate of 1 ml/min. The mixture was put into 1 ml cryogenic vials and these were stored at -80°C in styrofoam containers overnight. The next morning the vials were then transferred to liquid nitrogen.

6. Cloning, Expanding, Freezing

The cells were cloned by a single cell selection technique. Individual cells are picked up in a drawn out Pasteur pipette from a diluted sample of hybridomas. The field was viewed through an inverted microscope (40x) making sure that only one cell entered the pipette. The cell was transferred to a microtiter plate well that contained feeder cells (1 x 10^6 feeder cells/ml).

The clones were fed HT RPMI every 5 days the same way the hybridomas were fed. After 11 days the clones
were screened for anti-PLA antibody production using the same ELISA screening assay that was used for the hybridomas.

The positive wells containing only one colony were expanded to 24-well plates, then six-well plates and finally 240-ml flasks. The clones were frozen in the same manner as the hybridomas.

Working with the Monoclonal Antibodies

7. Antibody Subclass Identification

A class and subclass detection kit made by Chemicon International was used to determine the subclass of the monoclonal antibodies.

Microtiter wells of Dynatech Immulon 2 plates were coated with 50 μl of PLA in ELISA coating buffer (5 μg PLA/ml) and incubated at room temperature overnight. They were then aspirated and washed three times with 0.5M PBS, 0.1% Tween 20.

To block any remaining adsorption sites, the plates were incubated with 250 ul of 10% Normal Goat Serum in 0.12M PBS, 0.1% Tween 20 at 37°C for 1 hr and then aspirated and washed three times with 0.5M PBS, 0.1% Tween 20.

For each clone, 50 μl of its Grow to Death supernatant (supernatant from cells that have been allowed to grow until death) were placed in nine wells and incubated at 37°C for 1.5 hr. The plates were aspirated
and washed three times with 0.5M PBS, 0.1% Tween 20.

Fifty ul of Normal Rabbit Serum in 1% Bovine Serum Albumin-PBS and 50 ul of the rabbit antibodies to eight specific mouse immunoglobulin classes and subclasses (IgA, IgG1, IgG2b, IgG3, IgM, Kappa light chain, Lambda light chain), were added to the nine individual wells of each clone and incubated 1.5 hr at 37°C. The plates were aspirated and washed three times with 0.5M PBS, 0.1% Tween 20.

To each of the wells, 50 ul of Goat anti-rabbit IgG(H+L) were added and then incubated at 37°C for 1.5 hr. The wells were washed with 0.5M PBS, 0.1% Tween 20.

One hundred ul of the ABTS peroxidase substrate (section 4.1) were added and color development was allowed to proceed for 40 min before it was stopped with ELISA stopping solution (section 4.1).

The presence of the darkest green color indicates the class and the type of light chain the monoclonal antibody is.

8. Venom Cross-Reaction Assay

The monoclonal antibodies were tested to see if they would cross-react with venoms other than honey bee venom.

Each of the following was put into 24 microtiter wells of Dynatech Immulon 2 plates (50 ul/well): yellow
jacket venom, yellow hornet venom, white faced hornet venom all diluted 100 times in ELISA coating buffer, wasp (*Polistes*) venom diluted 10 times and PLA (5 µg/well). These were incubated overnight at room temperature. The plates were then aspirated and washed three times with 0.12M PBS, 0.1% Tween 20.

Any remaining adsorption sites were blocked by incubating the wells with 250 µl of 0.12M PBS, 0.1% Tween 20, 10% Normal Goat Serum at 37°C for 1 hr. The plates were aspirated and then washed three times with 0.12M PBS, 0.1% Tween 20.

Fifty µl of each of the clone GTD supernatants were placed in two wells of each of the 24-well group of venom coated wells and incubated at 37°C for 1.5 hr. They were then aspirated and washed three times with 0.12M PBS, 0.1% Tween 20.

Fifty µl of peroxidase labeled Goat anti-mouse IgG (Tago 6450) diluted 500 times with 0.12M PBS, 0.1% Tween 20, 10% Normal Goat Serum were added to the plates and incubated for 1.5 hr at 37°C.

One hundred µl of the ABTS peroxidase substrate (section 4.1) were then added and color development was allowed to proceed for 30 min before it was stopped with ELISA stopping solution.
RESULTS

1. Immunodiffusion Assay

Precipitin bands were formed with three out of the four immunized mice, indicating that these three mice were producing anti-PLA antibodies (Fig. 1).

