ANSWER KEY
for
"Resistance Is Futile ... or Is It? The Immunity System and HIV Infection"

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Part I – HIV and the Immune System

1. HIV is a retrovirus (a virus that uses reverse transcriptase).

a. What is reverse transcriptase?

Reverse transcriptase is an enzyme that is part of the HIV virus. It uses an RNA template to create a double-stranded DNA copy.

b. How is a retrovirus different from other viruses?

Retroviruses are encoded by RNA, not DNA. Their genome must first be reversed transcribed into DNA before it can be integrated into the host cell DNA. This last part (integration into the host cell chromosome) is another difference from most other viruses.

c. How does a retrovirus infect a cell and reproduce itself?

The life cycle of a retrovirus includes:

- Delivery of the virus into the host cell.
- Reverse transcription of the RNA viral genome into double-stranded DNA.
- Integration of the viral DNA into the host cell's DNA.
- Production of viral components (RNA and proteins) using the integrated viral DNA.
- Assembly of new viral particles.
- Budding of the new viral particles, which can infect other cells.

2. Review of the immune system (T cells).

a. What is a T cell?

b. What varieties of T cell exist? How are they functionally different?

c. What are their roles in the human body?

d. How is each T cell variety differentiated from the others (molecularly)?

A T cell is a type of white blood cell (also called a leukocytes or lymphocyte) important in immunity. They are called T cells because they are produced in the thymus. There are many different types of T cells, each with a different function in defending the body. Here are some examples:

- *T helper cell.* These are the general coordinating centers of the immune system. While they do not themselves remove foreign particles from the body, their activity is required for the function of other immune cells, such as cytotoxic T cells and B cells. They release cytokines, proteins which stimulate and direct the activity of other immune cells. On their cell surface is the identifying protein CD4.
- *Cytotoxic T cell.* This is a cell that kills other cells. These cells go around the body and recognize and destroy cells that have been infected. On their cell surface is the identifying protein CD8.
- *Memory T cell.* These cells "remember" how to recognize and fight pathogens that the body has previously encountered. Memory T cells are long-lived, in contrast to the short-lived effector T cells.
- *Regulatory T cell.* The role of these cells is to terminate an immune reaction at the end of infection.
- *Natural killer T cell.* These cells are the "front line" of our immune system,

playing a role in both killing infected cells and producing cytokines without responding to a specific antigen.

3. Immune system and HIV

a. Which type(s) of immune cells is/are targeted by HIV?

Cells that have the CD4 protein on their cell surface, such as T helper cells.

b. Why are other cells not targeted by the virus?

Other cells do not have the CD4 proteins on their cell surface. This protein is recognized by HIV and is required for infection.

c. How should cytotoxic T cells respond to the initial phase of HIV infection (when some T helper cells are still functioning)? Explain your reasoning.

While the immune system is relatively intact (only a few T_H cells have been infected and compromised), T_H are still producing cytokines to activate B cells and T_C. Thus, T_C should be functional and recognize and kill infected T_H.

d. As time progresses, why do the cytotoxic T cells stop responding to the HIV infection? Propose an explanation.

As HIV infection progresses, more T_H are compromised and their reduced numbers makes it difficult to coordinate the immune activity of the T_C. Eventually, there are too few functional T_H to direct the activity of T_C, and the immune system is compromised.

e. What happens to the immune system after HIV infection? Why? Can this account for the symptoms of AIDS (i.e. immunodeficiency, or the inability to defend against any foreign invaders like viruses and bacteria)?

When enough T_H are compromised by viral infection, the B cells and T_C cells no longer receive signals (cytokines) to direct their activity. At this point, the immune system crashes. AIDS is diagnosed when the T_H count is less than 200 T_H per milliliter of blood. At this point, the immune system is so compromised that a person is susceptible to infection by any other pathogen.

f. Why do you suppose that there is a delay between the time of HIV infection and the appearance of symptoms (and AIDS)?

The answer to this question is not fully understood and is the subject of current investigation. Some of the possibilities is that the HIV virus in the newly infect T_H cell may remain quiescent for some time. But it is unclear why there is such variability in the delay between HIV infection and the development of AIDS between individuals.

g. How does HIV evade the immune system?

HIV evades the immune system by disabling the coordinating center of the immune system (most other viruses target other types of cells and therefore are attacked by the immune system).

