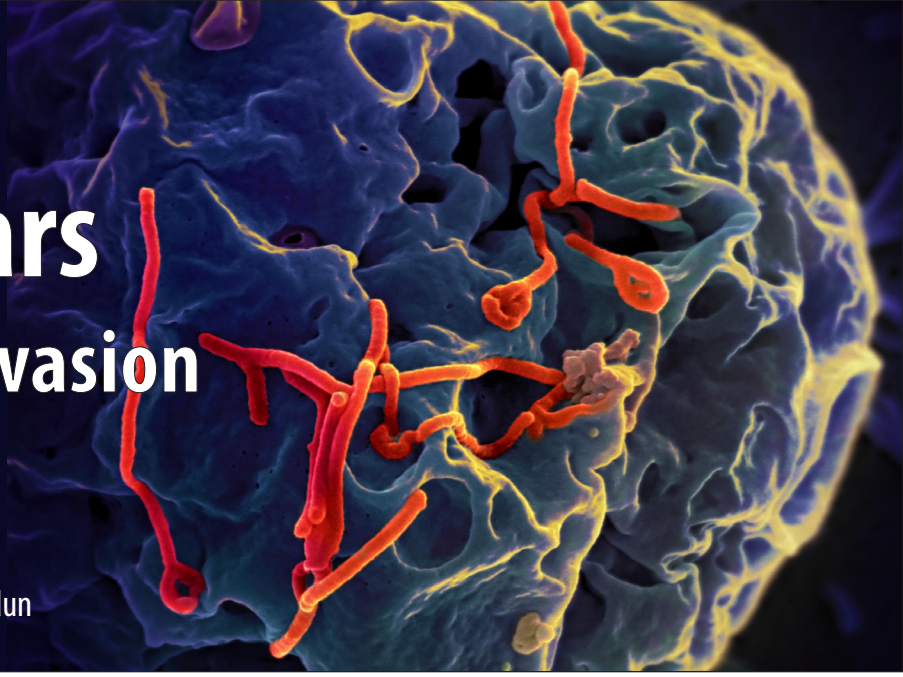
NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

The Ebola Wars
Mission Immune Evasion



by
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Alex J. Sadowski, Michael Petersen, and Rumtin Aidun

Phase I – Infection of *Homo sapiens*

[Day 0]

[Identity Confirmed: Ebola virus]

Incoming Message:

> *The goal of this operation is to infect a human so that you can replicate and increase our numbers. There are a variety of ways to accomplish this task but keep in mind that* Homo sapiens *will use its barriers from the first line of defense of the innate immune response to prevent you from entering any of its cells. Regardless, I have full confidence that you will complete this task. Good luck.*

"I hope that an opportunity arises for me to carry out this mission," thought Ebola virus. "I am ineffective unless I get into a cell. I will first need to overcome the innate immune response."

This Ebola virus particle was located on a used Kleenex tissue, covered with mucus and a spot of blood, that never made it into the garbage can in Michael's home. Michael, who had traveled abroad, initially had not known he was infected with the virus, recently fell ill and had been coughing for days. The tissue contained not only this one, but many other Ebola virus particles.

"This is my chance!" cried Ebola virus.

An unsuspecting friend named Jeff visiting Michael's home picked up the fluid-filled tissue from the floor and threw it into the trash can. "Yuck!!" Jeff exclaimed. "I realize that Michael is sick, but I wish he would clean up after himself," he muttered about his friend. However, he soon became distracted in conversation and never washed his hands after handling the tissue.

Jeff, who was a college lacrosse player, had a few small cuts on the surface of the skin on his hands from a particularly rough game earlier that day. "That is my way in," exclaimed Ebola virus.

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The sentinels of Jeff's innate immune system, macrophages and dendritic cells, were scanning the area near the wounds, but rather than being ingested and destroyed by the macrophages like other foreign entities, the Ebola virus directly attached to and infected one of these immune cells. The first phase of the mission had been completed successfully.

"I'm in! Now it's time to unleash my weapons!" exclaimed the Ebola virus.

