

Evolution and HIV

Why do we care?

1. HIV is an emerging/emergent virus.
2. HIV rapidly evolves to become drug resistant and to evade the immune system.
3. HIV is deadly.
4. HIV is responsible for 5% of all deaths worldwide (more than car accidents, malaria, war and homicides).

The natural history of the HIV/AIDS epidemic

Past major disease epidemics:

"Spanish" influenza of 1918
50-100 million people died in a few months.

Black Death (plague) – between 1346-1350
28 million people died, amounting to ~30% of Europe's population.

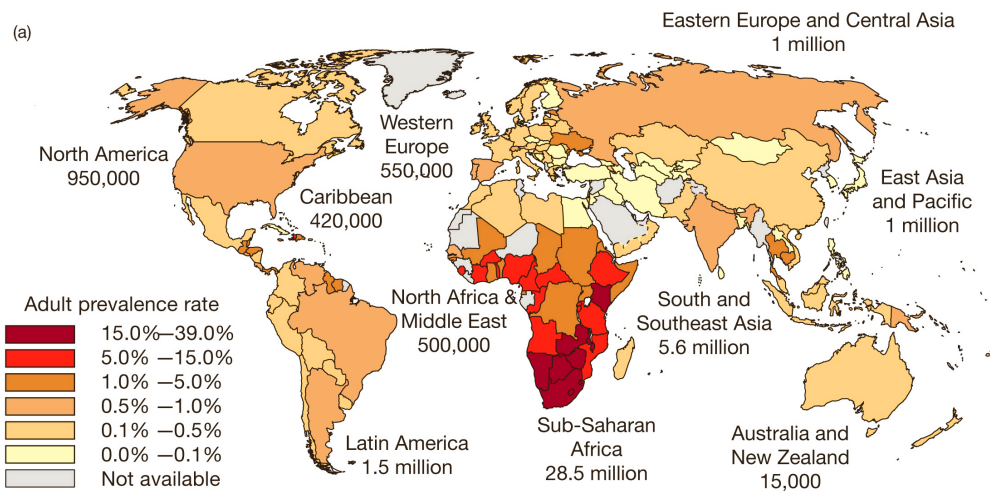
New World smallpox epidemic – ca. 1520
Decimated 25% of Native American populations on two continents.

Today, HIV/AIDS is a serious epidemic on the same level as these prior epidemics.

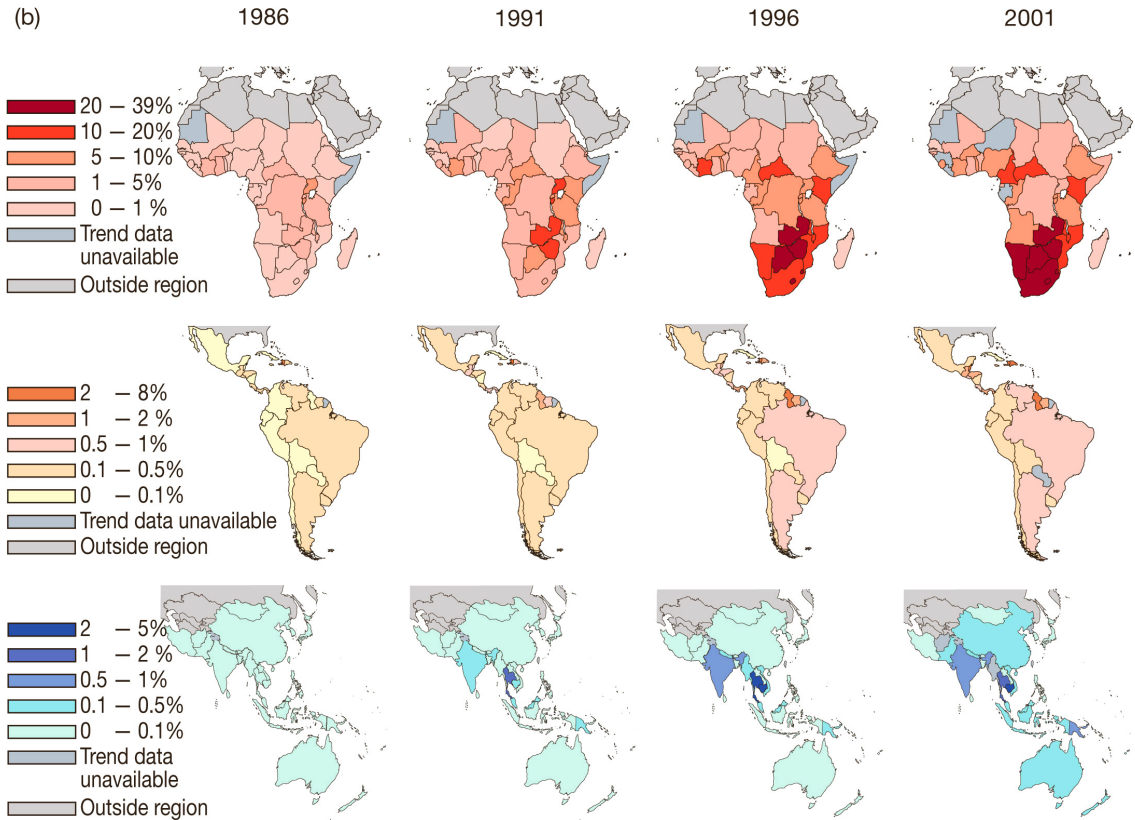
70 million have been infected, while one-half of those have died.

