Using the Wax Moth Larvae as a Model to Study the Immune System

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Table of Contents

Teacher Section

I. Science Background

The innate immune response of vertebrates is remarkably similar to the immune response in insects. One of the most common insect models for studying the innate immune response is the greater wax moth or honeycomb moth (*Galleria mellonella*). The larvae of *Galleria mellonella* (GML) are found in most regions of the world, from Europe to North America and Australia. Studies using the larvae have elucidated several positive correlations between virulence and host responses, and have proven to be a simple and reliable model system to study infections in this insect. Some of the microorganisms studied using GML

In this laboratory activity we propose to use an organophosphate insecticide as the inflicting agent. The most commonly presented hypothesis by the students may be one in which the insecticide will cause the death of the organism. However, the PO response is effective in protecting the GML and enhances its survival rate. Although the insecticide is an effective infectious agent that clearly initiates an immune response (PO activity), it does not cause death. Therefore, this kind of cellular hemocytic response will allow the students to investigate the survival rate of GML. Moreover, the response offers an opportunity to design an inquiry lab by modifying the protocol described here. The students, for example, may change treatments to observe variations of: 1) the survival rate of GML, and 2) the elicited production of PO in 3(ma)62(s)113(1))4-l3(aBMt1a0n3d2)a1n3d)1d3(c)ti4gB6a[(o(w)1v7eoih.sTcha002rdthsaide0n0t4(u)a1t3(vwe)D0f3(P4s)e9-3(/lT)1s5d4a0gThje)2e

D.

Supplies

Materials (for a class size 30-35 students)

- *Galleriamellonella* larvae
- 48 Petri Dishes
- 8 Syringes of 10 mL
- 70% ethanol
- Insecticide Dilutions: Demon WP (cypermethrin)
- Distilled Water
- 84 Test tubes
- 8 Automatic Pipettes P20 and P1000
- 42 Automatic Pipette tips
- Incubator
- Scissors
- PBS Buffer
- Cacodylate Buffer
- Cotton Swabs
- Centrifuge
- ELISA reader spectrophotometer used at 490 nm wavelength

VI. A. Materials and Equipment

Suggested for each group of 4 students

<u>Galleria mellonela Larvae</u> (24) <u>http://shop.speedyworm.com/wax-worms-p2.aspx</u>
(Wax Worms) Price: $8.50 1 cup = 250 worms
<u>Petri Dishes</u> (4) labeled two sets of plates. Each set will be composed of one each for 3 larvae each for Control and three for treatment of selected insecticide. The other set will have the same number of larvae, but will be used for the phenoloxidase analysis.
Fisher Scientific S08184 United Scientific Supplies Disposable Petri Dishes $1.90 Pack of 10 or S33580A Plastic Petri Dishes $129 Case of 500
<u>Insulin Syringe</u> (1) to inject insecticide: 148291B Fisher Scientific BD Micro-Fine™ IV Insulin Syringes $40.19/100
<u>Test tube</u> (1): Fisher Scientific S32168 Kimble™ KIMAX™ Plastic Test Tubes $20.90 Pack of 250
<u>Isopropyl Alcohol</u> 4ml (70%) to disinfect the larvae prior to injection: Local store $1 a bottle
<u>Cotton swabs</u> to be used to apply the Ethanol on the larvae: Local store $1
<u>Microcentrifuge tubes</u> (12) to recover hemolymph Microcentrifuge Tubes
Fisher Scientific S348903 $27.05 Pack of 500

Equipment for Entire Class

<u>Incubator</u>
Fisher Scientific Education Incubator Catalog No. S521081. See-through door allows for visual inspection without heat loss. $455.00 - $582.00
<u>Microcentrifuge</u>
Fisher Scientific Education S67601B Fisherbrand™ Standard Mini-Centrifuge $439
Note: The microcentrifuge will be used to extract the hemolymph. It should be spun for only 7 seconds at -touch-minimum speed. The gravitational force will make the hemolymph quickly flow down to the bottom of the microcentrifuge tube. The larva's body will be retained inside a pipet tip which is placed inside the microcentrifuge tube.
Pipette P23(r)1 /P <</A1(a) BT /TT0 1(r)414f* BT /TT0 1 Tf 0 Tc 0 Tw 132.36 1184f* [(P)4(i)-1 1 Tf 0C2_04 Tc -0.004 Tw 9 0

	2 mM riboflavin		
	0.1 mM EDTA		
	15 W fluorescent lamps		
	L-dopa (4 mg/ml)		

VII. Student Prior Knowledge and

Day 3: The immunity of invertebrate immunity
Ask the students to read take notes about the immune system from all of the following websites:

Materials

http://www.jimmunol.org/content/179/11/7209.full
"Invertebrate immune systems–Specific, quasi-specific, or nonspecific?" (2007) Rowley