PHASE I ACCOMPLISHED

The immune system's response to infection can be likened to a battle between host cells and pathogens. Pathogens often have weapons, or mechanisms to evade immune responses, against which host cells deploy a variety of defenses. The three major lines of defense of the immune system are outlined below. If a pathogen is successful, it can breach each of these lines of defense.

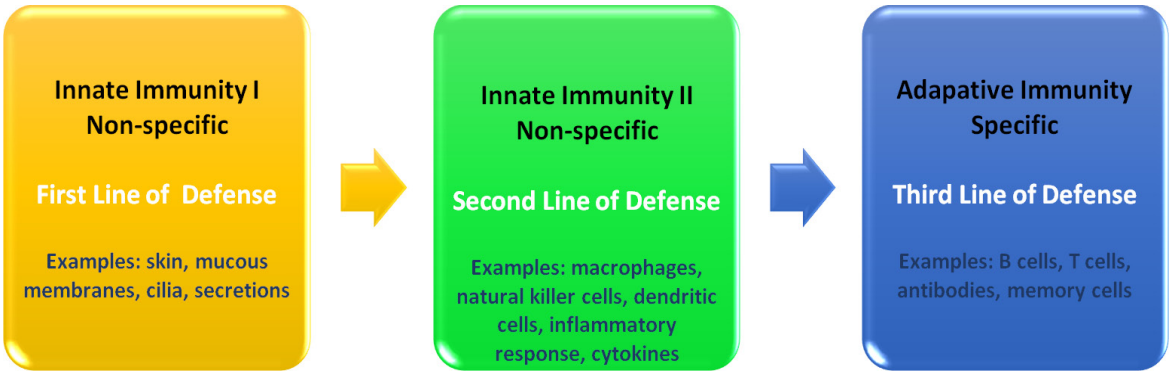


Figure 1. Three lines of defense of the immune response.

Questions

1. In the story so far:
   a. What weapons did the host cell deploy as part of the first line of defense?

   b. How did the Ebola virus evade the first line of defense?

   c. What second line of defense was deployed by the host?

2. Propose a few alternative means by which the Ebola virus could have evaded the first line of defense that were not mentioned in the story.

3. What might Jeff have done to prevent the breach of the first line of defense by the Ebola virus?

4. Start completing the tables (Table 1: Summary of Host Defenses, Table 2: Summary of Ebola Virus Weapons) at the end of the document to summarize the major host defenses and Ebola virus weapons described in the story and preparatory reading. You will be able to complete more of these tables as you progress through the case.

Phase II – Weapons Engaged

[Day 3]

Incoming Message:

> *You are ready for the next phase of your mission. Now that you have entered a human cell, you must trick the immune system into thinking that you are not present so that you can multiply and overcome the second line of defense. You have several key proteins that will assist you with this phase of your mission.*

"Of the seven genes in my genome, I have three that will be particularly useful for this operational phase to overcome the immune system's second line of defense: *VP35*, *VP24*, and *GP/sGP*," said Ebola.

Figure 2. The Ebola virus genome.