WHO estimates that by 2020, nearly 90 million will have died.

90% of infected people live in developing nations



Worldwide prevalence of HIV-1 infections



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What is HIV?

HIV (human immunodeficiency virus) is a lentivirus that can lead to *acquired immunodeficiency syndrome* (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening, opportunistic infections.

The HIV infection process

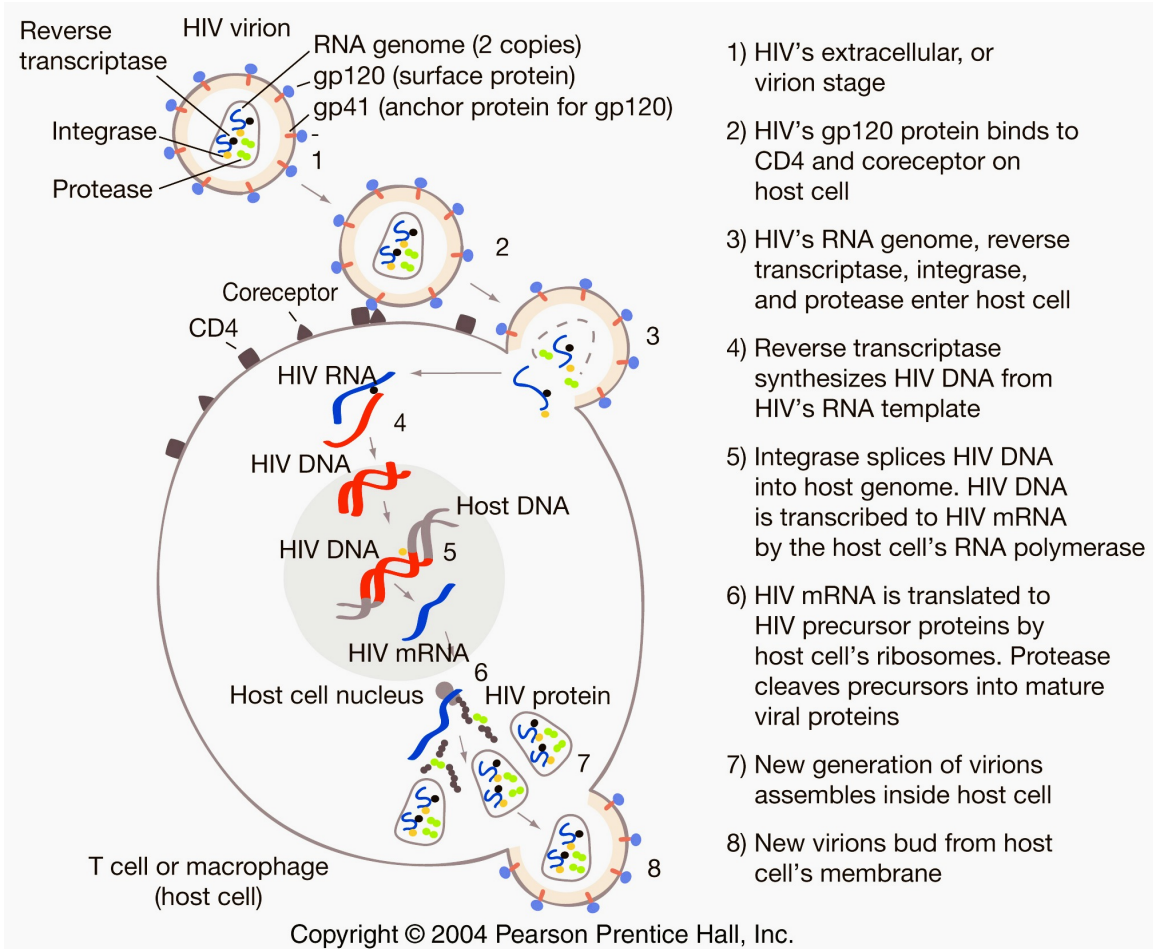
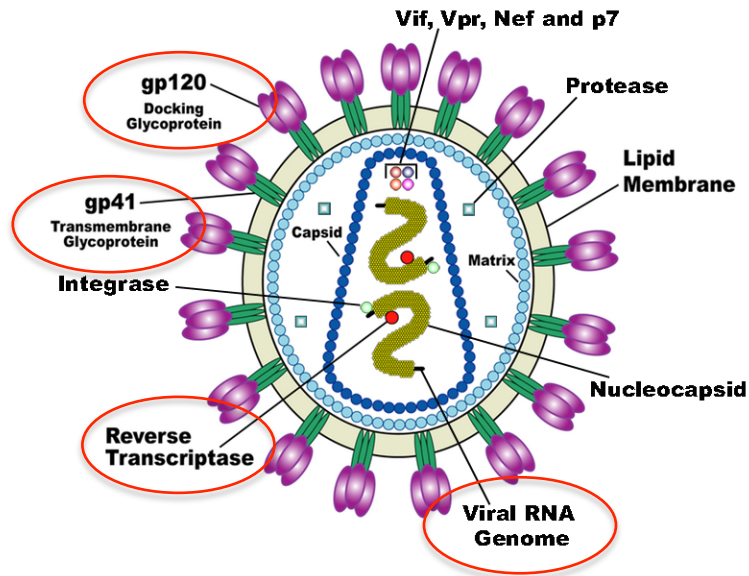
A virus is *not* alive

