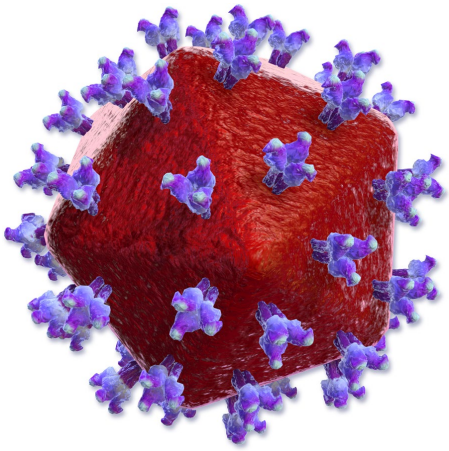
NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

The Unluckiest Man in the World?

An Examination of Immune System Function



by

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Part I – Introduction

The year was 2006 and Mr. Timothy Ray Brown was sitting in the doctor's office concerned about his health. Eleven years earlier, in 1995, he had experienced what he thought was the worst day of his life, sitting in another doctor's office. It was then that he had received the news that he was HIV positive. At that time he thought he was receiving a death sentence. There were no effective HIV treatments in 1995 and Mr. Brown was expected to live for only a few more years. Luckily for him, successful treatment using highly active antiretroviral therapy (HAART) with the first protease inhibitor drugs became available in 1996. Mr. Brown demonstrated only moderate side effects to the drugs, his German heath care coverage paid for their cost, and for over ten years he had been living a rather normal life. That was until eleven years later, when extreme fatigue and shortness of breath caused him to go back to the doctor.

After talking with Mr. Brown, the doctor decided to run a complete blood count (CBC) with differential, a $CD4^+$ T-cell count and an HIV RNA test to determine if his antiretroviral therapy had stopped working. A CBC measures the number of all the different cell types in the blood, while the differential specifically looks at the different types of white blood cells. An HIV RNA test measures the number of HIV copies there are in a milliliter of blood.

Question

1. Keeping in mind what you have learned about development of resistance to antimicrobials, what is one reason that Mr. Brown's therapy may no longer be successful even though it had been working for ten years?

While waiting for Mr. Brown's test results, the doctor spent time refreshing his knowledge of HIV infection in order to better answer questions that Mr. Brown might have. Figure 1 (see next page) illustrates the life cycle of the HIV virus. Use this figure to answer the following questions.

