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**Overview of Activity**  
**Adherence: What Sticks Can Make You Sick**

1. **Summary of Activity.**

Using visual diagrams, students explore the relationship of host surface cell receptors and adhesin molecules located on pathogen surfaces for adherence or the first step in infection. Using the process of scientific inquiry, students will propose their own investigations into pathogenic adherence using the laboratory technique of agglutination or the clumping of host cells to pathogens capable of adherence. To prevent student exposure to pathogens, a precipitation reaction has been used to mimic agglutination reactions.

2. **Intended Grade Level.**

Assignment 1 - Adhesins and Their Receptors is intended for high school biology classes. Assignment 2. Detecting Adherence through Agglutination and Assignment 3. Using Agglutination to Identify Adhesins or their Receptors are recommended for AP or advanced biology classes, or for anatomy and physiology classes.

3. **Recommended Prior Knowledge.**

Student understanding of the following terms would be helpful.

- virus
- bacterium
- immunity
- host
- pathogen

4. **Background information.**

Bacterial attachment to cells within our body is a crucial first step to causing infection or disease. Attachment is a very specific process. Microbes have specific molecules on their surface, called **adhesins**, which can bind to specific **receptors** on the surface of the host cell. The way that adhesins of microbial invaders bind to host cell receptors is analogous to a key fitting into a lock. The specificity of this fit determines the range of host cells susceptible to infection by a particular strain of pathogen. The Howard Hughes Medical Institute Holiday Lecture Series: *2000 and Beyond, Confronting the Microbe Menace* wonderfully illustrates this specificity in Lecture Three – “Outwitting Bacteria’s Wily Ways”.

The technique of agglutination utilizes the adherence of pathogenic adhesins to host cell receptors to determine the range of host cells susceptible to a particular pathogenic strain. When adhesins and receptors bind together, host cells and pathogens clump together in a manner that can be easily seen. These 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.

## 5. **Materials list.**

### **I. Assignment 1. Adhesins and Their Receptors**

- A. Student copies of Bacterial Adherence: Identifying Host and Tissue Specificity through Agglutination, drawings of Pathogens and Possible Host Cells, and questions for Assignment 1.
- B. An overhead transparency of Pathogens and Possible Host Cells is also very helpful. Pathogens and Host Cells can be kept on one page or cut into individual pieces for manipulating on the overhead projector.

### **II. Assignment 2. Detecting Adherence through Agglutination**

- A. Student Copies of Assignment 2.
- B. 500 mL of each of the following solutions
  - Solution A – 1 M MgSO<sub>4</sub>
  - Solution B – 0.5 M NaOH
  - Solution C – water
  - Solution D – 1 M HCl For each student group

Note: These same solutions are used for Assignment 3.

- C. For each student group:
  - Well plates or well depression slides with 6 wells
  - 6 toothpicks

- Dropper bottles with the following labels
- Suspension of Human Intestinal Cells
  - E. coli* Strain 1
  - E. coli* Strain 2
  - E. coli* Strain 3
  - E. coli* Strain 4
  - E. coli* Strain 5

Note – For a list of specific solutions in each dropper bottle, refer to Teacher Preparation instructions, below.

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

- A. Student Copies of Assignment 3.
- B. Solutions A, B, C, and D from Assignment 2
- C. For each student group:
  - Well plates or well depression slides with 6 wells
  - 6 toothpicks

- Dropper bottles with the following labels
- |                   |               |
|-------------------|---------------|
| <b>Scenario A</b> | Bladder cells |
|-------------------|---------------|

	<i>E. coli</i> with Type I fimbriae
	Fructose
	Galactose
	Glucose
	Mannose
<b>Scenario B</b>	Bronchial Cells (NCI-H292)
	Wild Type <i>Bordetella</i> (non-mutant)
	Fimbriae mutant
	Filamentous Hemagglutinin mutant
	Pertussis Toxin mutant
	Hemolysin mutant
<b>Scenario C</b>	Adenylate Cyclase mutant
<b>Scenario D</b>	Laryngeal Cells (HEp-2)
	HIV
	Anti-CD4
	Anti-CD3
	Anti-CCR5
	Anti-CXCR4
	T cells

Note – For a list of specific solutions in each dropper bottle, refer to Teacher Preparation instructions, below.