HIV (and all other viruses) is obligated to use the cellular machinery of host macrophages and T-cells for reproduction, killing the host cell in the process.

HIV is transmitted from person to person when bodily fluids carry the virus to the bloodstream or mucus membrane of another person.

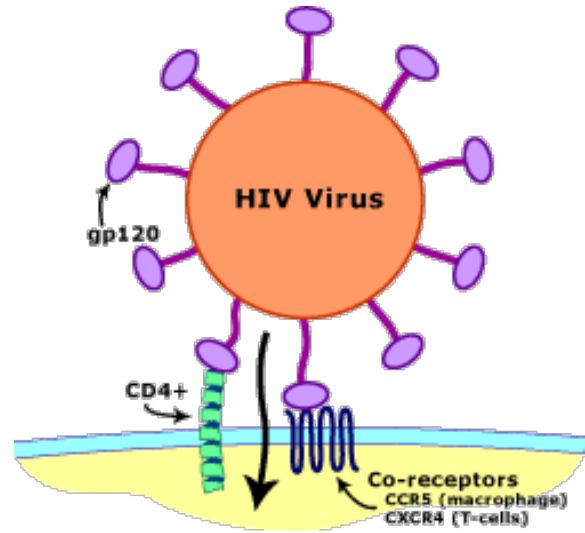
- Sexual activity
- IV needle sharing
- Transfusion of contaminated blood
- Childbirth
- Breastfeeding