Question

2. Is the HIV genome made of DNA or RNA?

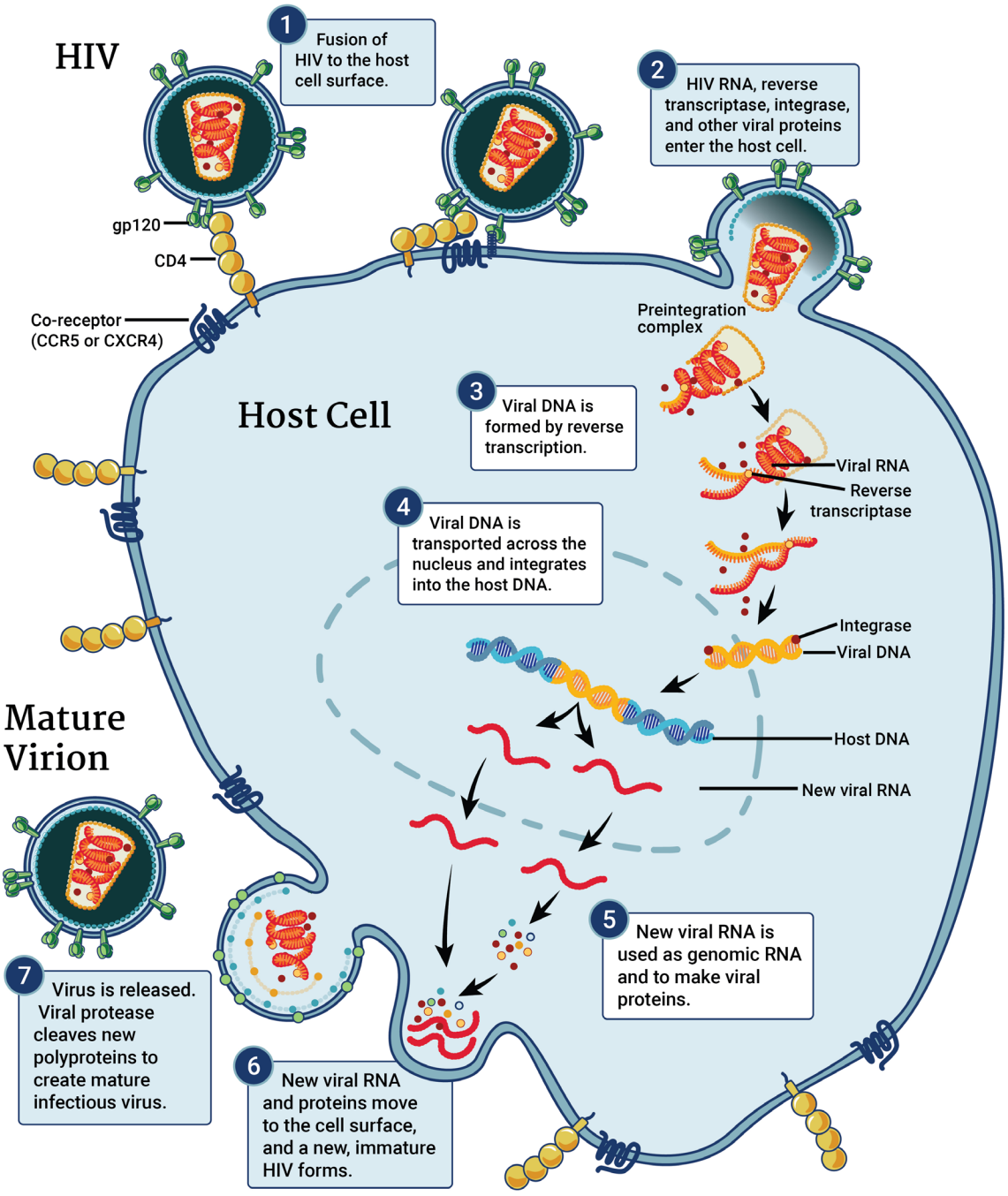

Figure 1. HIV replication cycle. *Credit:* NIH–NIAID, CC BY 2.0, https://www.niaid.nih.gov/diseases-conditions/hiv-replication-cycle.

3. HIV requires two host cell receptors for infection. The first allows attachment of HIV to the cell, the second allows membrane fusion for entry. Looking at the life cycle figure, which host cell membrane proteins are involved in these two steps?

4. Based on your answer above, what specific type of immune cell does the HIV virus infect?

5. When the virus leaves the cell to infect other cells, is the virus released from the cell through lysis or budding?

6. Based on its mechanism of exit, would you classify this as an enveloped or naked virus?

Once the virus enters the cell, the virus is able to replicate and the number of virus particles in the body increases. Look at the graph (Figure 2) to answer the questions below.

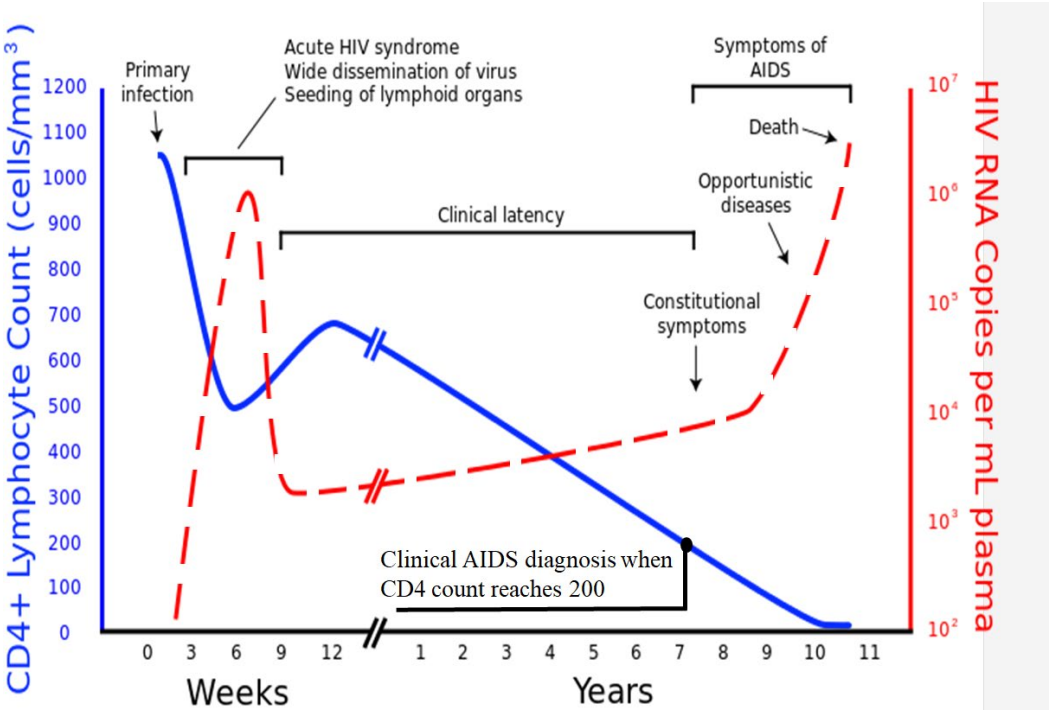

Figure 2. HIV viral load in relation to $CD4^+$ lymphocyte count.
Credit: Sigve, PD, <https://en.wikipedia.org/wiki/File:Hiv-timecourse_copy.svg>.

