



## **Student Activities**

### **Adherence: What Sticks Can Make You Sick**

Bacterial attachment to cells within our body is a crucial first step to causing infection or disease. In this exercise you will learn one of the ways scientists use to measure the attachment or adherence of bacteria to human cells. You will then use what you have learned to identify specific attachment sites (receptors) to which pathogens bind.

#### **Host Defenses**

Start by reviewing what you know about the defenses of the following parts of your body. What ways might your body use to try to keep microbes from infecting different body areas like the intestines and throat? You may want you to use the following web sites to help you.

##### Intestines

<http://www.colorado.edu/Outreach/BSI/k12activities/interactive/actidhpsstomach.html>

##### Throat and Bronchi

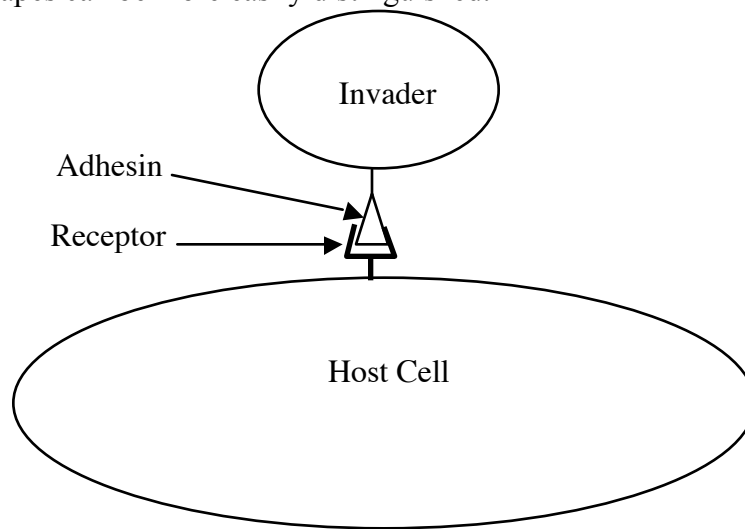
<http://www.colorado.edu/Outreach/BSI/k12activities/interactive/actidhpsmucociliary.html>

Note that both the intestines and the throat and bronchi have many defenses to prevent potential invaders from attaching. Our normal flora plays a big role in this. There are so many bacteria in our intestines that the entire surface is completely covered with bacteria leaving no space for potential invaders to get in. In addition, our intestines are constantly contracting in a process called peristalsis. Invading bacteria are then unable to attach to our cells and are immediately swept out of the intestines. Our upper respiratory tract is covered with a mucus layer to help prevent invading organisms from attaching to our cells. Cilia of bronchi and nose act as a mucociliary escalator pushing trapped bacteria up and out of our body.

Unfortunately for us, disease-causing bacteria sometimes do find a way to attach or adhere to the host's cells thereby completing the first step needed for an infection to occur. Scientists now have an explanation for how this attachment can occur. To better understand how a bacterium can become attached to its host's cells, watch the DVD *2000 and Beyond: Confronting the Microbe Menace*, Lecture Three – Outwitting Bacteria's Wily Ways, Chapters 4 through Chapter 22. Here you will see how a diarrhea causing, intestinal bacterium, *E. coli*, can become attached to our intestinal cells

## Attachment

Attachment is a very specific process. Potential invaders do not just bind randomly to any surface using a general sort of glue. This would be ineffective since microbes would then be stuck to surfaces other than the host they were trying to infect. Rather microbes have specific molecules on their surface, called **adhesins**, which can bind to specific **receptors** on the host cell. The way that adhesins of microbial invaders bind to host cell receptors is analogous to a key fitting into a lock. A specific adhesin (or key) will only recognize and bind to a specific receptor (or lock) that possesses the correct complimentary shape. The adhesin on the invader below has a specific triangular shape that fits into the receptor on the host cell. The cells and molecules in the drawings in this activity are not drawn to scale so that specific molecular shapes can be more easily distinguished.