1. Structure of HIV



2. HIV replication is dependent on **attachment** to two proteins on the surface of the host cell.

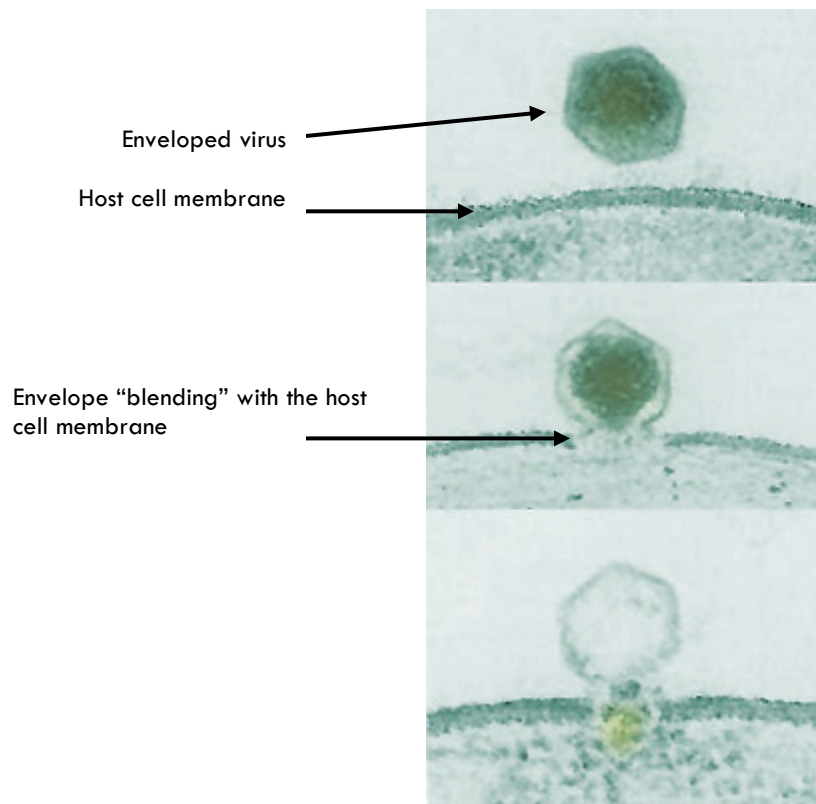
CD4 and a co-receptor



Affinity for immune cells is largely due to the specificity of *gp120* as it interacts with CD4 and the co-receptors.

3. Virus entry

Upon attachment, the viral envelope “blends” with the cell membrane, which opens the cell membrane so the viral components can enter.



The HIV genome (two identical RNA molecules) including the genes for three important proteins enter the cell.

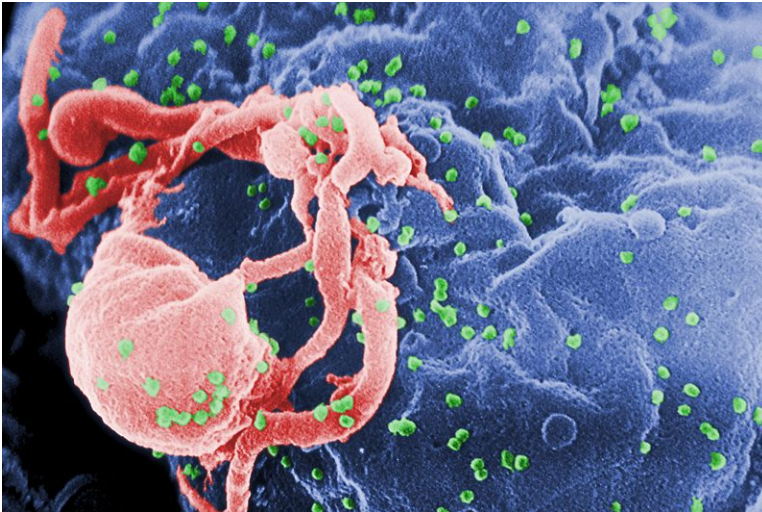
4. **Reverse transcriptase** transcribes RNA to DNA (*retrovirus*).
5. Host cells synthesize HIV **integrase**, which is a protein that splices the transcribed viral DNA into the host genome.

The host cellular machinery processes the viral DNA along with its own DNA.

Transcription, translation, protein synthesis.

6. **Protease** assists in cleaving viral precursor structures into new viruses.
7. Viruses assembly is completed inside the host.
8. New viruses leave the cell through a process called budding, and enter the bloodstream.

The release of HIV causes damage to the host cell, as the envelopes of the new viruses are actually parts of the host cell membrane.