Fig. 1. Diagram of Precipitin Bands
Antiserum from mice was allowed to react with different concentrations of PLA antigen. The antiserum was placed in the center well and different concentrations of PLA were placed in the surrounding wells: 11.62 µg PLA/ml, 20.324 µg PLA/ml, 30.0648 µg PLA/ml, 40.01296 µg PLA/ml, 50.002592 µg PLA/ml, 60.00052 µg PLA/ml.
2. Screening and Cloning the Hybridomas

Fifteen hybridomas were expanded to 24-well plates. Upon screening these 24-well plates, however, only five hybridomas were shown to be still producing antibodies (Table 1) and so only these five were eventually cloned. Clones producing anti-PLA antibodies were obtained from three out of these four hybridomas. From hybridoma 7E11 the three clones 7E11-1, 7E11-2, 7E11-3 were obtained. These clones from hybridoma 8B2 included 8B2-1, 8B2-2, 8B2-3, 8B2-4, and 8B2-5. Finally, three clones were obtained from hybridoma 9G8 including 9G8-1, 9G8-2, 9G8-3.

3. Antibody Subclass Identification

Table 2 shows the absorbance values (read at 415 nm) that were obtained from the assay. The highest absorbancy values are from antibodies that were incubated with rabbit anti-IgG1 and others that were incubated with the anti-Kappa light chains. These data show that all the monoclonal antibodies to PLA were of the IgG1 class and had Kappa light chains.

4. Venom Cross Reaction Assay

With the exception of the monoclonal antibodies that were incubated with PLA, all the absorbancy values shown in Table 3 are low. This indicates that the monoclonal antibodies do not cross-react with venoms other than honey bee venom.
Table 1. Results of Screening and Cloning the Hybridomas

<table>
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<th>1E8</th>
<th>1H5</th>
<th>3C4</th>
<th>4H2</th>
<th>5010</th>
<th>5F5</th>
<th>5H10</th>
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<td>Number of Antibody-producing Clones That Was Obtained</td>
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<td>6</td>
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The (+) indicates which hybridomas were expanded to 24 well plates and which hybridomas were cloned. The last row indicates the number of anti-PLA antibody-producing clones that were obtained.
<table>
<thead>
<tr>
<th>GTD Supernatant</th>
<th>NRS</th>
<th>IgA</th>
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<td>0.033</td>
<td>0.280</td>
<td>0.029</td>
<td>0.036</td>
<td>0.116</td>
<td>0.127</td>
<td>0.049</td>
</tr>
<tr>
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<td>0.038</td>
<td>0.037</td>
<td>0.261</td>
<td>0.029</td>
<td>0.034</td>
<td>0.111</td>
<td>0.128</td>
<td>0.048</td>
</tr>
<tr>
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<td>0.034</td>
<td>0.033</td>
<td>0.239</td>
<td>0.027</td>
<td>0.034</td>
<td>0.113</td>
<td>0.121</td>
<td>0.042</td>
</tr>
<tr>
<td>9G2-1</td>
<td>0.033</td>
<td>0.036</td>
<td>0.033</td>
<td>0.225</td>
<td>0.028</td>
<td>0.039</td>
<td>0.027</td>
<td>0.121</td>
<td>0.045</td>
</tr>
<tr>
<td>9G2-2</td>
<td>0.034</td>
<td>0.044</td>
<td>0.034</td>
<td>0.230</td>
<td>0.028</td>
<td>0.037</td>
<td>0.095</td>
<td>0.113</td>
<td>0.049</td>
</tr>
<tr>
<td>9G2-3</td>
<td>0.035</td>
<td>0.046</td>
<td>0.039</td>
<td>0.214</td>
<td>0.029</td>
<td>0.040</td>
<td>0.094</td>
<td>0.112</td>
<td>0.046</td>
</tr>
</tbody>
</table>

GTD supernatants from different monoclonal antibodies were put into rows of microtiter wells as labeled on the left side. Then rabbit antibodies specific for the different subclasses of mouse antibodies were put into columns of wells as labeled above and allowed to react. The wells with the highest absorbancy readings for each monoclonal antibody indicate the subclass and the light chain the monoclonal antibody is composed of.
### Table 3. Absorbancy Readings from Venom Cross-Reaction Assay