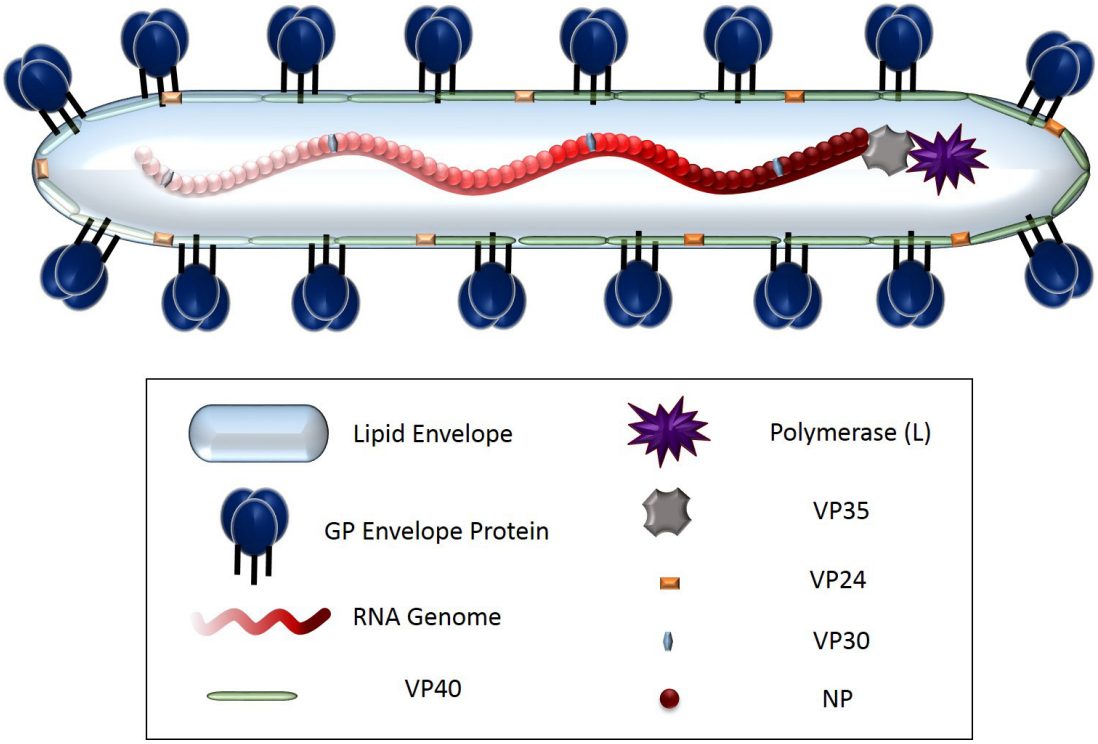


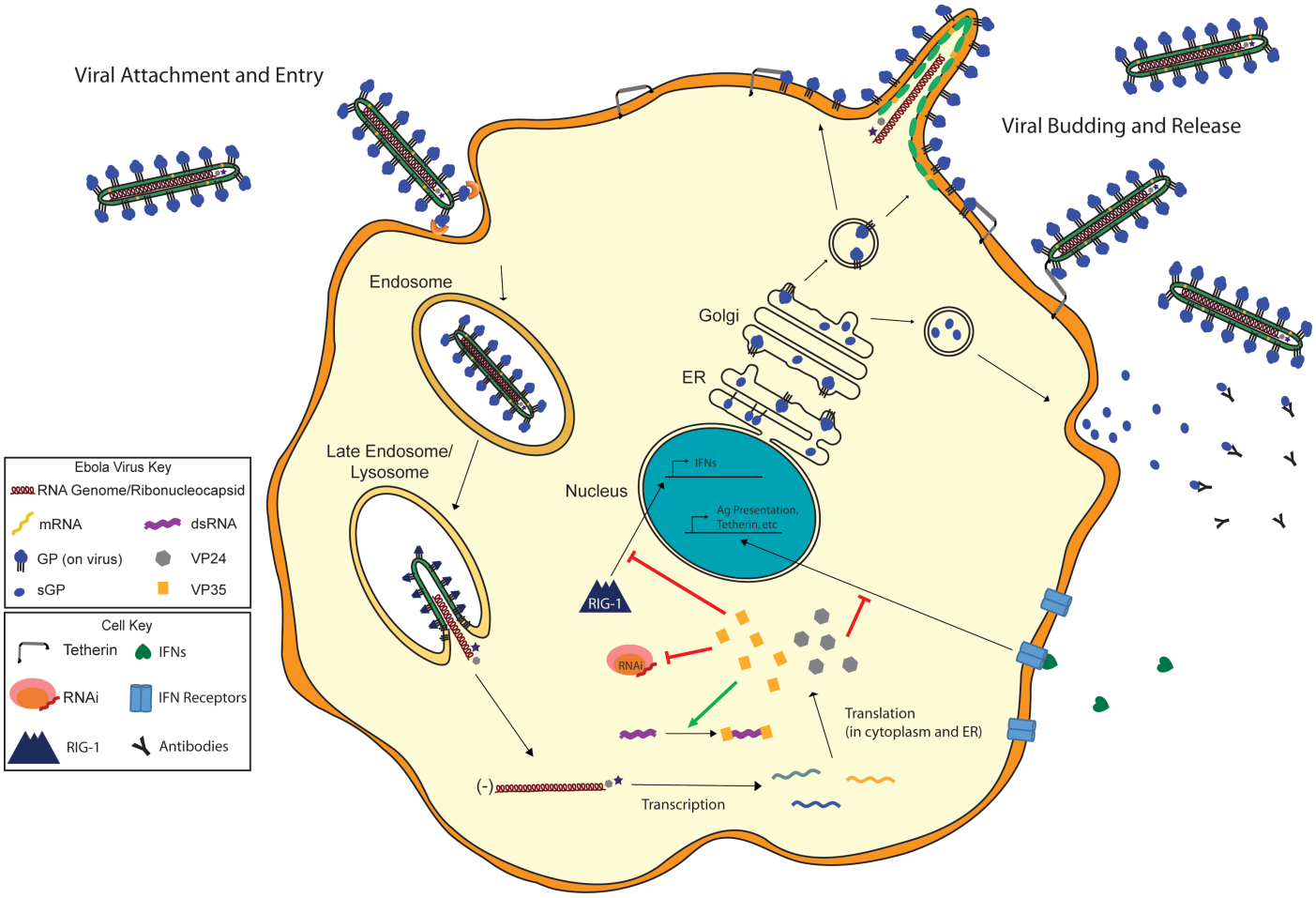
Figure 3. Ebola virus particle.