A key characteristic of molecules that serve as either adhesins or their receptors is that they have a very unique and specific three-dimensional structure that allows a lock-in-key fit between the two molecules. If the pathogen adhesin “key” fits the host receptor “lock”, then that pathogen binds to that host cell and the first step of an infection has been completed.

Adhesins on microorganisms are often proteins, although they can also be polysaccharides. Viral adhesins are most often proteins that are part of the viral capsid or protein shell. Bacterial adhesins are often fimbriae (sometimes called pili), which are long, thin, protein filaments. In bacteria, other surface proteins also serve as adhesins and rarely polysaccharides of the bacterial capsule may serve as bacterial adhesins as well. Adhesin molecules evolved specifically to allow a pathogen to bind to host cells.

Host cell receptors can also be either proteins or carbohydrates. However, host cell receptors did not evolve in order to allow pathogens to bind to them. Rather host cell receptors serve some useful function for the host cell, and the pathogen takes advantage of this receptor molecule in order to attach. For example, HIV attaches to a molecule called CD4 on the surface of T-cells. The CD4 molecule plays an important role in helping the T-cell function as an immune system cell by serving as a signal to other immune system cells with which the T-cell interacts.

Some examples of pathogens, the adhesins they use to attach, and the specific receptors they bind to are shown in the table below

Pathogen	Adhesin	Host Cell Receptor
Influenza virus	Hemagglutinin	Neuraminic Acid
<i>Plasmodium vivax</i> (malaria)	Merozoite protein	Duffy blood group antigen
Rhinovirus (common cold)	Capsid protein	ICAM-1
<i>Haemophilus influenza</i> (meningitis)	HifE fimbriae	Sialylyganglioside-GM1
<i>Klebsiella pneumoniae</i>	MrkD fimbriae	Type V Collagen
HIV	Gp120 protein	CD4

The specificity of an adhesin for a particular receptor in the attachment process can be a possible explanation for both host range and tissue tropism.

