D4P Discussion Guide

Joe Levin presents on whether SARS-CoV-2 re-infection could occur in primates.

Lack of Reinfection in Rhesus Macaques Infected with SARS-CoV-2 ·
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Researchers investigated whether the immune response to SARS-CoV-2 was sufficient to prevent reinfection in rhesus macaques. The monkeys in this study were first infected with SARS-CoV-2, allowed to recover, and then 4 weeks later were re-exposed to SARS-CoV-2. The purpose of this study was to see if the monkeys developed immunity to the virus.

Key Terms

- Immunological memory
- Three R's
- Histopathology and immunohistochemistry
- Neutralizing antibodies

questions for learners	discussion points for educator
1. *What is adaptive immunity, and how does this relate to immunological memory?*	Adaptive immunity is the part of the immunological response to a pathogen that mediates immunological memory. Adaptive immunity involves the production of specialized immune cells and molecules (antibodies) that are specific to a pathogen. The first time the immune system encounters a pathogen, it takes some time to produce these specialized immune cells and antibodies. Once the initial infection is cleared, these responses are archived as immunological memory. The next time that the immune system encounters the same pathogen, it can use the archived immunological memory to mount a stronger, faster response.
2. *Why was immunological memory explored in this study? What are the limitations researchers faced when trying to answer this question?*	Not all immunological memory is the same--the immune system is able to "remember" some pathogens better than others, and the efficacy of immunological memory determines whether reinfection of a pathogen can occur. Here, researchers set out to determine if monkeys possess immunological memory against SARS-CoV-2 after infection, and if this was effective enough to prevent reinfection. Understanding how the immune system responds to SARS-CoV-2 infection is critical for creating public health policies (i.e. when to lift rules around social distancing, or how a vaccine may work to keep a future outbreak from occurring). Time is the biggest limitation.. Since SARS-CoV-2 was only made known to humans in November of 2019, it is difficult to know how our immune systems respond to this virus on the long-term.
3. *What are neutralizing antibodies, and how did*	Neutralizing antibodies are produced by B cells, and function by binding to pathogens in a way that blocks ("neutralizes") their normal biological activity. In this study, a neutralizing antibody

the researchers determine neutralization?	assay was used. In this assay, the researchers took serum from the monkeys who had recovered from the initial challenge, heat inactivated the sera (to remove any virus), and then mixed them with SARS-CoV-2 virus and a cell line called Vero-E6 cells and left them for a week. Vero-E6 cells are a kind of monkey kidney cell line that, when exposed to a virus like SARS-CoV-2, undergo structural changes (called cytopathic effects) that are visual under a microscope. If the sera samples mixed with the virus and these cells prevented these cells from showing these viral cytopathic effects, then the antibodies in the sera from the monkeys were able to effectively neutralize the infectivity of the virus.
4. *What is a challenge? How did the researchers build a challenge into the design of their study?*	A challenge is another word for initial infection in an experiment in the lab. In this study, researchers challenged the monkeys with SARS-CoV-2 virus so that they would all get sick. Researchers studied the course of the monkeys' infections and allowed them to recover. After they had all recovered, 28 days after the initial challenge, the researchers again exposed the monkeys to SARS-CoV-2 to see if they would get sick again. This second exposure was a "rechallenge."
5. *How did the researchers track SARS-CoV-2 infection in this study?*	Viral infection was tracked in a variety of ways. Lungs of rhesus macaques were examined before, during, and after infection with SARS-CoV-2 using both X-rays (to look for physical instances of respiratory issues), as well as with histopathology, which is the study of the changes in tissues caused by disease. Similarly, the researchers looked for the presence of SARS-CoV-2 proteins (antigens) in lung tissues of rhesus macaques before, during, and after infection with SARS-CoV-2 using immunohistochemistry. This technique allows scientists to study the makeup of tissues using antibodies that specifically bind to specific components that might be found in those tissues. For these experiments, researchers added a tagged antibody, specific to the SARS-CoV-2 spike protein (the tag on the antibody allows scientists to "view" the reaction), as well as tagged antibodies to different cell markers to track where the virus was, and what cells it was interacting with.
6. *What are the three R's in animal research?*	The three R's in animal research are 1. Reduce, 2. Refine, and 3. Replace. All animal researchers keep these R's in mind when designing their experiments in order to reduce the numbers of animals used, refine the experiments to maximize animal well-being and minimize discomfort, and replace animals with cell lines, monkeys with mice, mice with fish, fish with insects etc. In research institutions, there are review boards made up of scientists, vets, and impartial outsiders who review and sign off on all animal experiments to ensure that researchers are using the three R's in their experiments.