#### IV. Teacher Preparation for Assignments 2 and 3

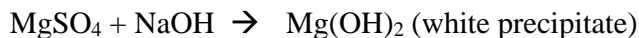
To prevent student exposure to pathogens, a precipitation reaction has been used to mimic agglutination reactions.

##### A. Prepare solutions

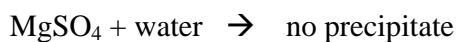
Make 500 mL of each solution below

Solution A – 1 M MgSO<sub>4</sub>

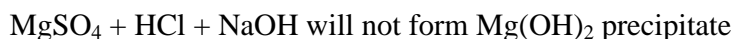
Solution B – 0.5 M NaOH



Solution C – water



Solution D – 1 M HCl



(A brief precipitate may form upon addition of NaOH before mixing with the toothpick)

### **B. Assignment 2. Detecting Adherence through Agglutination**

Prepare dropper bottles with the following labels and solutions for each student group.

<b>Bottle Label</b>	<b>Solution</b>
Suspension of Human Intestinal Cells	A - MgSO <sub>4</sub>
<i>E. coli</i> Strain 1	B - NaOH
<i>E. coli</i> Strain 2	B - NaOH
<i>E. coli</i> Strain 3	C - water
<i>E. coli</i> Strain 4	B - NaOH
<i>E. coli</i> Strain 5	C - water

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

1. Distribute solutions A-D into bottles as follows. Very little solution is needed in each bottle.
2. Label each bottle with the letter for each scenario and the name of the solution it is supposed to contain. **One** of each dropper bottle is needed.

#### **Scenario A**

<b>Bottle Label</b>	<b>Solution</b>
Bladder cells	A - MgSO <sub>4</sub>
<i>E. coli</i> with Type I fimbriae	B - NaOH
Glucose	C - water
Mannose	D - HCl
Galactose	C - water
Fructose	C - water

#### **Scenario B**

<b>Bottle Label</b>	<b>Solution</b>
Bronchial Cells NCI-H292	A - MgSO <sub>4</sub>
Wild Type Bordetella	B - NaOH
Fimbriae mutant	B - NaOH
Filamentous Hemagglutinin mutant	C - water
Pertussis Toxin mutant	B - NaOH
Hemolysin mutant	B - NaOH
Adenylate Cyclase	B - NaOH

### Scenario C

Bottle Label	Solution
Laryngeal Cells Hep-2	A - MgSO <sub>4</sub>
Wild Type Bordetella	B - NaOH
Fimbriae mutant	C - water
Filamentous Hemagglutinin mutant	C - water
Pertussis Toxin mutant	B - NaOH
Hemolysin mutant	B - NaOH
Adenylate Cyclase mutant	B - NaOH

### Scenario D

Bottle Label	Solution
T-cells	A - MgSO <sub>4</sub>
HIV	B - NaOH
Anti-CD3 antibody	C - water
Anti-CD4 antibody	D - HCl
Anti-CD5 antibody	C - water
Anti-CRCX4 antibody	D - HCl

## 6. Activity Outline.

- I. Assignment 1 - Adhesins and Their Receptors for all biology students:
  - A. Introduce bacterial adherence using handouts Bacterial Adherence: Identifying Host and Tissue Specificity through Agglutination, and HHMI Holiday Lecture Series: *2000 and Beyond, Confronting the Microbe Menace* DVD: Lecture Three – “Outwitting Bacteria’s Wily Ways”.
  - B. Use diagrams of “Pathogens and Possible Host” cells to answer questions. Students may answer questions individually or in groups. Another option is to prepare an overhead transparency of these diagrams and address questions through whole class discussion.
  
- II. Assignment 2. Detecting Adherence through Agglutination for all biology students:
  - A. Students read and follow the procedure on agglutination to determine which *E.coli* strain adheres to intestinal cells.
  - B. Discuss the value of controls in an experimental procedure.
  
- III. Assignment 3. Using Agglutination to Identify Adhesins or their Receptors for AP and advanced biology students and classes of anatomy and physiology
  - A. Students are assigned different scenarios for using the technique of agglutination to determine adherence between various host cells and

pathogenic adhesins. Students design experimental investigations in groups or individually.

- B. Using the technique of agglutination, students carry out their investigation and determine the specific host receptor – pathogen adhesin combination that leads to the first step in infection.
- C. Students analyze their results and present their scenario, experimental design, and results to classmates.