4. HIV protection.

a. Consider how HIV infects cells and reproduces. Also consider how the immune system fights off HIV infection. Humans differ by having mutations which result in slightly different proteins and immune function. Suggest as many hypotheses as possible to explain why some individuals might be protected against HIV infection. In other words, where and how might new viral infections be stopped? What could be different about the people who seem protected against HIV that caused viral replication to stop? Come up with at least 3 possibilities.

- Some individuals may have more active, more aggressive, or quicker-acting cytotoxic T cells. Alternatively, perhaps their cytotoxic T cells recognize different markers on the surface of cells that allows them to recognize infected T_H cells at an earlier stage of infection.
- Protected individuals have more effective B cells that produce antibodies faster or these antibodies are more effective at neutralizing HIV viruses.
- Protected individuals have previously been exposed to another pathogen that gives their immune system a boost in recognizing and fighting off HIV when it is first encountered (a vaccine-like hypothesis).
- T_H cells of the protected individuals are inefficiently recognized by the virus. This may be caused by a mutation in CD4 or other proteins utilized by the virus for cell recognition and entry.

- T_H cells of protected individuals are able to recognize and destroy viral RNA or proteins or DNA before it is integrated into their genome (perhaps a RNAi-like process, or inhibition of reverse transcriptase, etc.).
- T_H cells have the ability to suppress (down-regulate or silence) the expression of viral protein and RNA.
- T_H cells have a defect in budding.
- Other hypotheses are possible.

Part II – Paxton's Hypotheses about HIV-Protected Individuals

1. Classify each of your proposed hypotheses into the two categories proposed by Paxton and his colleagues.

Of the ones listed above:

"Super Cytotoxic T Cells" Hypothesis

a. Some individuals may have more active, more aggressive, or quicker acting cytotoxic T cells. Alternatively, perhaps their cytotoxic T cells recognize different markers on the surface of cells that allows them to recognize infected T_H cells at an earlier stage of infection.

"Super T Helper Cells" Hypothesis

b. T_H cells of the protected individuals are inefficiently recognized by the virus. This may be caused by a mutation in CD4 or other proteins utilized by the virus for cell recognition and entry.

c. T_H cells of protected individuals are able to recognize and destroy viral RNA or proteins or DNA before it is integrated into their genome (perhaps a RNAi-like process, or inhibition of reverse transcriptase, etc.).

d. T_H cells have the ability to suppress (down-regulate or silence) the expression of viral protein and RNA.

e. T_H cells have a defect in budding.

Other Hypotheses

f. Protected individuals have more effective B cells that produce antibodies faster or these antibodies are more effective at neutralizing HIV viruses.

g. Protected individuals have previously been exposed to another pathogen that gives their immune system a boost in recognizing and fighting off HIV when it is first encountered (a vaccine-like hypothesis).

2. How might you test each of your hypotheses? Propose an experiment. What are your controls? Experimental conditions?

Different student groups will have different answers depending on their hypothesis. As outlined in the Teaching Notes, the important aspect to point out to students is to design controlled experiments, not correlation studies.

For ethical reasons, the proposed experiments should require taking cells out of volunteers and analyzing them in culture.

Part III – Predictions from Paxton's Two Hypotheses

1. Design of the experiment:

a. Why were HIV and T helper cells mixed in the presence and absence of cytotoxic T cells?

In controls, you would expect HIV to infect T helper cells. The addition of cytotoxic T cells permits the experimenters to determine whether cytotoxic T cells really can fight off HIV infection by removing infected T helper cells.

2. For control individuals:

a. If you mix HIV and T helper cells in a test tube, what would you expect to happen? Why?

HIV should infect T_H cells, which become virus-producing factories. The amount of virus in the test tube should increase over time.

b. If you mix HIV, T helper cells, and cytotoxic T cells in a test tube, describe what you would expect to happen and why it occurs that way.

HIV should infect T_H cells, which become virus-producing factories. The amount of virus in the test tube should increase over time. However, cytotoxic T cells should recognize and kill infected T_H cells, slowing down the production of virus over time (compared to the absence of cytotoxic T cell).

3. For protected individuals:

a. Assuming that the "Super Cytotoxic T Cells" Hypothesis is correct, then when you perform the experiment using T helper cells and cytotoxic T cells from protected individuals:

i. If you mix HIV and T helper cells in a test tube, what would you expect would happen? Why?