7. Describe what occurs to the level of viral particles (viral load, measured by copies of RNA per mL) over the course of an HIV infection.

8. Describe what occurs to the levels of $CD4^+$ lymphocytes over the course of an HIV infection. How does this relate to the level of viral particles?

9. You should have noticed that during the first six weeks of infection the viral load rapidly increases, which is tied to a decrease in the number of CD4 T-cells. However, the virus is released from these cells by budding, thus only a portion of the $CD4^+$ cell death is caused by the virus itself. The decrease in cell number is more directly caused by the body's immune response to the HIV infection. Discuss with your group what you know about the adaptive immune system and how it combats viral infections. (You should discuss the role of the MHC molecules and T-cell responses.) After your discussion, define the role of each of the molecules and cells listed below and then hypothesize what could be leading to the decrease in CD4 cell numbers. Explain how that would occur.

   Role of MHC I:

   Role of MHC II:

   Role of TH cells:

   Role of TC cells:

   Hypothesis and explanation:

10. Once the disease has progressed to the AIDS stage you observe a large increase in viral load and a crash in the number of CD4 T-cells. However, AIDS patients do not die from the HIV virus itself; they typically die from a separate infection by a bacterial, viral, or fungal pathogen. Based on the normal function of the immune system, explain why AIDS patients die from these additional infections.

11. There is currently a clinical trial underway that is testing a protein subunit vaccine for HIV. Subunit vaccines consist of purified protein antigens that have been produced using biotechnology. They are based on the concept that humoral immune responses target specific localized regions of proteins so the entire pathogen is not required to mount an immune response. Discuss with your group how antibodies (one of the products of vaccination) function to protect the body. What are three ways that antibodies help protect the body from pathogens?

12. Look back at the life cycle of HIV. If you were working in a biotechnology company, what HIV protein would you use to develop your subunit vaccine? Explain you reasoning.

Part II – The Test Results

Three days after seeing the doctor because of his fatigue Mr. Brown's lab results were available. The first three tables below describe the normal cell ranges and results for each test. You will need to use these to draw conclusions regarding Mr. Brown's results.

HIV RNA Test

Number of RNA copies	*Evaluation*
100,000 +	High viral load; virus is actively replicating/making more copies of itself. The disease may progress quickly.
200–10,000	Lower viral load; virus is not rapidly reproducing but can indicate treatment failure.
20–75	Low levels, not causing disease symptoms.
< 20	Below level of detection, person is still infected but not showing symptoms.

$CD4^+$ T-Cell Count

This is the most important lab indicator of immune function and disease progression.

Number of $CD4^+$ T-cells	*Evaluation*
> 500 cells/ml	Optimal levels, continued annual measurements is optional.
300–500 cells/ml	Below normal, monitoring should continue on an annual basis.
< 200 cells/ml	Disease has progressed to a clinical diagnosis of AIDS, health will begin failing.

CBC Plus Differential

The table below provides the normal value of all the cell types found in a CDC plus dif test.

1. Fill in the cell function column of the table based on what you have learned in class. Refer to your notes as needed.

Cell type	*Normal value (cells/ul)*	*Cell Function/Definition*
Red Blood Cells	3.77–5.28 × 106/ul	
Platelets	150,000 – 379,000/ul	
White Blood Cells	3,400 – 10,800/ul	
Monocytes	100 – 900/ul	
Eosinophils	0 – 400/ul	
Basophils	0 – 200/ul	
Lymphocytes	700 – 3,100/ul	
Neutrophils	1600 – 7500/ul	
Myeloblasts (Immature Granulocytes)	0 – 100/ul	

2. The table below shows Mr. Brown's results for each of the tests described above. Compare his levels to the normal range and write if they are low, normal, or high in the conclusion column. For the HIV RNA test, state his HIV disease status.

Mr. Brown's Results

Test	*Mr. Brown' Results*	*Conclusion*
HIV RNA Copies	Too low to detect	
$CD4^+$ T-Cell Count	700 cells/ml	
Red Blood Cells	2.02×10^6/ul	
Platelets	20,000/ul	
White Blood Cells	35,600/ul	
Monocytes	1340/ul	
Eosinophils	100/ul	
Basophils	100/ul	
Lymphocytes	1680/ul	
Neutrophils	8400/ul	
Myeloblasts	22,120/ul	

3. Based on Mr. Brown's test results, would you conclude that his HIV infection has become resistant to his antiretroviral therapy? Why?