## **7. Teacher Answer Key**

### **Suggested answers to questions 1-21, Assignment 1. Adhesins and Their Receptors**

1. Which species will Pathogen A bind? Explain.

*Pathogen A binds only to Human cells because its adhesin is complimentary (fits) to the receptor of that cell.*

2. Which species will Pathogen B adhere or bind? Explain.

*Pathogen B binds to all species except the bird because its adhesin fits receptors on every cell but that of the bird.*

3. Which species will Pathogen C adhere?

*Pathogen C can also adhere to all species except the bird.*

4. Which pathogen has the broadest host range? Explain.

*Both pathogens B and C adhere to the greatest number of host cells so they have the broadest host range.*

5. Which pathogen has the most narrow host range? Explain.

*Pathogen A has the narrowest host range since it can adhere to only the human cells.*

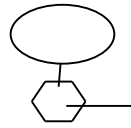
6. Can pathogen A infect mice? Explain your answer.

*Pathogen A cannot infect mice because the mouse does not have surface receptors for the adhesin of Pathogen A.*

7. Describe any interactions between bird cells and the given pathogens.

*The given pathogens would not infect the bird because none of the pathogen adhesins.*

8. Draw the pathogen that would infect the bird cells.



*Lower shape is critical to drawing.*

What factors determine the shape of this pathogen?

*The shape of the adhesin must fit into the shape of the receptor on the bird cell.*

9. Which species can be infected by the greatest number of pathogens? Explain.

*Human cells have receptors that can bind with the adhesins of pathogens A, B, or C so they can be infected by all three of these.*

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.

*Individual #2 has more surface receptors to the adhesin on pathogen A and would be more likely to get infected by this pathogen than individual #1.*

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	<i>Resistant</i>	<i>Susceptible</i>	<i>Susceptible</i>
#2	<i>Susceptible</i>	<i>Susceptible</i>	<i>Susceptible</i>
#3	<i>Resistant</i>	<i>Resistant</i>	<i>Resistant</i>

12. What factors influenced your decision to rank a host cell as resistant to a specific pathogen?

*A resistant host cell would have no receptor for a pathogen's adhesin.*

13. What factors influenced your decision to rank a host cell as susceptible to a specific pathogen?

*A susceptible host cell would have a surface receptor that matched a pathogen's adhesin.*

14. Compare pathogens B and C. Which would you consider more virulent and why?

*Pathogen B is more virulent since it has more adhesins on its surface.*

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?

*Since Human Cell #3 has no surface receptors that match the adhesins for these pathogens, this individual is unlikely to become infected and will have a greater chance for survival.*

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.

*Answers will vary, but generally those answers that state that individuals who lack a certain receptor are more likely to survive should have resistance to bacterial infections as support for their answers. Those students who state that these individuals will be less likely to survive should have inability of cells to respond to environmental changes or lack of necessary molecules as support for their position.*

17. Why would it be an advantage for a species to have a variability of surface receptors among its individuals?

*Some members of a species would survive if a particularly virulent or deadly infection were to occur.*

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	Infection by Pathogen		
	A	B	C
#1 lining nasal passage	<i>Can be infected</i>	<i>Can be infected</i>	<i>Can be infected</i>
#2 lining the lungs	<i>Can be infected</i>	<i>Can be infected</i>	<i>Can be infected</i>
#3 muscle cell	<i>Remains uninfected</i>	<i>Remains uninfected</i>	<i>Remains uninfected</i>

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.

*This individual will suffer symptoms of the nasal passage and lungs, with the lungs possibly having greater symptoms.*

20. Compare the symptoms in the same individual if infected by Pathogen B.

*This individual will also suffer from symptoms of the nasal passage and lungs, but this time the nasal passage may have greater symptoms.*

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.

*Drawings and descriptions should include shapes that are complementary to the triangular shape of this adhesin, thereby coating it and interfere with the "fit" of this adhesin to the host cell receptor.*

## Suggested answers to Assignment 2. Detecting Adherence through Agglutination

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

1. Which strains were able to adhere to intestinal cells?

*Strains 1, 2, and 4 adhered 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?

*The intestinal cells lacked any of the different bacteria, making it 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.