The T_H cells of protected individuals are no different than controls. Therefore, we expect T_H cells to be infected by the virus and produce new virus particles at the same rate as controls.

ii. If you mix HIV, T helper cells, and cytotoxic T cells in a test tube, describe what you would expect to happen and explain your reasoning.

With the "Super Cytotoxic T Cells" Hypothesis, it is presumed that T_C cells from protected individuals are "better or faster" at recognizing and destroying HIV-infected cells. Thus, these T_C cells should remove infected T_H cells more effectively. The quantity of virus produced in the test tube should accumulate at a slower pace than for controls.

b. Assuming that the "Super T Helper Cells" Hypothesis is correct, then when you perform the experiment using T helper cells and cytotoxic T cells from protected individuals:

i. If you mix HIV and T helper cells in a test tube, what would you expect to happen? Why?

According to the "Super T Helper Cells" Hypothesis, the T_H cells of protected individuals are not recognized or infected by HIV as effectively as controls. We would therefore expect little virus to be produced and to accumulate in the test tube over time.

ii. If you mix HIV, T helper cells, and cytotoxic T cells in a test tube, describe what you would expect to happen and explain your reasoning.

Since the "Super T Helper Cells" Hypothesis makes no assertion about the T_C cells of protected individuals, it is presumed that T_C cells will be able to remove infected T_H cells (if there are any) no faster than controls.

4. How is this experiment able to differentiate whether the mechanism of protection against HIV is through "Super T Helper Cells" or "Super Cytotoxic T Cells"?

The two hypotheses predict a different outcome to the above-described experiment. If some individuals are protected through the "Super T Helper Cells" Hypothesis, then HIV should not be able to infect T_H, and no virus should accumulate in the test tube (irrelevant of the presence or absence of T_C). If the "Super Cytotoxic T Cells" Hypothesis is correct, then the T_H cells of protected individuals should be as prone to infection as controls, but the addition of cytotoxic T cell should limit or reduce the production of virus particles in the test tube (more so than for controls).

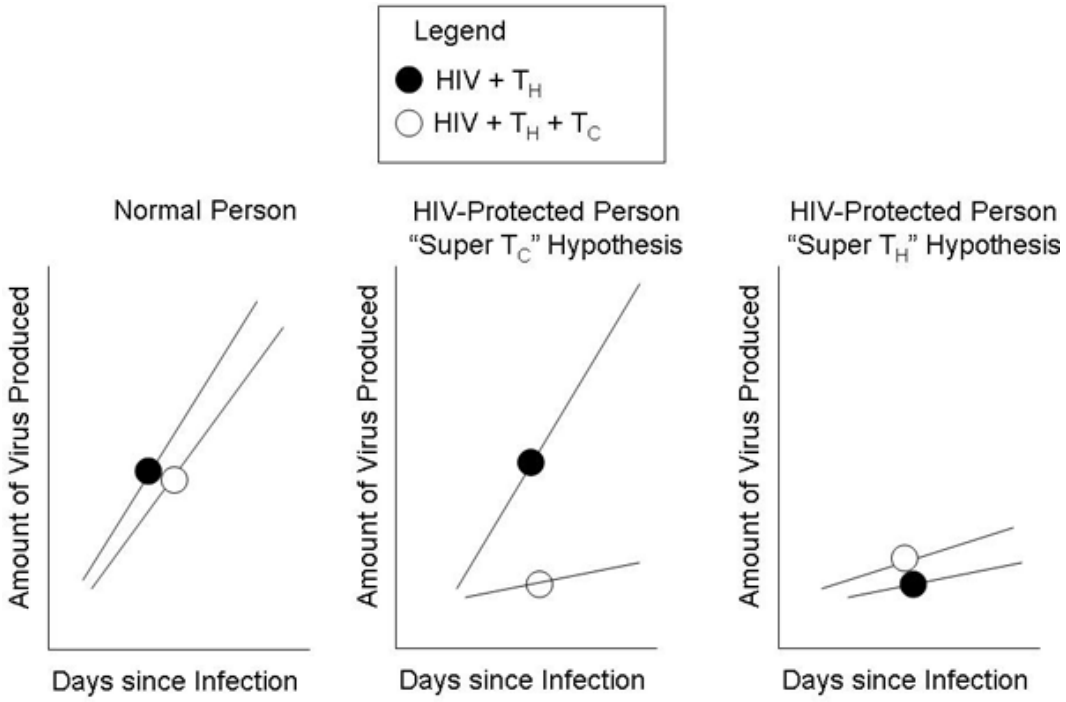
5. Use the graphic provided below to illustrate the results you would expect to obtain for

a. a normal/control person

b. a protected individual, assuming that the "Super Cytotoxic T Cells" Hypothesis is correct

c. a protected individual, assuming that the "Super T Helper Cells" Hypothesis is correct

Please note that each graph requires two lines (the two test tubes).