4. You should have observed that Mr. Brown's white blood cell (WBC) count was above normal. The WBC count is a sum of all the cells of the immune system, which were then counted separately during the differential count. Looking at the individual immune cell types, what cell type is most responsible for the higher than normal white blood cell count (i.e., which one is much higher than normal)?

5. Think about the normal function and location of the cell type you listed above. The CBC/dif detected 22,120 myeloblasts per µl of blood. Based on the normal function/location why might this be problematic? (In other words, where are these cells supposed to be located in order to do their function?)

Part III – A Pioneer Treatment

The doctor contacted Mr. Brown and gave him mixed news. His HIV was still controlled by his antiretrovirals, so HIV was not responsible for his symptoms. Instead he was diagnosed with acute myeloid leukemia (AML). AML is a cancer of the blood that begins in the bone marrow when the myeloblasts develop a series of mutations, which allow them to divide uncontrollably. The myeloblasts accumulate and crowd out the normal cells, eventually spilling out into the blood stream. These cells prevent normal blood cells, like red blood cells, from differentiating, leading to the anemia that caused Mr. Brown's fatigue symptoms. AML can progress rapidly and be fatal within months if left untreated.

Mr. Brown started chemotherapy to treat his AML immediately after his diagnosis. Unfortunately, the standard AML chemotherapy treatment did not successfully treat his cancer, so in 2007 he was scheduled for a hematopoietic stem cell transplant (bone marrow transplant). A bone marrow transplant replaces a person's own hematopoietic stem cells in the bone marrow with stem cells from donor marrow. In order to do this, the patient's own cells must first be destroyed through "conditioning," a process which uses an intense chemotherapy and radiation regimen to destroy nearly all cancerous and healthy bone marrow and immune cells.

In the 1980s doctors had speculated that a bone marrow transplant might be a cure for HIV because the infected $CD4^+$ T-cells would be destroyed during conditioning, and replaced by uninfected donor cells. During conditioning however, approximately 1% of a patient's T-cell population is not destroyed, leaving a small reservoir of virus still hiding in those cells. That small amount of virus is enough to infect the new donor cells after transplant. Mr. Brown's doctor remembered a paper he had read while in medical school that he thought might allow them to prevent any remaining virus from spreading to donor cells after transplant. The paper looked at the prevalence of a certain genetic mutation in the CCR5 gene in individuals who were at high risk of exposure to HIV. The mutation they were studying causes the CCR5 protein to be truncated and not expressed on the cell's surface. The researchers were trying to determine if this mutation affected people's susceptibility to HIV virus infection.

The data from the paper is shown below (Table 1). The "+" indicates a wild-type (normal) allele, the Δ32 (read as delta 32) is the mutated allele. Remember each person inherits two copies, or alleles, of a gene. The two alleles of the CCR5 gene are shown with a "/" separating them. Discuss the table with your group and use it to answer the questions below.

Table 1. CCR5 genotype distribution among HIV-1-seropositive and HIV-1-seronegative individuals in the same risk group. Modified from Table 2 in Dean et al. (1996). (*Note:* "CCR5" reflects current nomenclature for the chemokine receptor 5 gene/protein; in the original article it appears as "CKR5.")

			Number of patients (% total) with CCR5 genotype		
Risk Group	*HIV status*	*Total #*	*+/+*	*+/Δ32*	*Δ32/Δ32*
Homosexual men and hemophiliacs	Positive	1343	1148 (85)	195 (15)	0 (0)
	Negative	612	508 (83)	87 (14)	17 (3)*
* association of genotype with HIV-negative status p value = 2.5×10^{-8}					

Questions

1. What percentage of HIV-positive individuals are wild-type (+) for both of the CCR5 alleles? HIV-negative individuals?

2. What percentage of HIV-positive individuals are homozygous (have two copies) of the Δ32 CCR5 mutation? HIV-negative individuals?

3. Based on the data, does having theΔ32 CCR5 mutation increase or decrease your risk of contracting HIV?

4. Looking back at the life-cycle of HIV, why do you think the CCR5 mutation seems to prevent infection from the virus?

Mr. Brown's doctor decided he had to find Mr. Brown a stem cell donor that was not only a match for transplant purposes but who was also homozygous for the Δ32 protective mutation. Out of 232 tissue-type matches, donor number 61 tested positive for both copies of the Δ32 mutation. On the day of the transplant Mr. Brown stopped taking his antiretroviral drugs. Nine years after his stem cell transplant, Mr. Brown turned 50 years old. He has never restarted his antiretroviral therapy and remains HIV free. He was the first person to be cured of HIV.

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