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

Paxton and his colleagues had a few hypotheses about why some of the individuals exposed to HIV were protected against this virus.

$CD8^+$ lymphocyte inhibition of HIV-1 replication ("Super Cytotoxic T Cells" Hypothesis)

Perhaps the reason that some individuals were protected against HIV is because they had cytotoxic T cells that were better and faster at recognizing infected T helper cells. This ability allowed the immune system to rid the body of any HIV infection before the virus could replicate inside T helper cells and transform these cells into HIV factories.

$CD4^+$ infectibility and efficiency of viral replication ("Super T Helper Cells" Hypothesis)

Perhaps the T helper cells of the protected individuals were different, preventing the infection and replication of the virus inside the cell. There are many steps necessary for viral infection and replication inside T helper cells and any of them could be impeded.

Questions

1. Classify each of *your* proposed hypotheses into the two categories proposed by Paxton and his colleagues (*Note:* some hypotheses may fit into neither category).
2. How might you test each of your hypotheses? Propose an experiment. What are your controls? Experimental conditions?

Part III – Predictions from Paxton's Two Hypotheses

Paxton and his colleagues recruited 25 volunteers who claimed to have had repeated exposure to the HIV virus and yet were not infected with HIV. He also enlisted the help of nine individuals not exposed to the HIV virus (and who tested negative for the virus). This latter group is the control, whose response to HIV should be the same as the response of the majority of people.

Paxton and his colleagues wanted to identify which of their two hypotheses might be correct. The problem with working *in vivo* is that it is unethical to expose individuals to HIV. In addition, the human immune system is complex, with multiple interactions. To isolate the action of T helper cells, cytotoxic T cells, and the HIV virus, Paxton and his colleagues worked in test tubes.

Paxton isolated T helper cells and cytotoxic T cells from individuals in each group. He then performed the following experiments:

- In one tube, he mixed HIV virus and T helper cells.
- In another tube, he mixed HIV virus, T helper cells, and cytotoxic T cells.

He monitored the accumulation of virus in the test tube over time by measuring the amount of p24 proteins produced.

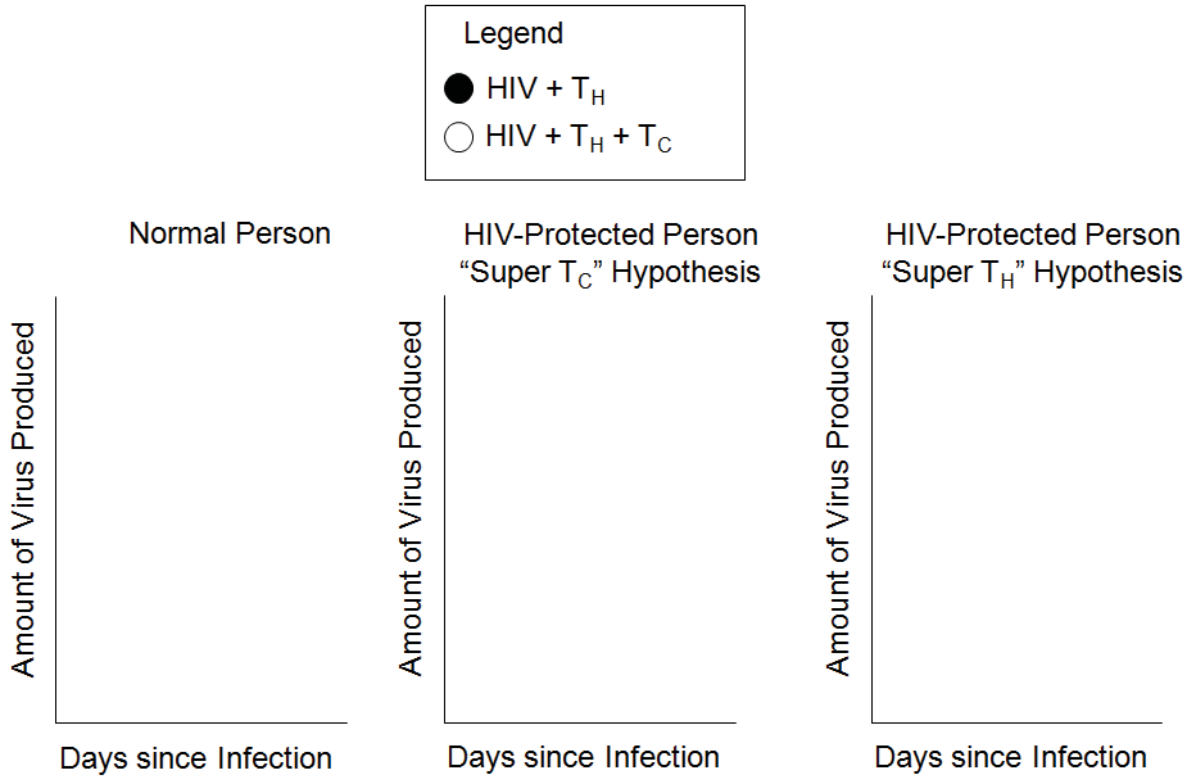
Questions

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

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"?