"One of my biggest challenges will be disabling the communication system used by the host immune system to coordinate a defensive response. My best bet is to subdue the chemical messengers called cytokines, specifically interferon, that alert other immune cells of an infection so that they can take action," thought Ebola virus.

To this end, VP24 was produced inside infected cells to block interferon signaling through the cellular STAT1 pathway and turn off any existing interferon alarms.

Next, VP35 blocked the production of new interferons by preventing the activation of a key protein called interferon regulatory factor 3. This antagonist action allowed Ebola virus to remain undetected by the immune system. "Ha! Interferon, you can't mess with the Ebola virus," said Ebola with an evil chuckle.

Figure 4. Immune evasion strategies of Ebola virus.



"However, being that I am a virus that infects cells, the phagocytes and complement will likely still be on the prowl and prevent me from accomplishing this phase of the mission. Also, the natural killer cells will try to eliminate the cells that I infect. I will fight back. They will not win!"

In another of its multiple roles, VP35 attached to and put a cap on viral dsRNA (double-stranded RNA) so that it would not be detected as an antigen. Thus, the host cell could not use its attacking RNAs (RNAi) to prevent Ebola virus from making more proteins. "I'm on a roll!" exclaimed Ebola.

Concurrently with this battle, inside the cell more and more viral components were being produced to form new Ebola virus particles. However, those components needed to be assembled and released from the infected cell if infection was going to spread. At the plasma membrane, a host protein called tetherin was waiting to "tether" new particles to the cell surface and prevent their budding. "GP to the rescue," shouted Ebola virus. The virus' glycoprotein (GP) was not only used to attach to new target cells when on extracellular virus particles, but it also had the ability to interact with and inhibit tetherin from preventing viral release. Through its action, new Ebola virus particles were created and released into the extracellular milieu at a high rate. They went on to infect other cells and repeat the cycle. The army was growing. More and more cells became infected. Infected dendritic cells could not produce stimulatory cytokines, and natural killer cells that target virus-infected cells started to die by apoptotic cell death.

PHASE II ACCOMPLISHED

The scenario above captures a multitude of mechanisms that Ebola virus employs to evade the second line of defense of the immune response through the actions of specific proteins. Review these by closely examining Figure 4. Then, using a laptop, play one round of the game *Operation: Ebola!* at **https://catlilli.itch.io/ebola** and answer the questions below. Be sure to take note of how you structure your genome because it can influence your gameplay. Also, be sure to record your score. Please note that although the setting of this game is the bloodstream, the Ebola virus can invade tissues and infect cells in other locations.