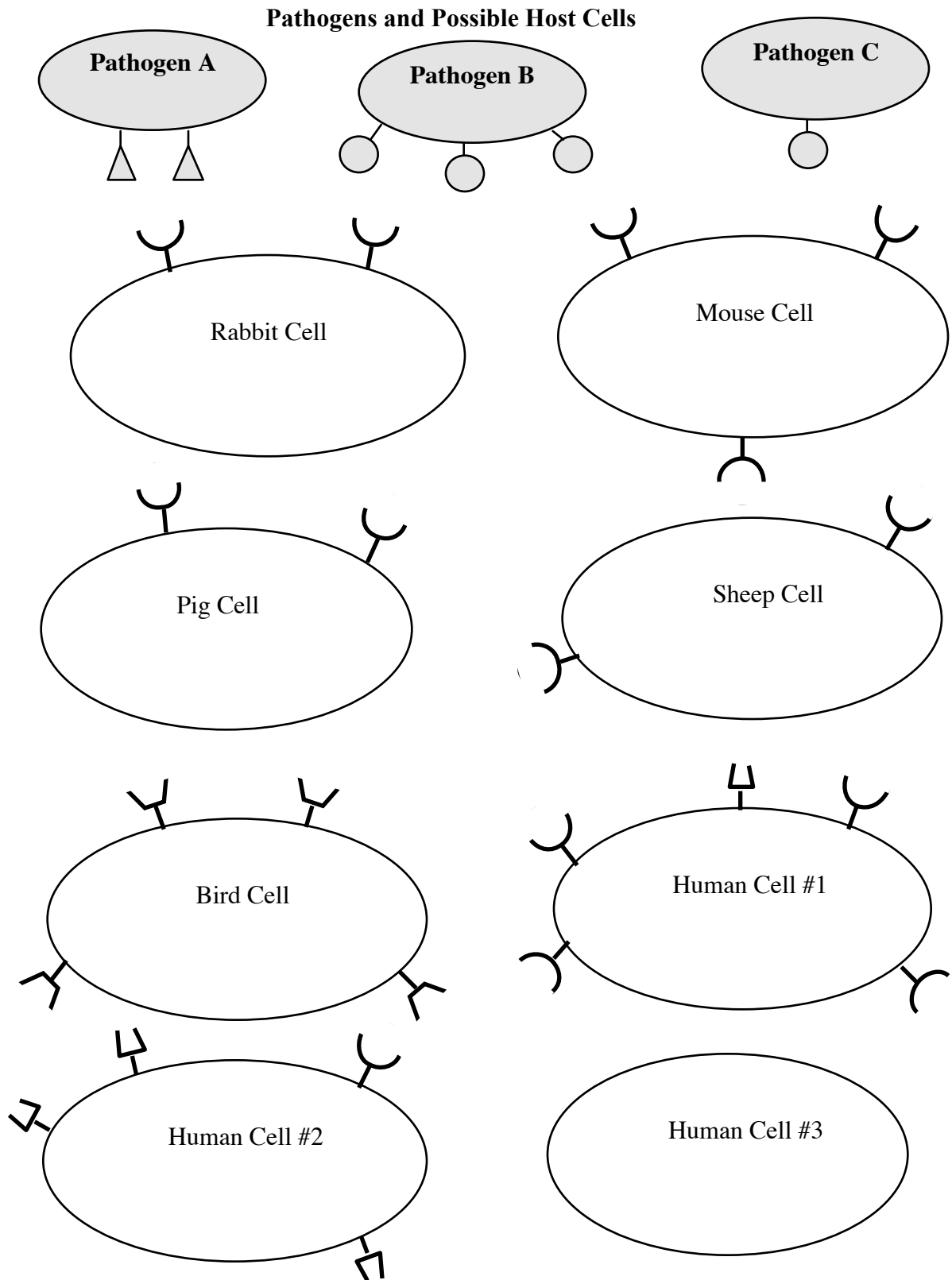
**Host range** refers to the different host species that a particular pathogen can infect. One of the factors limiting host range is the presence of the specific receptor to which the pathogen can bind. Some pathogens have a very **narrow host range** and can infect only one host species. Examples of diseases with a narrow host range infecting only humans are smallpox and polio. Other pathogens can infect many different host species and are thus said to have a **broad host range**. Examples of diseases that infect more than one species are *Yersinia pestis* (bubonic and pneumonic plague) which infects both humans and many different rodent species, and rabies virus which infects humans, dogs, and several different rodents.

Not all tissues within an infected individual are directly affected by a pathogen. **Tissue tropism** refers to the different tissues within a given host that are infected by a pathogen. For example, cold viruses infect only cells or tissues of the upper respiratory tract. Attachment is one of several factors that determine tissue tropism. Some pathogens can bind only one type of tissue. Other disease pathogens can attach to several different tissue types within given host.

Similarly, variations in both hosts and pathogens lead to differences in virulence or how sick we may become. For example, different human patients may produce different levels or amounts of receptor proteins on their cell surfaces. These different levels of expression for surface receptors for the same pathogen can lead to varying levels of susceptibility to that pathogen. Individuals completely lacking a given receptor will consequently be resistant to infection by that pathogen. Other individuals may express high levels of a receptor and thus be highly susceptible to infection by the pathogen with the complimentary adhesin. Similarly, different strains of the same pathogen may express different levels of adhesin on their surfaces leading to different levels of virulence for each strain. Adherence is one of several factors contributing to tissue tropism.

**Assignment 1. Adhesins and Their Receptors**

Study the adhesions and surface receptors on the cells in these diagrams, then answer the questions that follow.



Use the adhesions and surface receptors on the cells in the previous diagrams to answer these questions about host range.