Part IV – Paxton's Results

1. Do cytotoxic T cells provide protection from HIV in control individuals?

T_C cells are able to recognize and kill infected T_H cells, slowing down the rate of new virus production in some of the samples.

2. Try to identify patterns in the results. Can the individual experiments performed using cells from resistant individuals be grouped into categories? If so, how many? Classify each subject into the different categories.

There are three categories of protected individuals:

- EU1 is in a category on his/her own.
- EU2 and EU3 cells respond similarly to the HIV challenge.
- EU4, EU9, EU11, EU12, EU17, EU18, and EU23 cells also exhibit similar response to the experiment.

3. Compare these results with what you had predicted in the previous section.

a. Are the results of the controls as you expected?

Data are as expected. Students may be surprised by the negligible effect of T_C cells on viral accumulation in vitro in some subjects.

b. Which of Paxton's hypotheses seem to be validated by the results of the protected individuals? Why?

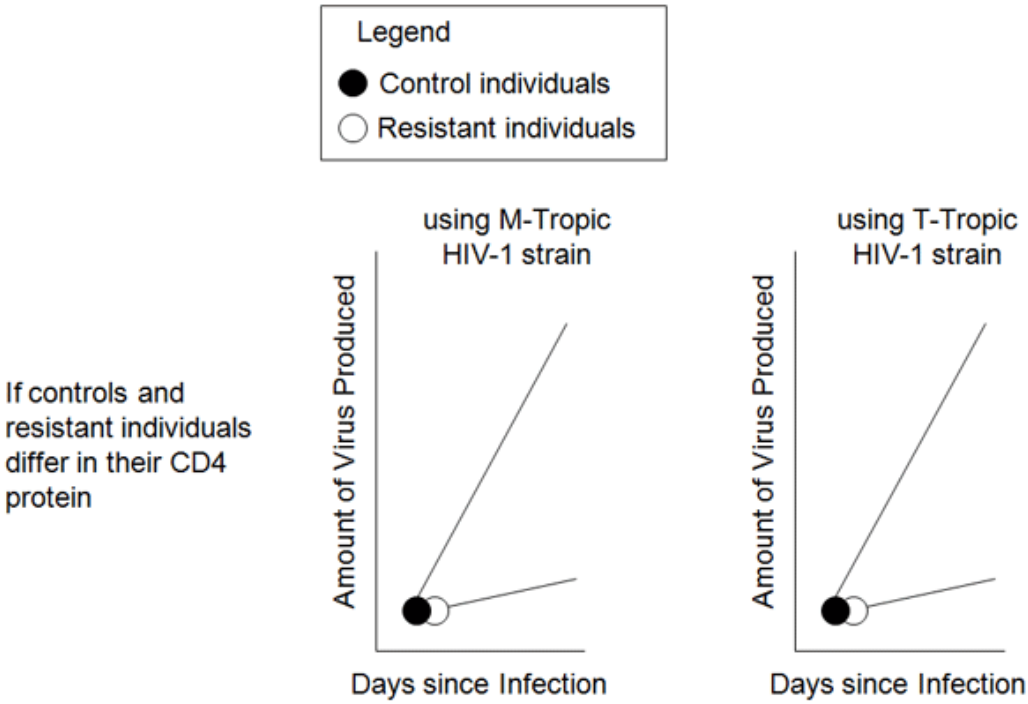
Among the population of protected individuals that Paxton's team has tested, some individuals (EU2 and EU3) appear to be resistant due to a "Super T Helper Cell" mechanism whereas others (EU4, EU9, EU11, EU12, EU17, EU18, and EU23) appear protected through a "Super Cytotoxic T Cell" mechanism.

c. What do you make of EU1? How do you account for his unusual response?

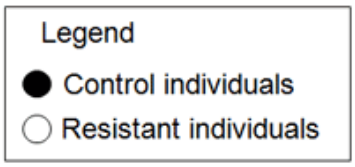
EU1 doesn't appear to be protected through a mechanism investigated by Paxton's team. Either EU1 has been very fortunate thus far (and is not resistant), or another mechanism of protection is at play (some possibilities were suggested in Question 4 of Part I and Question 1 of Part II).