Questions

5. In the story so far:
   a. In what order in the Ebola virus genome did you place the genes?

   b. How did each of the Ebola virus weapons *VP35*, *VP24*, and *GP/sGP* play a role in the game?

   c. What weapons did the host use to fight back? Which host response overcame the Ebola virus infection, if any?

   d. What was your score?

6. Propose what is likely happening clinically to an individual infected with Ebola virus during this time point. Note that the virus has already entered the host and is starting to deploy its arsenal of weapons.

7. Continue completing the Table 1 and Table 2 at the end of the case using information learned from the story, figures, and game.

The Final Phase – Evading Specific Immune Responses

[Day 10]

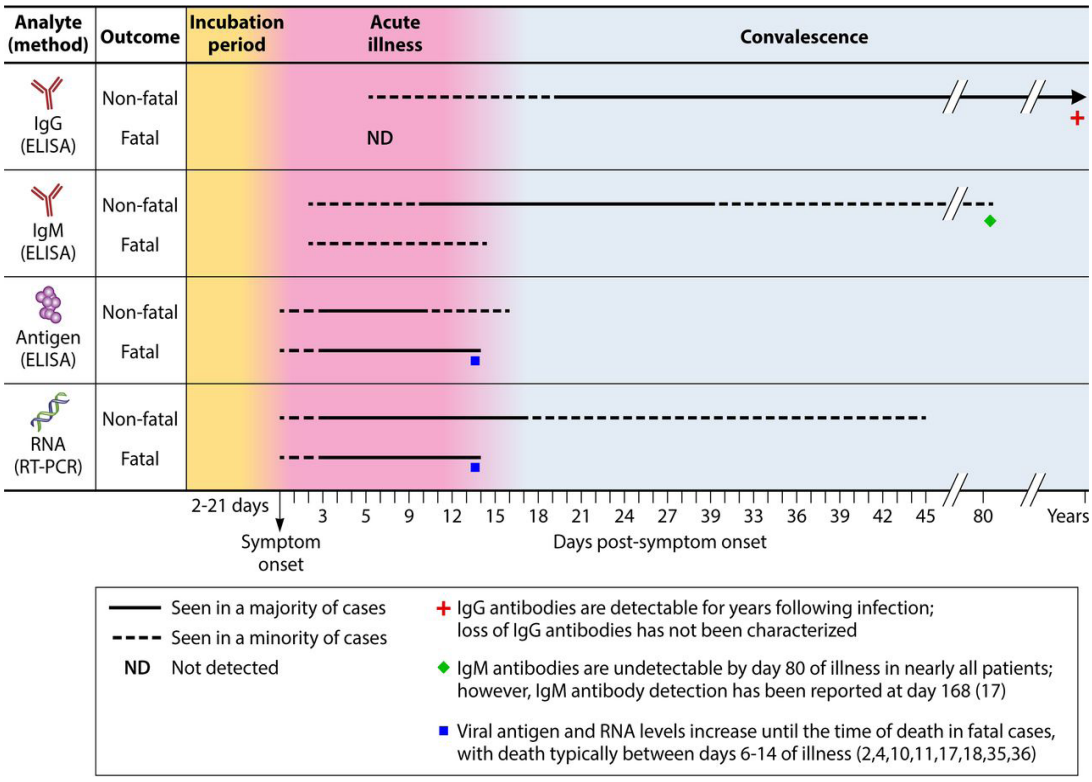
Incoming Message:

> *Those who survive viral infections may produce detectable amounts of antibodies soon after infection, and exhibit a more robust immune response. Your final phase of the mission is to evade the third line of immune defense by avoiding specific antibodies and cytotoxic T cells.*

"My lipid exterior will serve as fantastic camouflage against the immune system, as I will look like the host cells from the outside. However, it may not be enough to completely prevent detection," declared Ebola. "I also will produce secreted GP (sGP), a shortened form of my GP protein. Instead of embedding in the viral envelope, this weapon will be secreted from the cells. Since it looks almost identical to the GP on the virion surface, it will 'soak up' many of the neutralizing antibodies that are around, allowing my particles to slip by."