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

Below are Paxton's results (from Figure 1 of his paper). The graphs produced in the top part come from control individuals (each graph represents the results of experiments performed using cells from one person) (Note: LP = Leukopac Preparation, or blood obtained from random blood donors; LW = Laboratory Workers, i.e., people working in the lab). The bottom graphics show 10 selected results from people claiming to be protected against HIV infection (Note: EU = Exposed Uninfected individuals).

The filled circles (•) represent the results of experiments in which HIV was incubated with T helper cells, and the empty circles (o) represent experiments where HIV + T helper cells + cytotoxic T cells were mixed in the test tube.

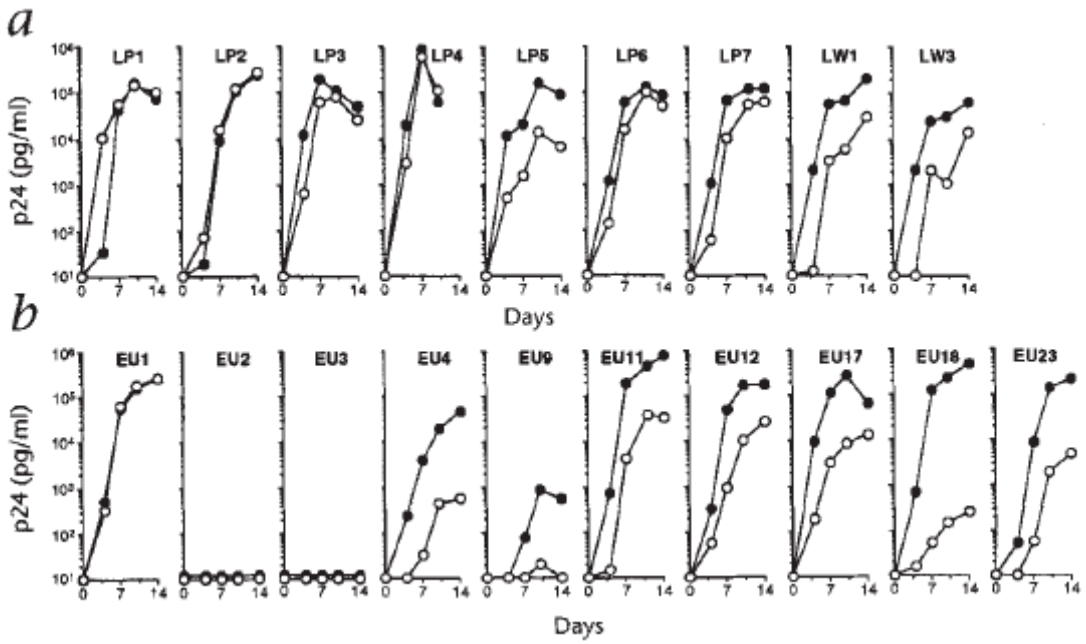


Figure 1 from: Paxton *et al.* (1996). Reprinted with permission of Macmillan Publishers Ltd: *Nature Medicine*, copyright 1996.

Questions

1. Do cytotoxic T cells provide protection from HIV in control individuals?
2. Try to identify patterns in the results. Can the individual experiments performed using cells from protected individuals be grouped into categories? If so, how many? Classify each subject into the different categories.
3. Compare these results with what you had predicted in the previous section.
   a. Are the results of the controls as you expected?
   b. Which of Paxton's hypotheses seem to be validated by the results of the protected individuals? Why?
   c. What do you make of EU1? How do you account for his unusual response?

Part V – The "Super T Helper Cell" Mechanism

From the results of this experiment, it is apparent that EU1 has either been lucky so far, or exhibits a mode of protection not anticipated by Paxton's team. EU2 and EU3 do not appear to be infected by the HIV virus at all ("Super T Helper Cells"). The remaining protected individuals exhibit different degrees of infection with very active cytotoxic T cells to slow down the progression of new infections ("Super Cytotoxic T Cells").

