Laboratory 5a The Specificity of Antibodies: A Simulation

Inspired by a lab developed by Fred Sculco, Noble and Greenough School, Dedham, MA.

Background

Antibodies recognize foreign molecules, called antigens. They tag and aggregate them for removal from the body (see Figure 5.1). All antibody molecules have a specific three-dimensional structure critical to their function of recognizing and clumping antigens. Each type of antibody has a unique variable region that matches only certain antigens.

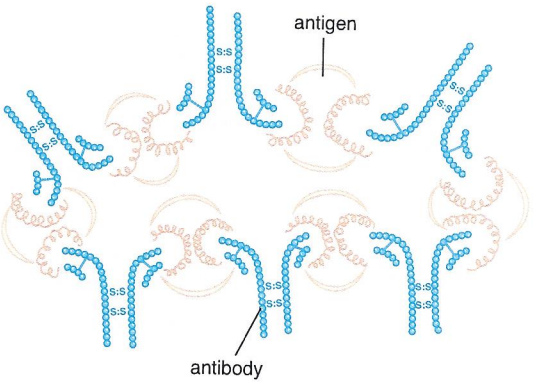

Figure 5.1. Agglutination. Antibodies recognize and clump antigens (agglutination), making it easier for white blood cells (WBCs) to remove the invading particles from the body.

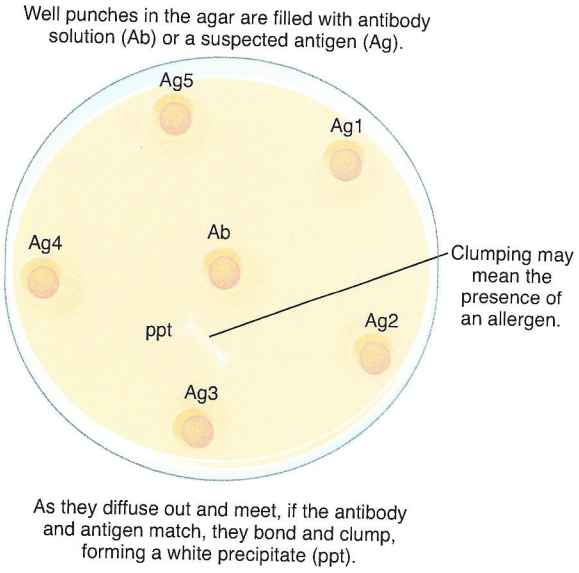

Figure 5.2. Ouchterlony Test. During an Ouchterlony test, a patient's serum (with his or her naturally occurring antibodies) is placed in the center well. Solutions with known antigens are placed in the outer wells. All molecules diffuse. If an antibody molecule finds an antigen, it will clump and fall out of solution (precipitate).

Antigens may be either free-floating proteins or carbohydrate molecules, such as those that cause allergic reactions. More often, antigens are molecules on the surface of cells or viruses that invade the body. Either way, specific antibodies bind with specific antigens and induce an increase in the number of those specific antibodies in the host organism.

Allergens are antigens that specifically induce the formation of immunoglobulin E (IgE) antibodies. An allergic reaction occurs when an excess of IgE molecules stimulate inflammatory response symptoms, such as swelling, redness, and itchiness. You are allergic to the specific antigens that cause this IgE inflammatory response in your body.

When an antigen binds to an antibody molecule, the complex is too small to be seen. However, when hundreds of antibodies bind to hundreds of allergens, they create a network of many millions of molecules (see Figure 5.1). Researchers have used this knowledge to produce tests to identify when a specific antigen is present in a solution.

One method researchers use to test for antigen-antibody binding is called the Ouchterlony test, or Ouchterlony method (see Figure 5.2). To do an Ouchterlony test, an agar matrix is poured into a Petri plate. A hole (well) is punched in the center of the agar, and an antibody-containing solution is added. Suspected antigens are placed in wells evenly spaced between the center and the edge of the plate. The solutions are allowed to diffuse outward from the center of the well.

When antibodies diffuse into antigens, they bind to them and to each other, causing an agglutination (clumping) reaction. The aggregated antibody-antigen precipitates out of solution and may be visible as a white or colored band at the interface of each diffusion front.

The Ouchterlony method may be used in several applications, including allergy testing, to identify a suspected allergen. This test can be used to screen blood serum for the presence of antibodies and, thereby, learn of prior exposure to an antigen. Screening by antibodies, for example, is used in human immunodeficiency virus (HIV) testing. Ouchterlony testing may be used to identify an antigen in a solution, when assaying for a protein in a mixture. In addition, the test may be used to determine whether an antibody will bind to a particular antigen. This technique would be useful if one were looking for an antibody to use for affinity chromatography, a method of protein purification.

Purpose

Rocky is scratching his skin raw because he has a rash. To which allergens does Rocky's blood serum have antibodies?

Photo by author.

Environmental Health and Safety Officer

Materials

LB agar Petri plates 60 × 15mm, sterile, 2/3 full
Permanent lab marker pens
Transfer pipets, 3 mL
Prepared antigen solutions
Rocky's blood serum antibody solution
Pipets, 1 mL and Pipet pump, blue, or P-1000 and tips
Caution: Wear goggles and gloves when using chemicals.