*Nothing! If intestinal cells alone adhered to each other, it would be impossible to tell whether the agglutination observed in the other wells was due to the bacteria, or simply intestinal cells adhering to each other.*

4. What additional controls would you add?

*It would be good to test the agglutination of each bacteria strain to itself. However, this control for the procedure is not absolutely essential since you can look at the bacterial cells in the test tube or dropper bottle and see that there is no agglutination.*

## Suggested answers to Assignment 3.

Using Agglutination to Identify Adhesins or their Receptors

Please note that the possible hypotheses, predictions and experimental designs are only a partial listing. Students may propose others.

### 1. Scenario A

### Question

Which sugar on the surface of bladder cells is serving as the receptor for *E. coli* with type I pili?

### Possible Hypotheses

Mannose is the receptor on bladder cells for *E. coli* with type I pili

Galactose is the receptor on bladder cells for *E. coli* with type I pili

### Prediction for first hypothesis above

If mannose is the receptor, it will inhibit agglutination.

### Experimental design

Bladder cells + mannose + *E. coli*

Bladder cells + galactose + *E. coli*

Bladder cells + glucose + *E. coli*

Bladder cells + fructose + *E. coli*

### Negative controls

Bladder cells alone

Each sugar alone

*E. coli* alone

### e. Positive control

Bladder cells + *E. coli*

Note – if you add the *E. coli* to the bladder cells before adding the sugar, the sugar will not be able to inhibit the reaction and agglutination will be observed.

### Answer –

***Mannose is the receptor for this type of fimbriae and should inhibit agglutination in this test.***

## **2. Scenarios B and C**

Note - The filamentous hemagglutination mutant is a strain lacking filamentous hemagglutinin and other possible adhesins.

The *B. pertussis* wild type strain is a strain with all possible adhesins present.

### Question

Scenario B – What adhesin is responsible for adherence of *B. pertussis* to bronchial cells?

Scenario C – What adhesin is responsible for adherence of *B. pertussis* to laryngeal?

### Possible Hypotheses

Filamentous hemagglutinin is responsible for the adherence of *B. pertussis* to bronchial/laryngeal cells.

Fimbriae are responsible for the adherence of *B. pertussis* to bronchial/laryngeal cells

### Prediction based on the first hypothesis

If filamentous hemagglutinin is responsible for the adherence of *B. pertussis* to bronchial/laryngeal cells then a mutant lacking filamentous hemagglutinin will not cause agglutination.

### Experimental design

Filamentous hemagglutinin mutant + bronchial/laryngeal cells

Fimbriae mutant + bronchial/laryngeal cells

Pertussis Toxin mutant + bronchial/laryngeal cells

Hemolysin mutant + bronchial/laryngeal cells

Adenylate Cyclase mutant + bronchial/laryngeal cells

### Negative controls

Each bacterial strain alone

Bronchial or laryngeal cells alone

### Positive control

*B. pertussis* wild type + bronchial/laryngeal cells

### Answers

***For Scenario B – binding to bronchial cells is mediated by filamentous hemagglutinin (no agglutination observed with the FHA mutant)***

***For Scenario C – binding to laryngeal cells is mediated by fimbriae and filamentous hemagglutinin (no agglutination observed with the FHA mutant or the fimbriae mutant)***

## **3. Scenario D**

### Questions

What molecule is serving as the co-receptor for HIV on T cells?

### Possible hypotheses

CD3 is the co-receptor for HIV on T cells

CXCR4 is the co-receptor for HIV on T cells

Prediction for first hypothesis

If CD3 is the co-receptor for HIV on T cells then antibodies to CD3 should inhibit agglutination.

Experimental design

T-cells + anti-CD3 + HIV

T-cells + anti-CRCX4 + HIV

Positive controls

T-cells + HIV

Negative controls

T-cells alone

HIV alone

Other controls

T-cells + anti-CD4 + HIV (expect no agglutination confirming that CD4 is part of co-receptor – ie both CD4 and another molecule serve together as the receptor)

T-cells + anti-CCR5 + HIV (expect no agglutination confirming that CCR5 is not involved in binding – this is expected since it is thought that there is no CCR5 on T-cells)

Note – if you add the HIV to the T-cells before adding the antibody, the antibody will not be able to inhibit the reaction and agglutination will be observed.

Answer

***CRCX4 is the co-receptor for HIV on T-cells (antibodies to CRCX4 inhibited agglutination).***