1. Which species will Pathogen A bind? Explain.
2. Which species will Pathogen B adhere or bind? Explain.
3. Which species will Pathogen C adhere?
4. Which pathogen has the broadest host range? Explain.
5. Which pathogen has the most narrow host range? Explain.
6. Can pathogen A infect mice? Explain your answer.
7. Describe any interactions between bird cells and the given pathogens.
8. Draw the pathogen that would infect the bird cells.

What factors determine the shape of this pathogen?

9. Which species can be infected by the greatest number of pathogens? Explain.
10. Examine Human Cells #1, #2, and #3. Let's assume (for questions 10 through 17) that these cells came from three different individuals. Which individual do you predict to be more susceptible to infection with pathogen A – individual #1 or individual # 2? Explain your answer.

11. Decide whether each individual is resistant or susceptible to the pathogens. Mark your decisions in this table.

Human Cell	Exposure to Pathogen		
	A	B	C
#1			
#2			
#3			

12. What factors influenced your decision to rank a host cell as resistant to a specific pathogen?
13. What factors influenced your decision to rank a host cell as susceptible to a specific pathogen?
14. Compare pathogens B and C. Which would you consider more virulent and why?
15. Examine Human Cell #3. In terms of infection, what advantage does this individual have over the other two individuals? How might this advantage affect the survival of this individual?
16. Remember that host cell surface receptors serve some useful function for the cell. These receptors may admit some necessary molecule into the cell or may detect molecules in their surroundings that help the cell respond to changes – like the need to divide if a slight injury occurs. Will an individual who lacks a specific receptor be more likely or less likely to survive? Explain.
17. Why would it be an advantage for a species to have variability in the number and types of surface receptors among its individuals?
18. Reexamine Human Cells # 1, #2, and #3, only this time assume that each is from different organs of the same individual. Let's assume that Human Cell #1 is a cell lining the nasal passages, Cell #2 is a cell lining the lungs, and Cell #3 is a muscle cell.

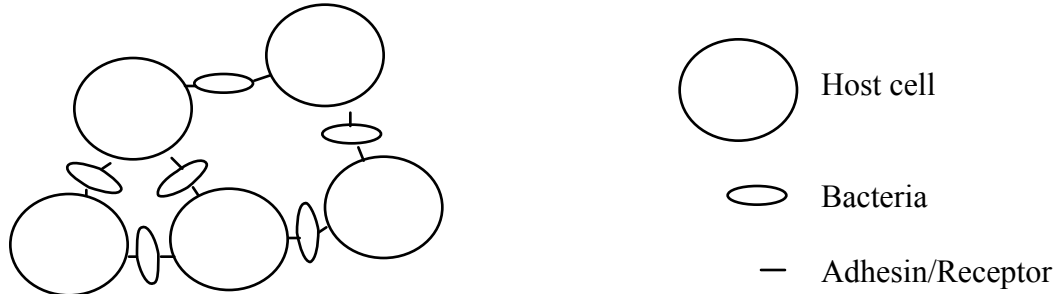
According to what you know about adhesins and receptors, decide whether each cell type can become infected or will remain uninfected when exposed to each pathogen. We will also assume that each cell can be infected once adhesion occurs. Note your decisions in this table.

Human Tissue Cell	Probability of infection by Pathogen		
	A	B	C
#1 lining nasal passage			
#2 lining the lungs			
#3 muscle cell			

19. If you will recall, individuals may exhibit tissue tropism – different organs may be infected by a pathogen depending upon the receptors that they possess. Describe the symptoms of this individual if infected with Pathogen A.
  
20. Compare the symptoms in the same individual if infected by Pathogen B.
  
21. Today many researchers are using computers to model the shape of important biological molecules, like adhesins and receptors, in order to predict their functions and interactions within living organisms. Let's say that you work for a drug company that is interested in developing a treatment to combat Pathogen A. Draw and describe the shape of a molecule that you would investigate as a possible drug to interfere with the adhesin of this pathogen.