Procedure

1. Obtain three Petri plates containing agar. Label them each with your initials and Trials 1, 2, and 3, respectively.
2. Use a transfer pipet to poke through the agar to plate No. 1 (the bottom plate). Apply a slight suction by compressing it with your fingers (see Figure 5.3). Bore five holes around the edge of the Petri plate (see Figure 5.2). Bore one hole in the middle. Label the wells on the plate bottom with the antigen to be added (see step 4).
3. Repeat Step 2 with the other Petri plates.
4. Obtain the antibody and antigen solutions, one with Rocky's blood serum (containing antibodies) and four with extracts of suspected puppy allergens (such as flea extract, Itchless flea powder, Puppystew dog food, Cleantooth dog biscuits, or Fluffy dog shampoo).
5. Using a different sterile, 1-mL pipet or P-1000 micropipet, fill the five outer wells with the suspected puppy allergen extracts. Fill the central well with Rocky's blood serum.
   Note: Try to use the same volume of antigen and antibody in each well (ie, 200 µL). However, do not overfill the wells since this will cause the samples to mix on top of the agar.
6. Leave the plates, undisturbed, overnight. After 24 hours, a precipitin line will appear between one or more of the puppy allergens and Rocky's serum.
7. Record the results of the Ouchterlony test in the form of scale drawings of the Petri plates and a numerical value (5 = strong precipitation; 0 = no precipitation). Calculate average results.
8. Determine which allergens, if any, appear to give a reaction that could cause Rocky's rash.

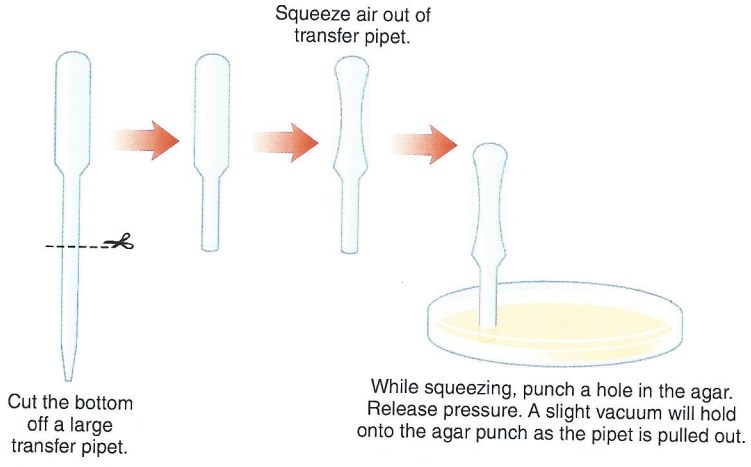

Figure 5.3. Punching Wells for Ouchterlony Test.

Data Analysis/Conclusion

Based on the results of the Ouchterlony test, what recommendations would be made to Rocky's owner? Give evidence for these recommendations. Identify some of the errors in the experimental procedure that could lead to fallacious data. What can be done to decrease the likelihood of these errors occurring? Discuss how antibody-antigen recognition and binding may be used in other applications besides allergy testing.

Thinking Like a Biotechnician

1. How likely is it that one Ouchterlony test will give results that lead to the understanding of an organism's allergic response? Explain.
2. Why is the speed of agglutination or precipitation not a valuable piece of data in this experiment?
3. Setting up an Ouchterlony test may be time consuming. Why not just mix two solutions together to see if they clump? Suggest an advantage to having the molecules diffuse through and precipitate in the agar.

Laboratory 5b The Action of Different Enzymes on Apple Juice Production

Inspired by labs by Louann Carlomagno, formerly of Genencor International, Inc.

Background

Many industries use enzymes to create better products (see Table 5.1). As you know, the dairy industry uses enzymes to speed the curdling of milk in cheese production. Both naturally occurring enzymes, such as rennin from calf stomachs, and genetically engineered enzymes (eg, chymosin) are used now. These enzymes create desirable products, which are sometimes cheaper, faster, and of higher quality than uncatalyzed products. Speeding up the changes that occur during the curdling process increases cheese production. Of course, this means increased sales for the cheese company, and greater profits for the owners and shareholders.

As in all industries, apple juice producers want a cheaper, higher-quality product. One goal of juicers is to extract as much juice as possible from every apple. In the 1980s, scientists at the biotechnology company, Genencor International, Inc., found two enzymes that they believed might possibly increase the amount of juice released from apple cells. The enzymes, called pectinase and cellulase, were created in nature by two different fungi. However, neither fungus grew well in the lab. The scientists decided to genetically engineer some fungi, which do grow

Table 5.1. Examples of Marketed Biotechnology Enzymes

amylase	breaks down starch to sugar; used by fabric and beverage industries
pectinase	degrades the cement between plant cells and softens plant fibers; used in paper-making, coffee/tea extraction
cellulase	decomposes cellulose in plant fiber and breaks down cells; used in the paper-making and textile industries
subtilisin	protein-digesting enzyme; used in detergents to remove protein stains
Purafect® Prime L protease (Genencor International, Inc)	protein-digesting enzyme
rennin	protein-digesting enzyme; curdles milk for making cheese