Paxton's team was particularly interested in protected subjects EU2 and EU3 and in investigating the mechanism of action of their protection against HIV. To investigate this, they performed an experiment where they mixed purified T helper cells from control or protected individuals with different strains of HIV-1. The goal was to determine whether all HIV-1 strains could infect the T helper cells from protected individuals. HIV-1, the most common form of the virus and the one responsible for the pandemic, can be classified into two different types:

- M-tropic (also called non-syncitia-inducing (NSI) or R5 HIV-1) strains, and
- T-tropic (also called syncitia-inducing (SI) or X4 HIV-1) strains.

This turned out to be a very informative experiment. About the same time, two other papers were published that clarified some of the differences between these two strains of virus.

- M-tropic HIV-1 strains must bind to two cell surface proteins to enter and infect a cell (Dragic et al., 1996):
  - the *CD4 protein* and
  - the beta-chemokine receptor *CCR5*.
- Conversely, T-tropic HIV-1 strains use slightly different proteins to enter and infect a cell (Feng et al., 1996):
  - the *CD4 protein* as well as
  - the alpha-chemokine receptor *CXCR4* (at the time called fusin).

Armed with this information, we can look back at the experiment performed by Paxton's team and investigate whether CD4, CCR5, CXCR4, or another protein is mutated and "different" in individuals that are protected against HIV.

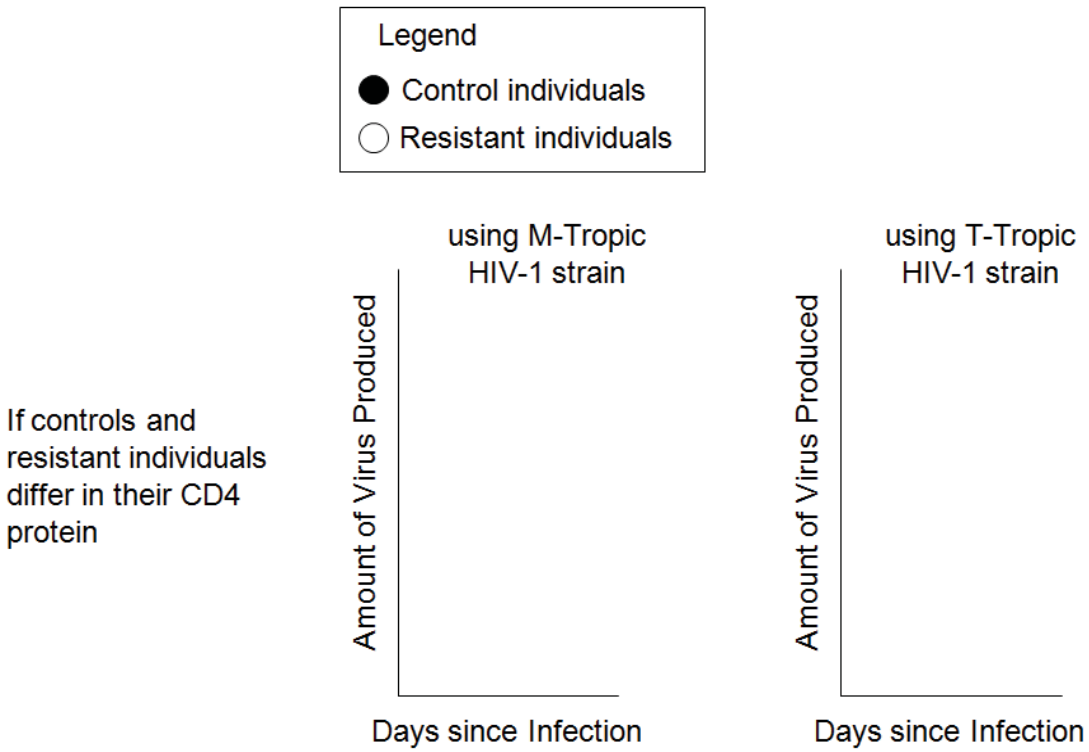
Here is the design of this experiment:

- In one tube: Mix HIV-1 (T-tropic strain) + T helper cells from a control person.
- In another tube: Mix HIV-1 (T-tropic strain) + T helper cells from a protected person.
- Monitor the appearance of p24 in the test tube (i.e., production of new virus) over time.