Part V – The "Super T Helper Cell" Mechanism

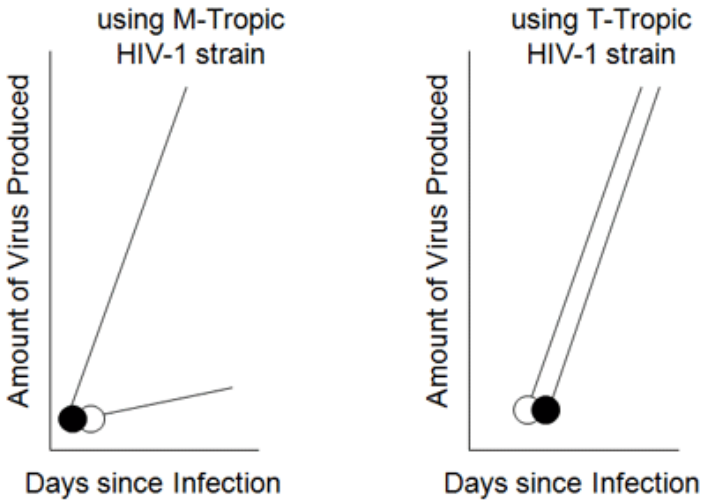
1. Let's assume that protected individuals have an altered CD4 protein (a mutation in the CD4 gene) compared to controls that renders the protein unrecognizable by gp120. Use the graphs below to draw the results you expect to obtain from the above-mentioned experiment. Remember that each graph should have two lines, and review which proteins are required for infection by the two strains.



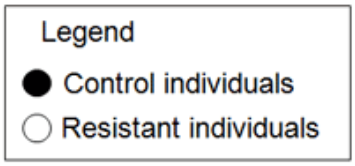
2. Let's assume that protected individuals have an altered CCR5 protein (a mutation in the CCR5 gene) compared to controls. Use the graphs below to draw the results you expect to obtain from the above-mentioned experiment. Remember that each graph should have two lines, and review which proteins are required for infection by the two strains.



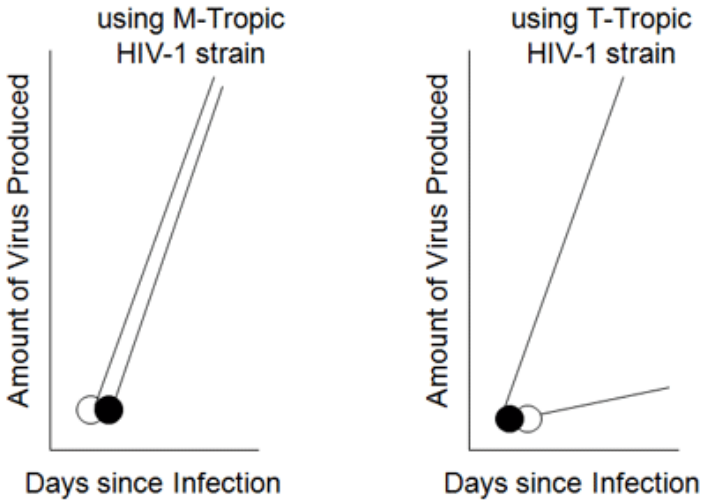
If controls and resistant individuals differ in their CCR5 protein



3. Let's assume that protected individuals have an altered CXCR4 protein (a mutation in the CXCR4 gene) compared to controls. Use the graphs below to draw the results you expect to obtain from the above-mentioned experiment. Remember that each graph should have two lines, and review which proteins are required for infection by the two strains.



If controls and resistant individuals differ in their CXCR4 protein



Part VI – Why Some People are Protected Against HIV

1. Infection:

a. Which strain(s) of HIV-1 can infect and replicate in the T_H cells of protected individuals?

T-tropic strains of HIV-1 can infect T_H cells from protected individuals.

b. Which co-receptor is used by this strain(s) of HIV-1 to infect these cells?

T-Tropic strains use CD4 and CXCR4 proteins to recognize and infect T_H cells.

2. No infection:

c. Which strain(s) of HIV-1 can not infect and replicate in the T_H cells of protected individuals?

M-tropic strains of HIV-1 can not infect T_H cells from protected individuals.

d. Which co-receptor is used by this strain(s) of HIV-1 to infect the cells?

M-Tropic strains use CD4 and CCR5 proteins to recognize and infect T_H cells.