Scanning electron micrograph of HIV-1 (in green) budding from cultured lymphocyte (in blue). Multiple round bumps on the cell surface represent sites of assembly and budding of virions. From: Centers for Disease Control and Prevention

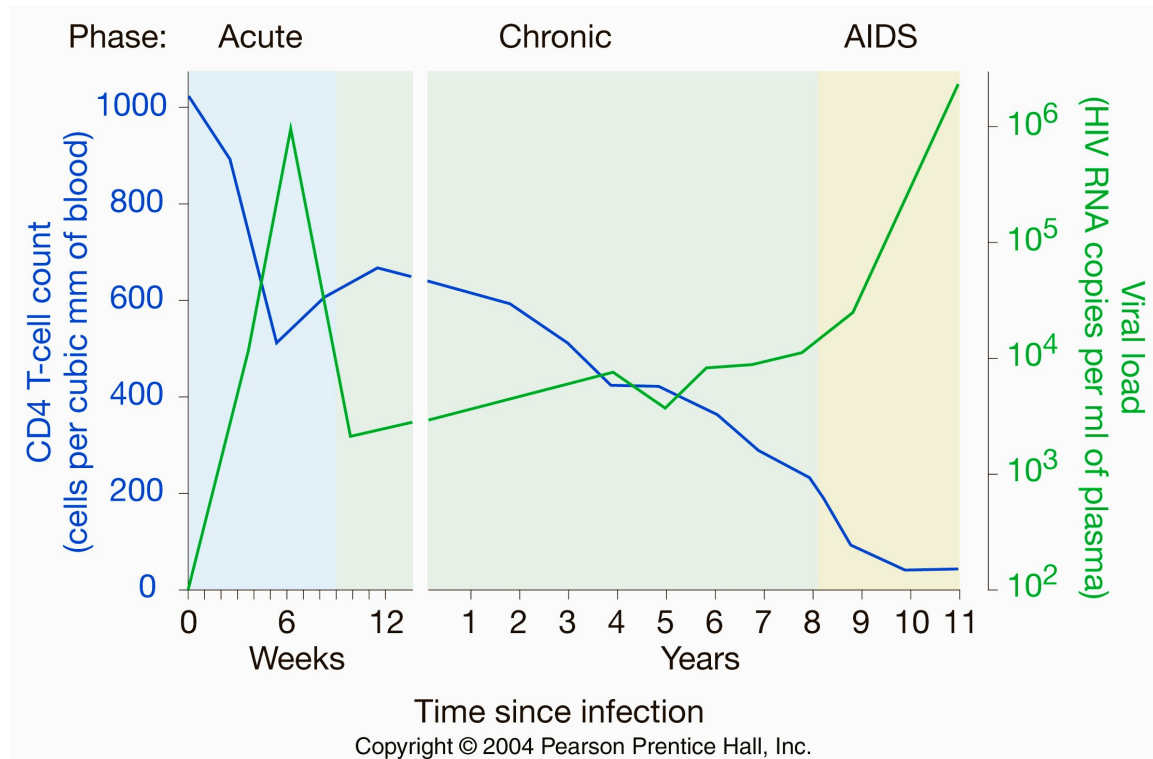
It is important to recognize that the host cellular machinery (polymerases, ribosomes, tRNAs) is used in almost every step of the infection process. Therefore, therapies that target these steps to stop HIV replication often have deleterious side effects on the host.

How does HIV cause AIDS?

The host responds to HIV infection by destroying (i) viruses in the bloodstream and (ii) its own infected cells before new viruses are assembled and released.

Problem – Destroying infected T-cells and macrophages destroys the cells necessary to prevent further infection in the host. The immune system collapses.

Progression of the HIV infection (three phases)



- 1. Acute phase** – HIV enters the host and replicate rapidly, resulting in a decreased number/concentration of CD4 helper T-cells.

The acute phase ends when the immune system mobilizes against the infection, causing the virus concentration to fall and the CD4 T-cell count to recover, but not fully.

- 2. Chronic phase** - HIV replication continues at a slow rate, but consistently increases the viral load while the CD4 T-cell counts decrease.

HIV is killing some T-cells and the immune system is killing other infected ones.

The chronic phase ends when the T-cell count falls below $200 \text{ cells mm}^{-3}$.

3. AIDS – The immune system no longer functions, and the victim eventually succumbs to infections that are easily cleared by a healthy immune system.