## Assignment 2. Detecting Adherence through Agglutination

In this activity you will test the ability of different strains of *E. coli* to adhere to human intestinal cells. The type of reaction you will be performing is called an agglutination reaction. If bacteria can attach to the host cell, it will cause what is known as **agglutination**, or clumping of the host cells. Since the shape of the bacterial adhesin compliments the shape of the host receptor, large complexes of bacteria and host cells adhering to each other (see the sketch below). Agglutination produces clumps that are visible to the naked eye. Agglutination reactions are used as a good first test for determining whether a particular bacterial strain or species can bind to a particular host species or cell type within a host.



### Procedure

1. Obtain a well slide or well plate with circular indentations or wells. Number each well from #1 through # 6 to identify each.
2. In each of the six wells, place one drop of the solution labeled “Suspension of human intestinal cells”.
3. Add a drop of bacteria #1 to well #1. Mix with a toothpick.
4. Repeat by placing bacteria #2 through #5 into their assigned wells. Mix with a different toothpick each time.
5. Note your results in the table below. If the bacteria were able to attach to the intestinal cells, they will cause them to clump.

<i>E.coli</i> Strain	Agglutination Observed? (yes or no)	Ability to Attach (yes or no)
1		
2		
3		
4		
5		
None (negative control)		

1. Which strains were able to adhere to intestinal cells?
2. Experiments may include different controls. **Positive controls** generally include all of the variables being tested while **negative controls** exclude these variables. A **procedural control** may be used to ensure that any observed differences are due to the variables instead of the experimental procedure. Why were the intestinal cells of well #6 considered to be the negative control?
3. If you had observed agglutination in well 6 (intestinal cells only), what would you be able to conclude about the attachment of the different *E. coli* strains to intestinal cells? Explain your answer.
4. What additional controls would you add?

In the following exercise you will use the agglutination reaction to identify the specific molecule acting as the host cell receptor for a pathogen or to identify which adhesin/receptor combination is responsible for adherence.

### **Assignment 3. Using Agglutination to Identify Adhesins or their Receptors**

You will be assigned one of four scenarios. For each scenario read about a pathogen and what is known about its adherence, adhesins, and receptor molecules. Each scenario also explains techniques used to identify receptors. Your job is to design an experiment to identify the host cell receptor or its adhesin, perform the experiment, analyze the data, and present your results to the class.

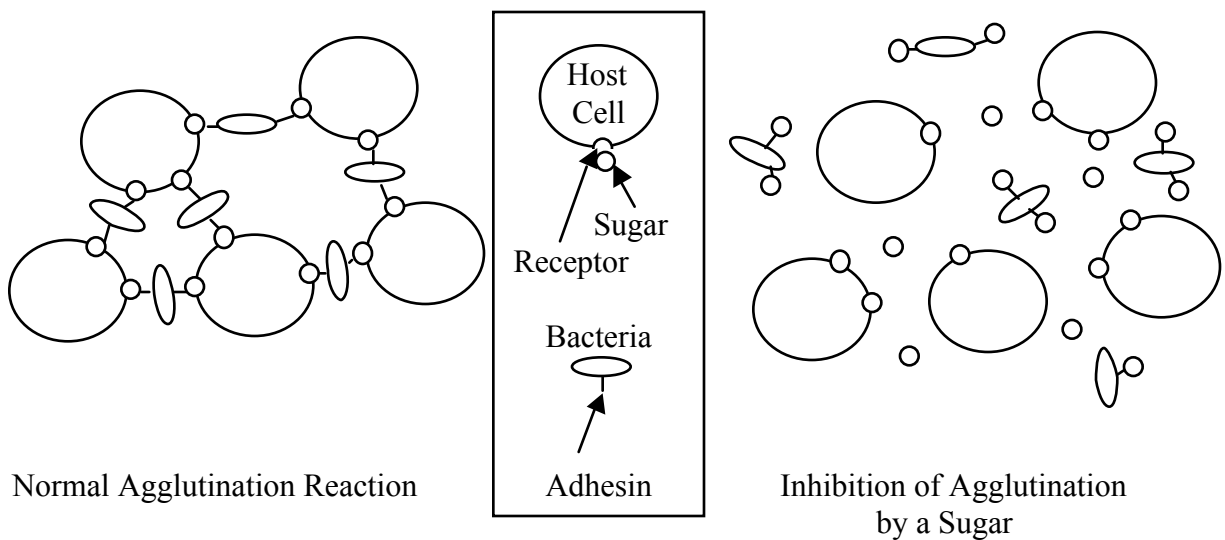
You will use the scientific method when performing these experiments. A review of the scientific method follows Scenario D. Place the answers to these questions on a separate sheet of paper.