3. Prepare the PO analysis buffer mixture in a microcentrifuge tube that contains 150 µl of distilled water, 20 µl of PBS Buffer, 20 µl cacodylate.
4. Each day after the first 24 hours one larva from the second set of plates will be used for the PO analysis. One larva from each plate, both control and treatment groups, will be used to extract the hemolymph.
5. Use scissors to slightly cut the larvae from the end side of its lower half of its body.
6. Insert the larva into the yellow pipette tip, which is inside the blue tip, within the microcentrifuge tube.
7. Make sure the larvae are fully inserted inside the tip with the fissure to the bottom.
8. Start the centrifuge for a maximum of seven seconds. If spun any longer the organs will flow down in the test tube along with the hemolymph. The extracted hemolymph should be at the bottom of the microcentrifuge tube; it should look clear yellow.
9. Transfer 10 µl of hemolymph into the previously prepared PO analysis buffer mixture microcentrifuge tube.
10. M EMCb4xtthe aphle

101

IX. Summative Assessment

Based on 2007 AP® BIOLOGY FREE-RESPONSE QUESTIONS (Form B)
BIOLOGY
SECTION II
Directions: Answer all questions.
Answers must be in essay form. Outline form is not acceptable. Labeled diagrams may be used to supplement discussion, but in no case will a diagram alone suffice. It is important that you read each question completely before you begin to write. Write all your answers on the pages following the questions in the goldenrod booklet.

1. Without adaptive behaviors, animals would not survive.
(a) Describe the common physiological responses to infection in mammals. Explain how these adaptive physiological responses increase the relative fitness for an individual's survival.
(b) Compare and contrast the immune system of vertebrates and invertebrates.

2. The defenses of the human body to the entry and establishment of a pathogen (disease-causing organism) can be divided into nonspecific responses and specific responses.
(a) Explain at least how THREE types of nonspecific defenses can prevent the entry and/or establishment of a pathogen in a person's body.
(b) Discuss how the immune system responds to an initial pathogenic exposure, and how this initial exposure can

developed to assist the beekeepers in controlling pests. In order to do so, it is essential to comprehend concepts of immunology. *Galleria mellonella* contain a complex and essential network of immune cells which play a crucial role in sustaining health throughout the body. In insects, Phenoloxidase is an enzyme that aids in the response to infection, hemolymph coagulation, cellular phagocytosis, oxidase-based melanization and some other examples of responses of the immune system to defend the body against any harm. For instance, insecticides are a substance that the immune system would recognize as foreign and leading to an immune response. Therefore, in this experiment you will study the larva's immune response to an insecticide.

Basic vocabulary in immunology

These are some important terms that will come up frequently in immunology:

- Immunity is defined as resistance to infectious disease.
- Immune system is the collection of cells and tissues that protects the body from infection.
- Immune response is the coordinated reaction of the cells of the immune system to a pathogen.
- Antigen is a general term that applies to molecules that bind to antibodies or T cell receptors. Antigens come in many forms. For example, small molecules in the environment and a huge array of bacterial and viral surface proteins might all act as antigens. Many times, you will see the terms antigen and microbe used interchangeably, since most antigens are derived from larger pieces of a microbe.
- Lymphocytes are cells found in the blood, lymphoid tissues and most organs of the body that express receptors for specific antigens and mediate immune responses. The lymphocytes that we will talk the most about are B cells and T cells. (B cell = B lymphocyte; T cell = T lymphocyte)

Pipette:

1) Input the amount of volume needed by adjusting it in the caliber pipette line.
2) Apply pipette tip.
3) To obtain the specified liquid, press down the pipetting plunger to the first stop with the thumb.
4) To extract the liquid, hold the tip against the side of the receiving container.
5) Push down the pipetting plunger to the first stop and then to the second stop to release all the liquid.

Centrifuge:

1) When setting the test tubes, make sure they are balanced.
2) Make sure to balance the rotor by placing eq/MCID9pf /LBody2e -0.004 Tw 9 0 0 9 72 619.2 Tm [(C)6(ent)2(r)4((l)-1(ea)13(s)-3(

1) Larvae will be injected once.
2) At least one group will use an organophosphate insecticide.
3) The control will consist of the same kind of injection, but with distilled water.
4) Wax moth larvae will be kept in groups of individuals in 90-mm Petri dishes at 28°C.
5) The insect's survival rate will be recorded over the period of 10 days.
6) To study immunity parameters in the insects, 24 h after the initial exposure to insecticide concentration larvae will be tested to determined activities of phenoloxidase.

C. Recording insect mortality images for Kaplan-Meier graph*

1) The larval mortality will be checked regularly every 24 hr. for a seven day period. Dead larvae are inert and usually turn black.
2) The standardized pathology assay looks at a stage where the larvae stop feeding and the coloration begins to darken and turn eventually black. This stage usually lasts around 5 days.
3) Mortality data will be graphed.

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