Without treatment, patients die within 2-3 years after progressing to AIDS.

Evolution and HIV: The story of AZT

Antiviral treatment – AZT (azidothymidine) was developed as one of the first anti-HIV therapies (1980s).

Works in the short run, but soon fails. **Why?**

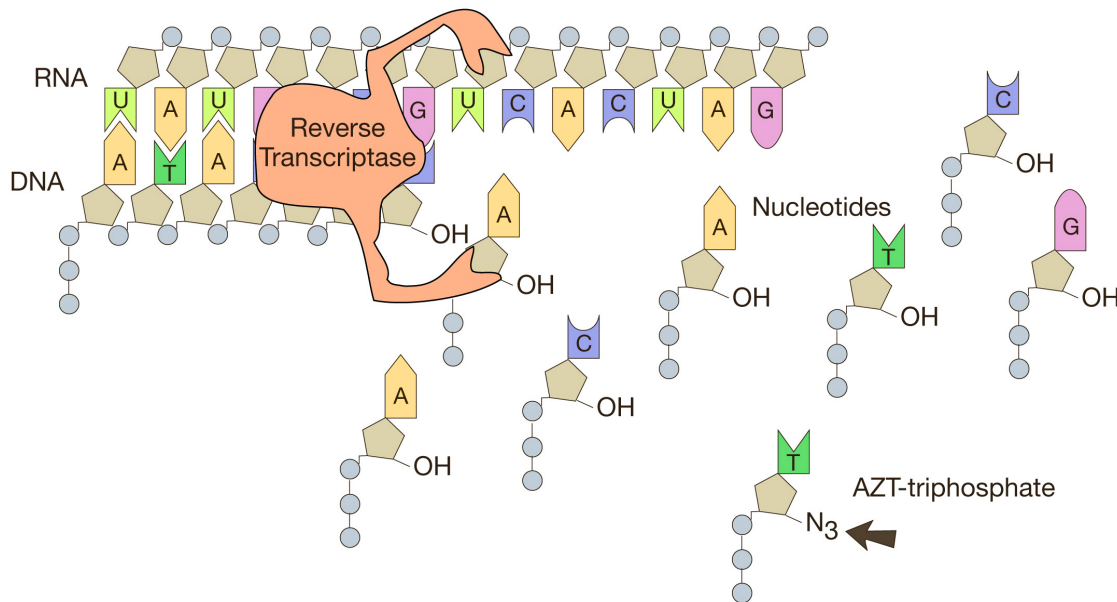
HIV is one of the fastest evolving entities known. Two factors drive this:

1. HIV reproduces quickly, as millions of HIV copies can be produced following the infection of a single HIV virion in a single day.
2. It reproduces sloppily, resulting in the accumulation of many mutations during DNA replication.

To combat viral infections drugs are used that target enzymes special to the virus.

For example, a drug that blocks reverse transcription of viral RNA to DNA would effectively limit replication.

This is the rationale behind AZT therapy.



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AZT stops reverse transcription by fooling the reverse transcriptase into selecting a nucleotide that contains an azido-group (N3) instead of a hydroxyl (OH).

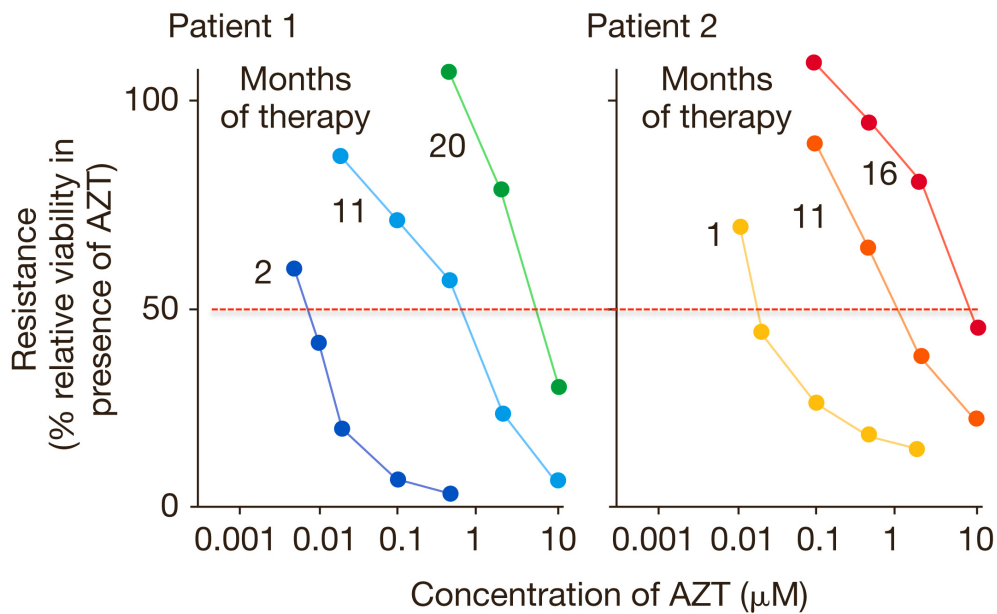
Additional nucleotides cannot be added to the nucleotide containing N3 group, and reverse transcription of DNA is halted.