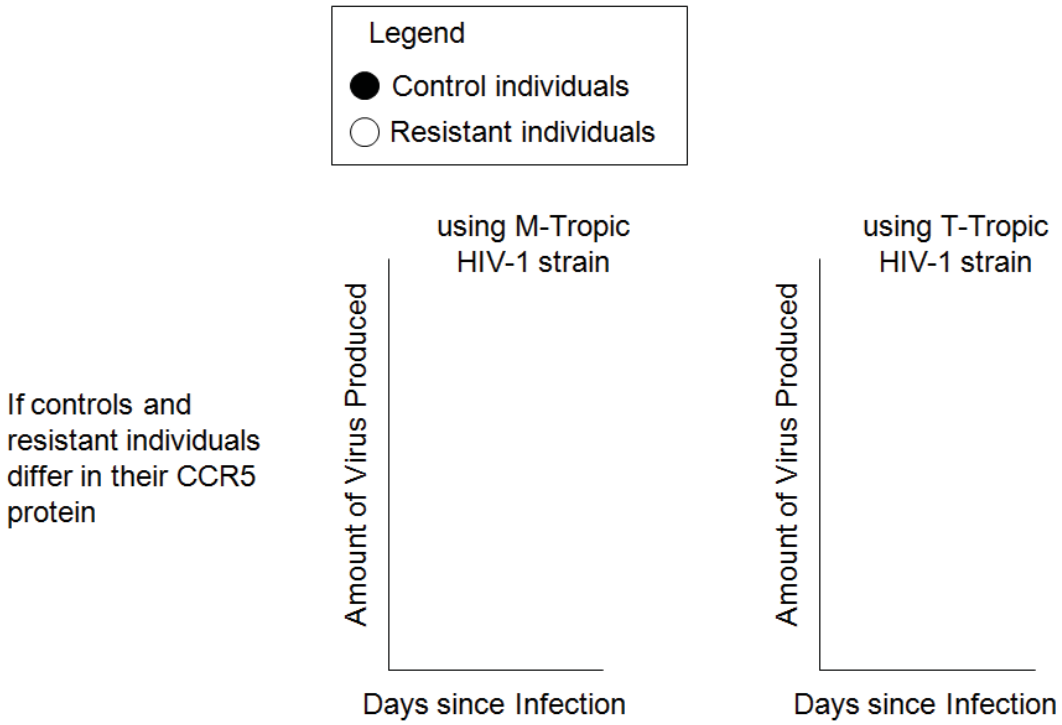
- In one tube: Mix HIV-1 (M-tropic strain) + T helper cells from a control person.
- In another tube: Mix HIV-1 (M-tropic strain) + T helper cells from a protected person.
- Monitor the appearance of p24 in the test tube (i.e., production of new virus) over time.