3. Which of your theorized graphics do the results most resemble?

The data should mirror the predictions expected if the protected individuals have an altered CCR5 protein on the surface of their T_H cells (graphics produced in answer to Question 1 of Part V).

4. Based on this information, what is the mechanism of HIV protection in EU2 and EU3?

EU2 and EU3 are protected against HIV-1 because they have a mutation in the CCR5 gene, which causes the protein to be altered or not produced on the surface of T_H cells. As a consequence, the HIV-1 virus cannot effectively recognize and infect T_H cells and the individuals appear protected against HIV-1 infection despite repeated exposure.

5. Are these people protected against all forms of HIV out there? What are the implications?

It is clear from these experimental results that some forms of HIV-1 (namely the T-tropic strains) could infect such protected individuals. Thus, such protected individuals should not engage in high risk behavior without fear of infection.

Part VII – Societal Implications of HIV Protection

1. It is a relatively simple procedure to test the genotype of a person at the CCR5 gene to determine whether they have the CCR5Î”32 mutation. Should a person wishing to have their genotype tested be allowed to do so? What are the arguments for and against genotype testing of the CCR5 gene?

This could be used as an excellent classroom discussion. Here are some potential answers.

Pro:

- Individuals have a right to knowledge of their own genome.
- Individuals may wish to know their risk of infection following exposure to HIV.
- Encouraging individuals to take preventative measures when they realize they are not protected against HIV.

Con:

- Knowledge of homozygous CCR5Î”32 phenotype may give false sense of security and encourage individuals to engage in high risk activities, increasing their odds of infection. As discussed, they remain susceptible to some forms of HIV.

2. This mechanism of protection against HIV seems to rely on continued exposure to maintain the immunity. However, the mechanisms causing the protection are not well understood, and despite relative immunity these people could still be infected. What would you recommend to a person engaged in high risk activity that appears to exhibit protection against HIV-1? What lead you to make these recommendations?

This is an excellent question for classroom discussion. The issues to consider are that any activity that puts an individual in contact with the virus increases the probability of infection. However, once an individual has engaged in the high risk activity and seems to exhibit protection, the immunity only lasts as long as the individual engages in the high risk behavior. Thus, there may be an argument that if the person foresees exposure to the virus in the future, it is better to have continued exposure than to withdraw from the high risk activity and engage in it once again at a later time (once the tolerance has dissipated). Note that this is highly contentious.

3. A recent article in a popular science magazine (Wallace, 2009) reported on the study that uninfected partners of HIV-infected men who practice oral sex have higher levels of HIV-specific antibodies in their saliva. The title and subtitle of the articles were: "HIV resistance through oral sex: A new study suggests that repeated exposure can help produce resistant antibodies." Discuss the accuracy of this title. Does it represent what's known about this field of investigation appropriately? Why or why not? What sort of effects might this title have in our society?

The title of this article seems to imply that anyone can become protected against HIV by engaging in oral sex with HIV-infected individuals. This, of course, is false. It should be pointed out that the complete article does a good job of describing the study and the conclusions reached. However, someone who is simply browsing the magazine and only reads the headline might come away with an erroneous impression of these research findings. These erroneous impressions could lead to bad decision-making in regards to their own health.

4. In biology, the terms "resistance" and "immunity" have different meanings. Resistance is a pre-existing mutation in an organism that confers protection against a threat or challenge such as a virus. "Resistance" is used in the same manner as "antibiotic-resistance" in bacteria. "Immunity" refers to an active response of the immune system to the challenge of foreign particle that confers protection upon the organism. You have investigated many forms of protections against HIV. Which of these constitute resistance and which of them constitute immunity?

This case presents people who are protected against HIV by the two different mechanisms:

- Some people are *resistant* because they have a preexisting CCR5 mutation. (this is the essence of the "Super T Helper Cell" hypothesis).
- Others seem *immune* because they have a more aggressive immune system, and despite repeated exposure to the HIV virus they remain seronegative. (This is the essence of the "Super Cytotoxic T Cells" Hypothesis). This case presents several possible examples of this, such as the sex-workers with unusual HIV-specific cytotoxic T cell and interferon activity, as well as the HIV-antibodies described in the next section. Since the detailed mechanism by which the immunity against HIV works are not fully understood, it is possible that these examples of "HIV immunity" are based on a pre-existing genetic component which would make them "resistance" mechanisms of protection.

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