Early uses (mid-1980s) of AZT therapy were successful, but within a few years of use, patients stopped responding to treatments. **Why?**

Perhaps the viruses within an infected patient changed to become resistant to the disruption by AZT.

Samples collected over time from infected patients showed that this was, in fact, happening.

(a) Resistance of HIV in two patients, followed over time



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Data showed that:

1. In every case, increased AZT decreases the viability of HIV.

AZT works, somewhat, but...

2. The virus was changing, such that it took greater AZT doses (1000-fold more in some cases) to limit HIV replication.

What was happening to the susceptible virus to allow it to resist treatment?

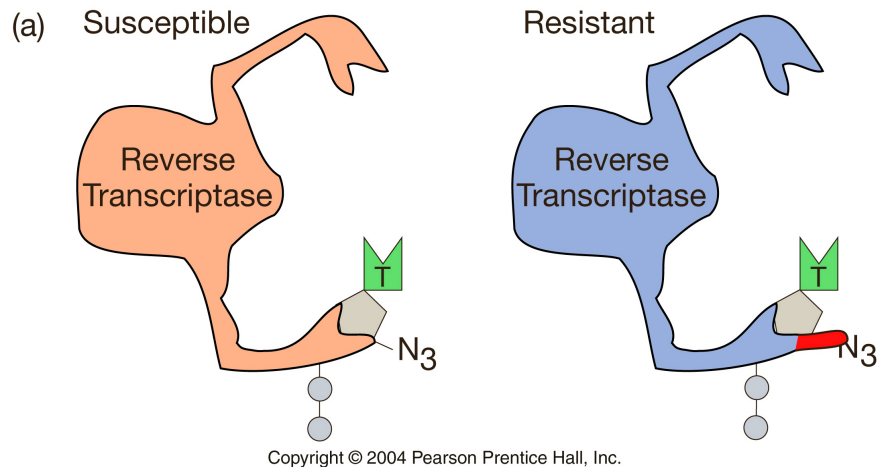
The reverse transcriptase genes of HIV collected from the patients were sequenced to determine if any mutations had occurred that might confer resistance to AZT.

Viral strains present late in treatment were genetically distinct from those present in the same individual before treatment.

Mutations in the reverse transcriptase gene led to specific amino acid substitutions in the active site of the reverse transcriptase.

Allowed the reverse transcriptase to discriminate between azidothymidine and normal thymidine.

The mutant HIV could now replicate in the presence of AZT.