Questions

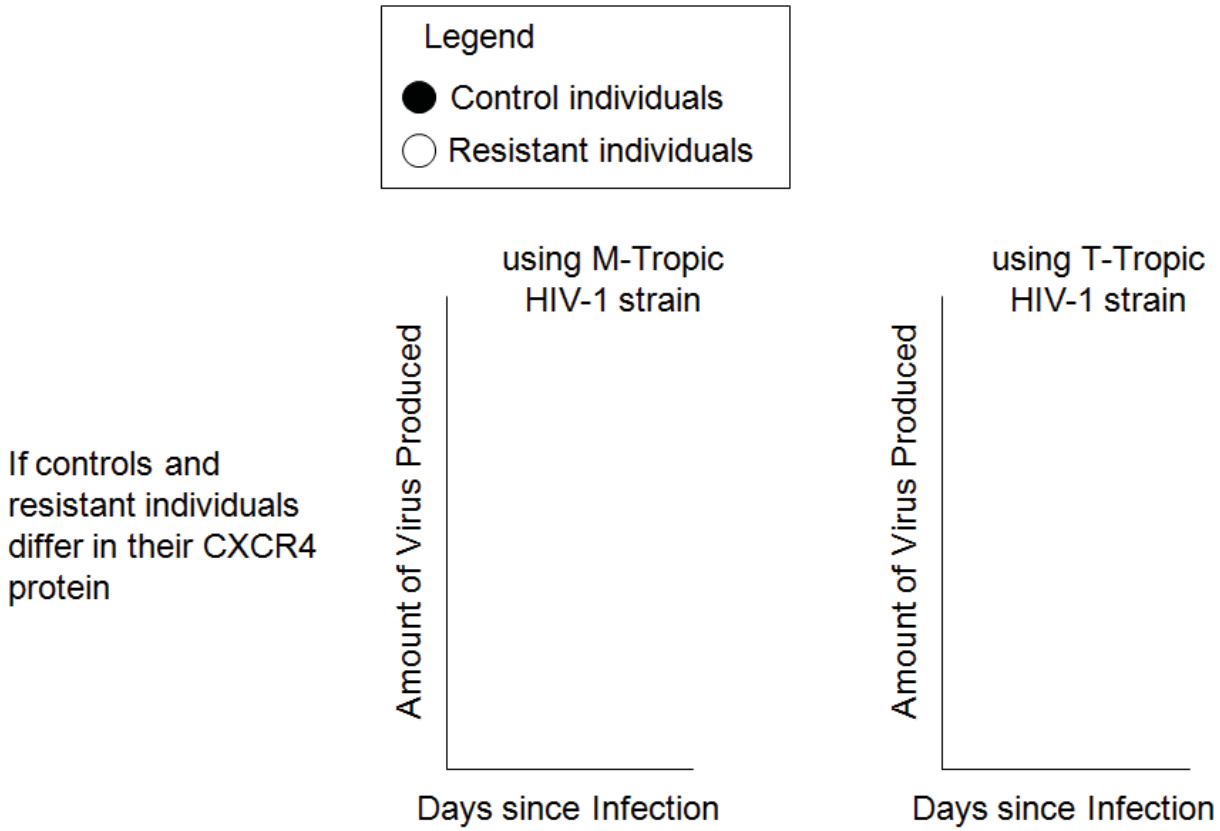
1. Let's assume that protected individuals have an altered CD4 protein (a mutation in the CD4 gene) compared to controls that renders the protein unrecognizable by gp120. Use the graphs below to draw the results you expect to obtain from the above- mentioned experiment. Remember that each graph should 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.



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.



Part VI – Why Some People are Protected Against HIV

Here are Paxton's results from this experiment. The filled circles (•) represent results using T helper cells from controls, and empty circles (o) using T helper cells from protected individuals. The letters and numbers above each graph show the name of the HIV-1 strain used in the experiment.

M-Tropic strains:

- JR-CSF
- GT
- SF162
- AD-6
- 92US657

T-Tropic strains:

- NL4-3
- SF2
- SF162dbl
- SF162 R3H

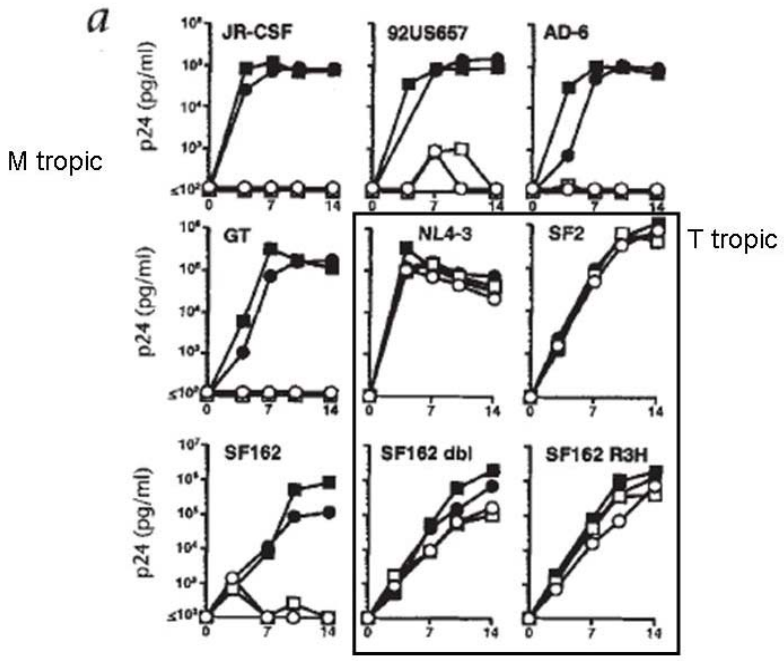


Figure 4 from: Paxton *et al.* (1996). Reprinted with permission of Macmillan Publishers Ltd: *Nature Medicine*, copyright 1996.

Questions

1. Infection:
   a. Which strain(s) of HIV-1 can infect and replicate in the T_H cells of protected individuals?
   b. Which co-receptor is used by this strain(s) of HIV-1 to infect these cells?
2. No infection:
   a. Which strain(s) of HIV-1 can not infect and replicate in the T_H cells of protected individuals?
   b. Which co-receptor is used by this strain(s) of HIV-1 to infect the cells?
3. Which of your theorized graphics do the results most resemble?
4. Based on this information, what is the mechanism of HIV protection in EU2 and EU3?
5. Are these people protected against all forms of HIV out there? What are the implications?