1. According to your scenario, what question will your investigation address?
2. Write your hypothesis (or hypotheses).
3. Write your prediction(s).
4. Design an experiment to answer your question.
5. Make a table to record your results, and then perform the experiment.
6. Below write a brief paragraph summarizing how your results support or falsify your hypothesis. If necessary, include how you would repeat or redesign your experiment to answer the intended question.

### Scenario A – Identifying the receptor for *E. coli* on human bladder cells.

Strains of *E. coli* that cause urinary tract infections have Type I fimbriae that are known to be the adhesin by which these bacteria attach to bladder cells. The specific part of the fimbriae involved in this attachment is called FimH. It is known that FimH binds to a sugar containing receptor. Your goal is to identify the sugar that is serving as the receptor.

A technique commonly used to identify structures serving as receptors is **agglutination inhibition**. In this type of experiment, molecules that may be a part of the receptor are tested for their ability to prevent agglutination. Sugars that are part of the receptor will inhibit agglutination. When added to the agglutination reaction, these sugars bind to the pathogen's adhesin, making the pathogen unable to bind to the receptor of the host.



As shown above, sugars that serve as the receptor on the host cell will inhibit agglutination by binding to all the adhesin on the bacteria. Once all the adhesin is bound, it can no longer bind to the receptor on the host cells, and will no longer be able to agglutinate the host cells.

You will design an experiment to test which of the four following sugars – fructose, galactose, glucose, or mannose – is serving as the receptor for attachment of *E. coli* to bladder cells.

You have the following solutions to work with:

- Bladder cells
- E. coli* with Type I fimbriae
- Fructose
- Galactose
- Glucose
- Mannose

Other available supplies:

- Toothpicks
- Trays with indentations

## **Scenario B – Identifying *Bordetella pertussis* adhesins that bind to bronchial cells.**

*Bordetella pertussis* causes whooping cough. Studies using techniques similar to agglutination tests have shown that many of the molecules on the surface of this bacterium bind to various receptors on human cells.

<u>Adhesin</u>	<u>Receptor(s)</u>
Fimbriae	VLA-5
Filamentous Hemagglutinin integrin	carbohydrates
Pertussis Toxin	heparin
Hemolysin	unknown
Adenylate Cyclase	unknown

Although it is known that many of molecules found on the surface of *B. pertussis* can bind to host cells, it is not known which of these several molecules is most important for attachment. To study the relative importance of the different surface molecules of *B. pertussis*, scientists have generated mutant strains of *B. pertussis*, with each strain lacking one potential adhesin. By comparing the binding of these different mutants, scientists can determine which adhesin(s) is(are) most important. Your goal is to determine which of the potential adhesins *B. pertussis* uses to adhere to bronchial cells.

You have the following solutions to work with:

- Bronchial Cells (NCI-H292)
- Wild Type *Bordetella* (non-mutant)
- Fimbriae mutant
- Filamentous Hemagglutinin mutant
- Pertussis Toxin mutant
- Hemolysin mutant
- Adenylate Cyclase mutant

Other available supplies:

- Toothpicks
- Trays with indentations

When you have completed your study, compare your results to those with students working with Scenario C - Identification of adhesin responsible for the binding of *Bordetella pertussis* to laryngeal cells.

