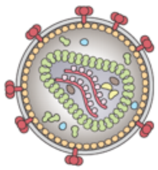

HIV: RECEPTORS AND CO-RECEPTORS

OVERVIEW

This demonstration is part of a series of activities and demonstrations focusing on various aspects of the human immunodeficiency virus (HIV) life cycle. It focuses on the first step of infection: the binding of HIV to the cells it infects.

HIV infects and destroys one specific class of cells of the immune system called **helper T cells**, which work by orchestrating and activating other immune cells. HIV weakens a person's ability to fight other infections and diseases. Acquired immunodeficiency syndrome (AIDS) occurs when a person's immune system is severely compromised. Without treatment, most people with AIDS die.

HIV has a single-stranded **RNA genome** surrounded by a protein shell called a **capsid**. An outer membrane, referred to as the **envelope**, covers the capsid. The envelope is derived from the host cell membrane and contains viral envelope proteins embedded in it. For HIV infection to occur, two protein subunits of the viral envelope protein (called Env) must bind with a receptor and co-receptor on the surface of the helper T cell.

Helper T cells have several protein receptors on their surface. Receptors allow cells to receive signals from other cells and tissues in the body and respond to those signals. **CD4 receptors** are unique to helper T cells and are one of the two receptors that HIV binds to. Binding of HIV-1 to CD4 is necessary but not sufficient for infection. HIV must also bind to a co-receptor: either **CCR5** or **CXCR4**. Depending on the strain of HIV, it will either bind to CD4 and CCR5 or CD4 and CXCR4.

This demonstration models how HIV initiates infection by binding to both receptors on the surface of the helper T cell: in this case, the CD4 receptor and the co-receptor CCR5. (The same concepts apply to the binding to CD4 and CXCR4.) Binding of HIV to both receptors causes fusion of the viral envelope with the cell membrane and deposits HIV inside the cell.

The exploration of receptor and co-receptor binding between HIV and its host cell is of importance in the designing of new drugs to block HIV infection. If a drug can be designed to block the receptor or co-receptors on immune cells, then HIV cannot enter cells and cause infection.

KEY CONCEPTS AND LEARNING OBJECTIVES

- Students will be able to model how the two subunits of the Env protein of HIV bind the CD4 receptor and co-receptor to infect helper T cells.
- Students will also model how the alteration or absence of a co-receptor inhibits HIV binding and subsequent infection.

CURRICULUM CONNECTIONS

Curriculum	Standards
NGSS (2013)	HS-LS1-2
AP Biology (2013)	3.C.3; 4.B.1

KEY TERMS

Virus, cell surface receptors (CD4 and CCR5 co-receptor), helper T cell, envelope, capsid

TIME REQUIREMENTS

This activity was designed to be completed in 10 minutes of classroom time. Educators will need to set aside at least 30 minutes for preparation the first time the activity is done.

SUGGESTED AUDIENCE

This activity is appropriate for all levels of high school biology and introductory college biology.

PRIOR KNOWLEDGE

Students should know what viruses are and that they infect cells. Some prior knowledge of viral structure would be helpful; the HHMI activity "Virus Explorer," at https://www.hhmi.org/biointeractive/virus-explorer, is a useful resource for this background information.

MATERIALS

- Rectangular plastic food storage container and lid, such as Ziploc 9-cup size, representing the helper T cell (may also use a shoebox)
- Small round food storage container and lid, such as Glad ½ cup size, representing HIV
- Two thin nails with no head or a very small head about 2.5 cm long, representing the viral RNA genome
- Knife, scalpel, or scissors to cut slits in both containers large enough for the nails to slip through
- Two wooden or plastic spools of different sizes, representing the two Env protein subunits
- Glue or lab tape (2 different colors)
- Barbeque or kabob skewer 16 cm (6") long or an orange wood manicure stick, representing the CD4 receptor on helper T cell surface
- Pipe cleaner, representing the CCR5 co-receptor on helper T cell surface

- Optional: markers or spray paint to color the spools

TEACHING TIPS

- If students are not familiar with the HIV life cycle, an animation is available at http://www.hhmi.org/biointeractive/hiv-life-cycle. The steps illustrated in this activity are from the beginning of the animation (time 0) to time 1:25 min. Additional activities in this series illustrate other steps in the life cycle.
- Students can be introduced to viral structure by exploring the HHMI "Click & Learn" interactive "Virus Explorer" at https://www.hhmi.org/biointeractive/virus-explorer.
- Students can be introduced to and/or review the immune system using a video clip from the **HHMI Holiday Lecture "AIDS: Evolution of an Epidemic,"** Lecture 1, *"From Outbreak to Epidemic," Chapter 14, "Cells of the immune system."* This chapter (42 seconds long) is located at http://media.hhmi.org/hl/07Lect1.html (click on chapter 14 on the right-hand side of the screen) and provides a brief overview of the immune system and its associated cells.
- A more extensive review of the immune system, which combines the above video clip and others, is available as a "**Click and Learn**" resource entitled "*Cells of the immune system*" located at http://www.hhmi.org/biointeractive/cells-immune-system. A student worksheet associated with this resource is also available at this website and can be used as homework prior to executing the demonstration in class.

PROCEDURE

1. Build the HIV model using a round plastic container and lid with two different-sized wooden or plastic spools attached to opposite sides. The large and small spools represent the two protein subunits of the HIV envelope protein (Env). They bind to the CCR5 co-receptor and CD4 helper T cell receptor, respectively. The two thin nails represent the RNA genome of HIV.