Part VII – Societal Implications of HIV Protection

Since this study, much has been learned about the mechanisms of protection against HIV. Here are some highlights.

"Super T Helper Cells"

In sexually transmitted HIV, the M-strain HIV-1 is the infectious agent 90% of the time (Ahmad, 2002). Thus, in most infections, the CD4 and CCR5 proteins are used by HIV to gain entry into T_H and infect the person.

Most of the individuals that are resistant through a "Super T Helper Cells" mechanism harbor the same mutation in their CCR5 gene. This is a deletion of 32 nucleotides that causes a frameshift in the reading sequence (Liu et al., 1996). Consequently, the cells of these individuals harbor no functional CCR5 protein. This does not appear to have any effect on the health of individuals. Since this mutation is found predominantly in populations of European decent, and since the mutation is first thought to have appeared in the population around 700 years, it has been hypothesized that the mutation confers resistance to *Yersinia pestis,* the infectious agent of the bubonic plague (Martinson et al., 1997). Others have suggested that the CCR5 mutation confers resistance to smallpox, and others still that this allele has spread in the population through neutral evolution (Sabeti et al., 2005). In populations of northern European descent, the frequency for CCR5Δ32 homozygous individuals is 1–3%, for heterozygotes it is about 14%, and for homozygote wild-type it is 83% (Sampson et al., 1996; Martinson et al., 1997).

Recent studies have shown that individuals homozygous for the CCR5 mutation are more prone to West Nile Virus infection (Glass et al., 2006). In addition, the lack of CCR5 protein makes mice more prone to hepatitis infection (Jefferys, 2006). These findings suggest that CCR5 might have a role in fighting other types of infections. This is an interesting finding, particularly in light of the fact that some experimental HIV therapies try to inhibit the expression of the CCR5 protein in healthy individuals.

As you probably have guessed from your answer to the questions in the previous section, some homozygous CCR5Δ32 individuals have tested positive for HIV infection (Biti et al., 1997; O'Brien et al., 1997).

Question

1. It is a relatively simple procedure to test the genotype of a person at the CCR5 gene to determine whether they have the CCR5Δ32 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?

"Super Cytotoxic T Cells"

Looking back to Figure 1 of Paxton's paper (Part IV of this case study), it seems that subjects EU4, 9, 11, 12, 17, 19, and 23 remained HIV negative despite repeated exposed to the virus by a mechanism that did not involve "Super T Helper Cells." In fact, starting in the early 1990s, there were reports of an exposed child, health care workers, and Kenyan prostitutes, all of which sustained repeated exposure, but who remained uninfected (Rowland-Jones et al., 1993; Pinto et al., 1995; Rowland-Jones et al., 1998). While luck may have played a part, the studies revealed that such individuals had unusual HIV-specific cytotoxic T cell activity. In fact, in the case of the immune Nairobi sex workers, it seems that their T_C are more active, respond to different signals, and are involved in the production of more interferon molecules than normal (Kaul et al, 2000; Kaul et al., 2001a; Kebba et al., 2004; Alimonti et al., 2006). Interferons are proteins released by an infected cell to warn other cells of the infection. The warned cells then take defensive measures

to protect themselves against infection. Similar results were found in studies of intravenous drug populations and partners of HIV-infected individuals (Biasin et al., 2000; Makedonas et al., 2002; Lo et al., 2003; John et al., 2004). Interestingly, it seems that repeated exposure is required for this form of immunity and that it is reduced when uninfected individuals reduce the frequency of their risky behavior (Kaul et al., 2001b; Yang et al., 2002).

Question

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 leads you to make these recommendations?

"Super B Cells and Antibodies?"

The body's first line of defense against HIV are the antibodies secreted in the mucosal surfaces (mouth, vagina, urethra). HIV-specific antibodies have been isolated in the mucus of resistant individuals engaged in oral, vaginal, or anal sex with HIV-infected individuals (Hirbod et al., 2008; Hasselrot et al., 2009). Control subjects did not produce this antibody response in their secretions. These antibodies appear to recognize and inactivate HIV virus in a test tube. Whether these antibodies help protect the uninfected individuals is an active area of study.

Questions

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?
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 particles 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?

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