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You have the following solutions to work with:

- Laryngeal Cells (HEp-2)
- Wild Type *Bordetella* (non-mutant)
- Fimbriae mutant
- Filamentous Hemagglutinin mutant
- Pertussis Toxin mutant
- Hemolysin mutant
- Adenylate Cyclase mutant

Other available supplies:

- Toothpicks
- Trays with indentations

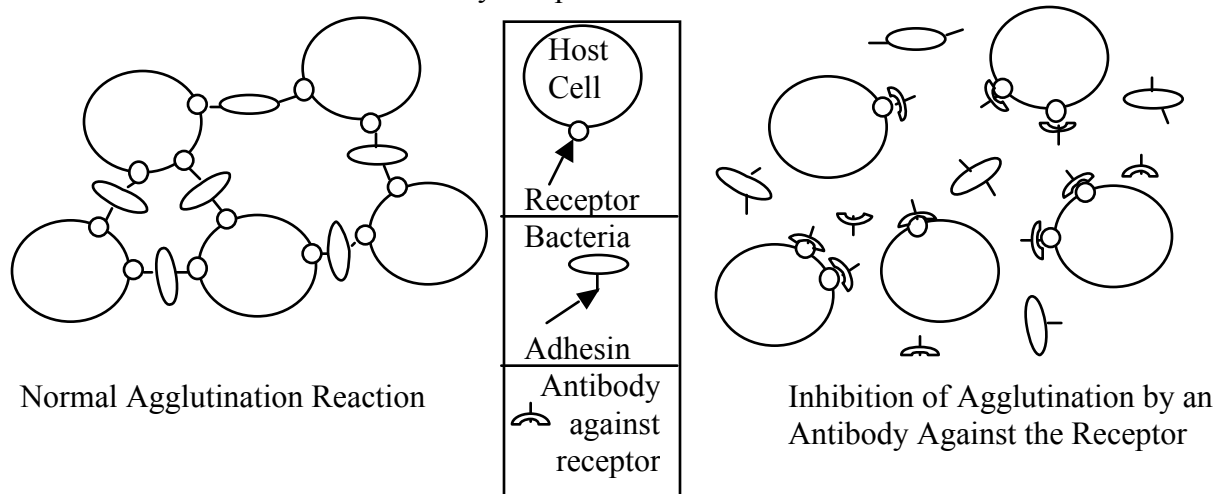
When you have completed your study, compare your results to those with students working with Scenario B - Identification of adhesin responsible for the binding of *Bordetella pertussis* to bronchial cells.

### **Scenario D – Identifying a coreceptor using antibodies to inhibit agglutination.**

It is known that early in infection with HIV, the virus infects primarily macrophages. As infection progresses, the HIV gradually infects more T cells and less macrophages. As more and more T cells are infected and destroyed, the patient's immune system collapses.

It is also known that the receptor for HIV is CD4, a molecule found primarily on a subclass of T cells. This molecule is also found on macrophages. It is also known that additional molecules serve as a receptor for HIV. By studying individuals at high risk for HIV who did not seem to become infected with the virus, scientists identified a coreceptor molecule – a second molecule to which HIV binds. This molecule, CCR5 (chemokine receptor 5), is found on macrophages but not T cells. Your goal is to determine what molecule is serving as the HIV coreceptor on T cells.

One method scientists use to identify receptors is to block adherence with antibodies.



As shown in the second drawing, antibodies that are specific for the molecule serving as the receptor on the host cell will inhibit agglutination by binding to all the receptor sites on the host cells. Once all the receptors are bound, they can no longer bind to the adhesin on the pathogen. Thus, the pathogen will no longer be able to agglutinate the host cells.