<table>
<thead>
<tr>
<th></th>
<th>GTD Supernatant</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Yellow Jacket</td>
<td>0.141</td>
<td>0.157</td>
<td>0.130</td>
<td>0.125</td>
<td>0.146</td>
<td>0.139</td>
<td>0.148</td>
<td>0.133</td>
<td>0.100</td>
</tr>
<tr>
<td>Venom</td>
<td>0.156</td>
<td>0.172</td>
<td>0.147</td>
<td>0.149</td>
<td>0.167</td>
<td>0.165</td>
<td>0.161</td>
<td>0.148</td>
<td>0.117</td>
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<tr>
<td>Yellow Hornet</td>
<td>0.165</td>
<td>0.183</td>
<td>0.160</td>
<td>0.166</td>
<td>0.177</td>
<td>0.178</td>
<td>0.171</td>
<td>0.165</td>
<td>0.132</td>
</tr>
<tr>
<td>Venom</td>
<td>0.165</td>
<td>0.189</td>
<td>0.184</td>
<td>0.171</td>
<td>0.175</td>
<td>0.178</td>
<td>0.177</td>
<td>0.164</td>
<td>0.141</td>
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<tr>
<td>White Faced</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hornet Venom</td>
<td>0.167</td>
<td>0.187</td>
<td>0.178</td>
<td>0.182</td>
<td>0.186</td>
<td>0.178</td>
<td>0.175</td>
<td>0.171</td>
<td>0.133</td>
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<tr>
<td></td>
<td>0.160</td>
<td>0.179</td>
<td>0.169</td>
<td>0.177</td>
<td>0.188</td>
<td>0.170</td>
<td>0.175</td>
<td>0.169</td>
<td>0.147</td>
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<td>PLA</td>
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<td>2.727</td>
<td>2.547</td>
<td>2.421</td>
<td>2.265</td>
<td>2.375</td>
<td>2.455</td>
<td>2.457</td>
<td>2.547</td>
</tr>
<tr>
<td></td>
<td>2.736</td>
<td>2.669</td>
<td>2.515</td>
<td>2.481</td>
<td>2.326</td>
<td>2.212</td>
<td>2.401</td>
<td>2.492</td>
<td>2.425</td>
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<tr>
<td>Wasp (Polistes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Venom</td>
<td>0.010</td>
<td>0.018</td>
<td>0.006</td>
<td>0.007</td>
<td>0.003</td>
<td>0.005</td>
<td>0.007</td>
<td>0.019</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>0.006</td>
<td>0.017</td>
<td>0.004</td>
<td>0.003</td>
<td>0.008</td>
<td>0.007</td>
<td>0.007</td>
<td>0.005</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Different venoms were put into rows of microtiter wells as labeled on the left. Then the GTD supernatants from different monoclonal antibodies were put into columns of wells as labeled above. A high absorbancy reading indicates the antibody reacted with the venom.
DISCUSSION AND CONCLUSION

Monoclonal antibodies have been used to deliver drugs to cancer cells (18), identify bacteria and other microorganisms (11), deliver toxins on the T-lymphocytes that cause rejection in organ transplants (21), and identify, purify, and quantify a variety of substances. The objective of my research was to produce monoclonal antibodies to phospholipase A$_2$ (PLA) and this was achieved. These antibodies are of the IgG$_1$ subclass and they do not cross-react with phospholipase components of other bee venoms. Monoclonal antibodies to PLA are a valuable reagent. Like all other monoclonal antibodies, they are very specific and their supply is endless. They can be used to identify and quantify PLA, to learn more about PLA itself, and to gain general scientific knowledge.

Assays (such as ELISA assays) can be developed using anti-PLA monoclonal antibodies for detecting the presence of PLA and then quantifying it. They can also be used to purify PLA using an affinity column. Because monoclonal antibodies are so specific, very pure solutions of PLA can be obtained. This would be useful in labs studying cell membrane structure since PLA breaks certain bonds in phospholipids which are major components in cell membranes (22).
Undesired PLA can be removed from a solution using the same affinity column technique.

More uniform assays relying on monoclonal antibodies with uniform affinities instead of the heterogeneous mixture of antibodies found in conventional antisera can be developed for quantifying PLA. This is especially desirable in allergy labs where allergen solutions of specific concentrations are prepared.

Analyzing the structure of PLA and how it interacts with antibodies is another use for these antibodies. More knowledge can be gained for allergists who are interested in studying allergens and how these relate to the immune system. A detailed analysis of the determinants on PLA can be made.

General science knowledge can also be acquired using these antibodies to PLA. One can determine the affinity constant (how tightly the antibody binds to its antigen) for the antibodies. A study can be made on general antigen-antibody interaction. Monoclonal antibodies to PLA may behave a little differently from other antibodies. These monoclonal antibodies can also be tested against PLA from other animals and from the results of these tests (whether or not the antibodies reacted with the PLA) evolutionary information might be gained.

There is one property monoclonal antibodies do not have and that is they cannot be used in assays involving precipitin band formation or agglutination unless the
determinant they bind to is repeating. This is because an antibody that binds to a non-repeating determinant can at most only bind two antigenic molecules. The resulting complex is not large enough to cause precipitation or agglutination. Many assays involve precipitation or agglutination. A test would have to be run to determine if the anti-PLA antibodies reacted with a repeating determinant. If it did not, the problem could perhaps be solved by using two different monoclonal antibodies, each reacting to a different determinant on PLA.

Monoclonal antibodies are an advantageous reagent in any lab. They are specific, have uniform affinity, and their supply is limitless. The monoclonal antibodies to PLA that I produced could be a useful reagent in many labs.
LITERATURE CITED