Although the Ebola virus had evaded the first two lines of defense, and seemed prepared for the third, its next steps were not enough. Jeff's body fought back and mounted a successful adaptive immune response against the virus. This was a specific response against Ebola viral antigens carried out by specialized lymphocytes, namely T and B cells. T helper cells activate other immune cells while cytotoxic T (Tc) cells eliminate infected cells by poking holes in them with perforins. Jeff's Tc cells targeted Ebola-infected cells by releasing cytotoxic factors that prevented the virus from replicating. Ebola virus is also thought to induce apoptotic cell death in T cells. As a result, infected individuals can have reduced T cell counts. Jeff's T cell counts had indeed decreased. His B cells produced antibodies in quantities that overcame the sGP levels and sequestered Ebola viral particles for destruction. First IgM antibodies specific to the Ebola virus antigens were produced, and subsequently, through class switching, IgG antibodies were produced to enhance and maintain the antiviral action. Much to Ebola virus' dismay, Jeff's immune system was able to eliminate the viruses from his body, and he eventually recovered from the infection. Michael, whose tissue had been the source of Jeff's infection, sadly did not have a robust immune response against the virus, and like approximately half of those that are infected with Ebola virus, succumbed to the infection.

Figure 5. Figure 1 from Broadhursta *et al.* (2016), reproduced with permission from American Society for Microbiology.



[Incoming Message]

> *Because you were unable to overcome the third lines of defense of all those infected, I am sorry Ebola virus, but this is a failed operation.*

MISSION FAILED

Questions

8. How was the third line of immune defense involved in the story?

9. What are the roles of IgM and IgG?

10. What does Figure 5 suggest about how the body fights off infection in Ebola survivors?

11. Had the outcome of the infection been fatal for Jeff, what do you propose would have been his test results?

12. Synthesizing what you have learned thus far through this case, what makes the Ebola virus so deadly?

13. The Ebola virus outbreak of 2014 caused devastation in West Africa with many lives lost. This case study is written from the perspective of the Ebola virus to enhance understanding of how this deadly virus can evade the immune response. When the immune system wins the Ebola virus loses, and vice-versa. Consider rewriting the case and redesigning the game from the perspective of the immune system. What would you change?

14. Replay the game *Operation: Ebola!* Design the viral genome differently than you did for Question 5.
    a. In what order in the Ebola virus genome did you place the genes?

    b. How did your genome changes affect the gameplay?

    c. What was your score?

    d. Compare your gene orders and scores with one or two other students. Are there any common trends? What genome order seems to produce the weakest and strongest virus, respectively? Why do you think this is?

15. Propose other ways to weaken the effects of the virus that do not include modifying its genome.

16. Finish completing Table 1 and Table 2, wrapping up major elements of what you have learned today about the weapons the Ebola virus uses to evade the immune response and the defenses of host cells.

Table 1. Summary of Host Defenses

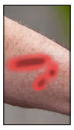
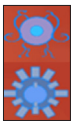
Host Denfense	*Related Images from Game*	*Role in Defending Against Pathogens*	*Line of Defense/Type of Immunity*
Skin			
		Immune sentinels searching for foreign invaders.	Second / Innate
Cytokines (e.g., interferons)	N/A		
	N/A	Embedded in the membrane, this protein prevents budding viruses from releasing from the cell surface.	Second / Innate
Natural Killer Cells			
	N/A	Help activate and regulate various cells of the immune system (B cells, cytotoxic T cells, and phagocytes).	
B Cells	 (cell product)		
		Detect and destroy cells in an Ebola-virus specific manner.	

Table 2. Summary of Ebola Virus Weapons

Ebola Virus Weapon	*Role in Evading the Immune Response*
Lipid envelope originating from the host cell that has GP attachment proteins.	
VP35	
	Turns off existing interferon alarms by disrupting STAT1 signaling pathway.
	Counteracts tetherin to allow viruses to bud from the cell.
sGP	
Mechanism unknown	Induces bystander apoptosis of immune cells (especially T lymphocytes and NK cells).

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