To assemble the HIV model:

- Measure the length of one of the two nails.
- Make a small slit in the bottom of the round plastic container large enough for the nails to slip through when the model virus binds the helper T cell model.
- Tape or glue the two spools to opposite sides of the container. If you use lab tape, you can then label each receptor. It would be helpful for the two spools to be different colors. You can color them with permanent marker or spray paint, or cover them in colored tissue paper.

2. Build the helper T-cell model using the rectangular plastic container or shoebox. We will show two receptors on the helper T-cell surface: the CD4 receptor (represented by the barbeque/kabob skewer or orange stick) and the CCR5 co-receptor (represented by the pipe cleaner).

To prepare the helper T-cell model:

- Place the HIV model near one end of the bottom of the rectangular box. Use a marker and mark the position and size of the slit in the HIV model. Also mark where the holes in the two receptors are located.
- Use a knife or whatever tool works best for you to cut a slit in the bottom of the box. It should be the same length and width as the slit in the HIV model.
- Make a hole in the location you marked for the wooden stick representing the CD4 receptor.
- Slide the HIV model on the stick and align the two slits. Check that the position you marked for the CCR5 co-receptor is accurate.
- Make a hole for the CCR5 co-receptor and slide one end of the pipe cleaner through it. Slide the HIV model onto the wooden stick and pipe cleaner.
- Mark the location for a series of six evenly spaced dots. They should be about 1/3 of an inch apart.
- At each of these locations, make a small hole just large enough for the pipe cleaner to pass through.

Figure 1. Helper T-cell and HIV models. The round plastic container represents HIV; the large (red) and small (blue) spools represent the two protein subunits of the HIV envelope protein (Env), which bind the CCR5 co-receptor and CD4 helper T cell receptor, respectively. The two nails inside the round plastic container represent the viral RNA genome. The rectangular plastic container represents the helper T cell; the wooden skewer represents the CD4 receptor and the pipe cleaner the CCR5 co-receptor.

3. Use these two models to demonstrate the binding of HIV to the host cell. The goal of this demonstration is to show that the viral RNA, represented by the nails inside the HIV model, cannot be released into the helper T cell until both receptor and co-receptor are attached to the HIV. When both receptor and co-receptor are properly aligned, the two membranes fuse, allowing the RNA (nails) to enter the T cell (Figure

2). If the helper T cell receptor and co-receptor and HIV do not align, then the viral RNA cannot enter the cell.

Figure 2: Model of HIV bound to helper T-cell receptors. The round plastic container represents HIV, and the two nails inside it are the viral RNA. For the demonstration, you may want to cover the container representing HIV with a lid to show that the virus is covered by an envelope. The lid was not included in this photo in order to show the two nails inside; when you demonstrate to the class you should cover the round plastic container with a lid.

EXTENSION RESOURCES

- It may be interesting for students to know that people who are homozygous for a mutation in the CCR5 gene are resistant to HIV infection. The mutation makes it more difficult for the HIV Env protein to bind. (For more information, visit Nature's Scitable website http://www.nature.com/scitable/blog/viruses101/hiv_resistant_mutation?isForcedMobile=Y.) Scientists are exploring different strategies to affect CCR5 production in HIV-infected individuals. If the CCR5 co-receptor is mutated, then the virus cannot successfully attach to and infect helper T cells.
- To demonstrate how HIV entry into immune cells may be blocked using this model, bend the end of the pipe cleaner that would bind with the HIV and change its shape. If the shape of the pipe cleaner (CCR5 co-receptor) is altered, then HIV cannot attach to the CCR5 receptor. The helper T cell and HIV will not correctly align, and the viral RNA (nail) cannot be released into the helper T cell.
- Note that several other mechanisms can contribute to HIV resistance and/or slow progression of HIV infection to AIDS in addition to receptor mutation. They include cytotoxic T cell activation, inhibition of HIV receptor expression, and/or inhibition of HIV replication by cytokines.

- Discuss with students why it is important to understand how HIV binds to host cells. To help guide the discussion, you may watch a clip from the lecture *"Drugs and HIV evolution," Chapters 17 through 19.* http://media.hhmi.org/hl/07Lect3.html (Click on chapter 17 on the right-hand side of the screen and watch until time 21:10.) The clip describes the discovery and approval of the drug Maraviroc in 2007. Note that most HIV strains bind to CD4 and CCR5, but some strains bind to CD4 and CXCR4. Ask students whether this drug would protect everyone from HIV infection.
- Students may have questions about the two protein subunits of the HIV envelope protein. These two subunits are called gp120 and gp41. One HIV gene, the *env* gene, codes for a protein (gp160) that is 160 kDa in size. This protein is then cleaved by a host cellular protease into two protein subunits 120 kDa and 41 kDa in size. Another activity in this series ("HIV and Protease Inhibitors") focuses on the cleavage of another HIV polyprotein.

AUTHORS

Written by Patricia Nolan Bertino, Scotia-Glenville High School, and Anthony Bertino, Canandaigua Academy. James Blankenship, PhD, Cornell University, advised on the project. Photos and additional writing by Mary Colvard.

Edited by Leah M. Cataldo, PhD, Buckingham Browne & Nichols School; Karen Gulliver, consultant; and Laura Bonetta, PhD, HHMI. Reviewed by Melissa Csikari, HHMI.

Scientific review by Allen Bateman, PhD, Debby Walser-Kuntz, PhD, Carleton College, Munir Syed, PhD, Hartwick College.