Your goal is to determine which of the three following molecules – CD3, CCR5, or CXCR4 - is serving as the receptor for attachment of HIV to T cells. CD3 is an immune system marker found on T cells. CCR5 and CXCR4 are both chemokines, small molecules that signal the immune system.

You have the following solutions to work with:

- HIV (fake of course)
- Anti-CD4
- Anti-CD3
- Anti-CCR5
- Anti-CXCR4
- T cells

Other supplies provided:

Toothpicks

Trays with indentations

## **A Review of the Scientific Method**

The following review of the scientific method may help you design your experiment. The scientific method follows the steps listed below.

1. Choose a question.
2. Design a hypothesis to test a possible answer to that question.
3. Make testable predictions based on the hypothesis.
4. Design an experiment to answer the question and see whether the predictions are met.
5. Perform the experiment and collect data.
6. Decide whether the data support or falsify the hypothesis.
7. Present your scenario to the class.

### **Step 1 – Choose a question**

In each case, your question has already been chosen for you by the scenario that you have been assigned. Read your scenario carefully before proceeding to the next step. Base all the following steps on your scenario.

### **Step 2 – Design a hypothesis**

Design a hypothesis related to your question. Remember that a hypothesis is a possible answer to the question. A hypothesis is also often described as an educated guess. A guess, because you do not know in advance whether your hypothesis will be correct or not, and educated because you base it on the knowledge you already have.

You can also design a series of hypotheses (different possible answers) for the question. This technique is often used because one way of adding support to a hypothesis is to falsify other possible hypotheses (answers).

For example, for the previous experiment in Assignment 2, you used agglutination reactions to determine which *E. coli* strains can adhere to intestinal cells. Some possible hypotheses for this experiment would be:

*Strain 1 will adhere to intestinal cells.*

*Strain 1 will not adhere to intestinal cells.*

*Strain 2 will adhere to intestinal cells.*

### **Step 3 - Make predictions based on your hypothesis.**

Predictions are experimental outcomes that will be true if your hypothesis is correct. Predictions can easily be written as if – then statements.

For example for the first hypothesis above a prediction would be

*If E. coli strain 1 can bind intestinal cells, then agglutination will be seen when intestinal cells are mixed with E. coli strain 1.*

#### **Step 4 – Design an experiment to answer your question**

In this case you will design an experiment using an agglutination reaction to answer your question. Be sure to include controls that will show that your reagents are good and that the procedure is working.

For example, for the experiment presented in part I – good controls would be:

1. *Intestinal cells alone (negative control to show that intestinal cells alone do not agglutinate)*
2. *E. coli strain 1 alone (negative control to show that E. coli alone do not agglutinate)*
3. *Intestinal cells plus an E. coli strain known to bind intestinal cells (positive control to show the agglutination reaction works for a strain known to adhere).*

#### **Step 5 – Perform you experiment and collect your results.**

Make a table similar to the one used in Assignment 2 to note the results of this experiment, including results to all controls.

#### **Step 6 – Decide whether your results support or falsify your hypothesis.**

The results of an experiment never prove a hypothesis. Hypotheses can never be proven. Instead, experimental results support hypotheses.

Hypotheses can, however, be falsified. Results can definitely show that a hypothesis is false. Note that it is not considered bad or a failure to prove a hypothesis false. By proving a hypothesis false, you eliminate one possible answer to your question.

Often the results of an experiment neither support nor falsify a hypothesis. There are many ways in which this happens.

- Controls show that one or more of the reagents were not working.
- Controls show that the experiment was not designed to answer the intended question.
- Results show that the experiment was not designed to answer the intended question.

When the results of an experiment neither support nor falsify a hypothesis it is necessary to repeat the experiment (if there is a problem with the reagents), redesign the experiment (if it is not answering the intended question), or sometimes even to rethink the question and hypothesis. As was the case with falsifying hypotheses, this is not bad science or a failure. Rather it is good science to recognize the problems with experiments and questions and to repeat and redesign them as necessary.