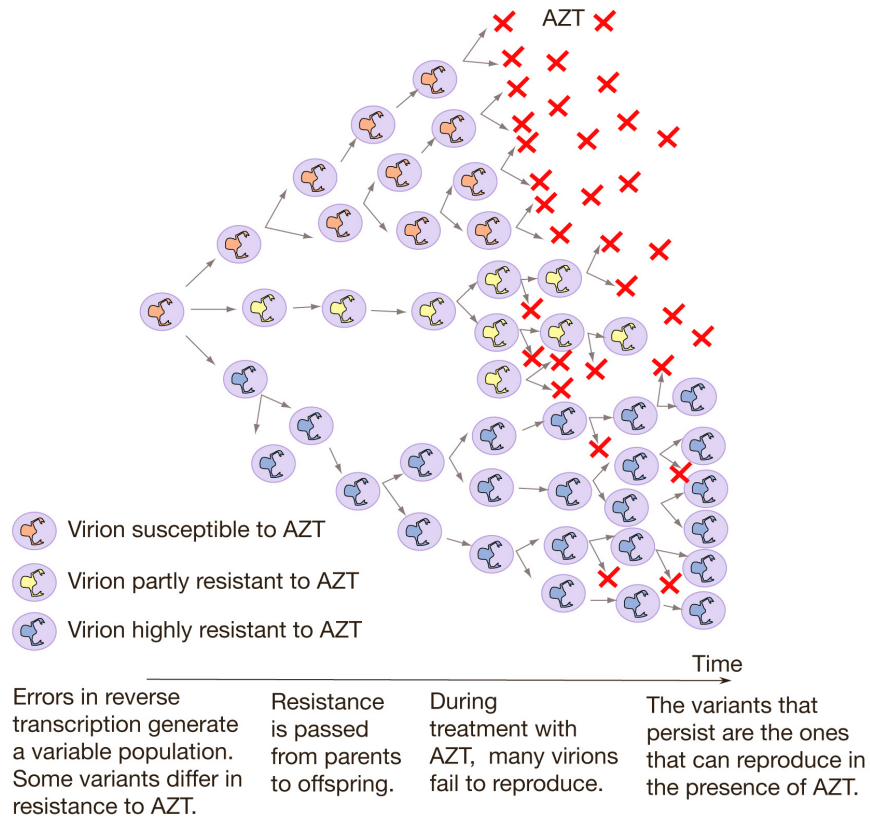
How did this happen?...Error-prone enzymes.

HIV has highest mutation rate of any virus observed to date.

During the course of an infection, a single strain of HIV can produce hundreds of different variants in any given protein/enzyme, including reverse transcriptase.

Over one-half of the DNA transcripts produced by HIV reverse transcriptase contain at least one mistake in the nucleotide sequence.

AZT therapy would actually increase the proportion of mutants able to discriminate against it because the "wild-types" would be killed, leaving behind the mutants.



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This is evolution by natural selection.

AZT has now been abandoned as an stand-alone HIV therapy.

Why is HIV successful?

After the *Acute Phase* of the infection, host antibodies and T-cells begin to recognize HIV and HIV-infected cells because of short viral proteins (epitopes) on the surface of the virus or infected cells.

Epitopes are encoded in the genes of HIV. Therefore, mutations in these genes (remember, *sloppy enzymes*) can change the "look" of the epitopes → poor recognition by host.

HIV with altered epitopes grow in number and persist.

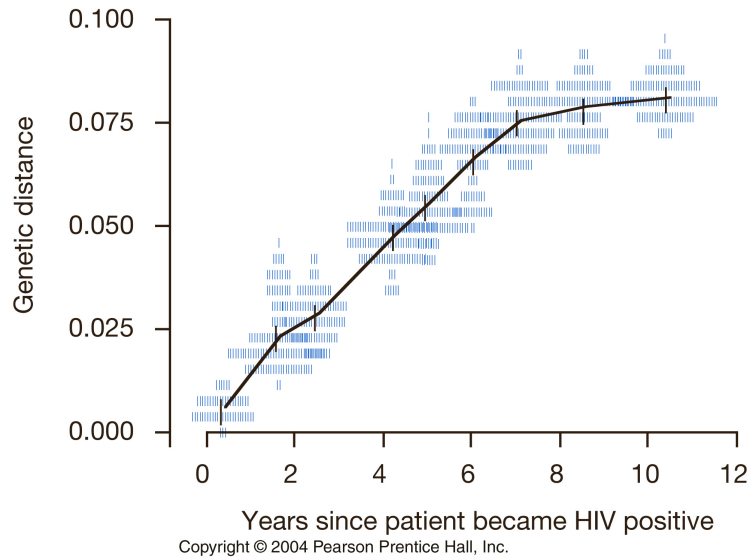
This process continues effectively throughout the *Chronic Phase*, as millions of new viruses are produced each day.

Research showed that throughout an infection (12 years), the sequence of the gene encoding gp120 (an HIV epitope) diverged by nearly 8%.

gp120 is a protein on the HIV envelope that is responsible for attachment to the host cell, as well as for recognition by the host.

For context, the entire genomes of humans and chimpanzees are 2% divergent.

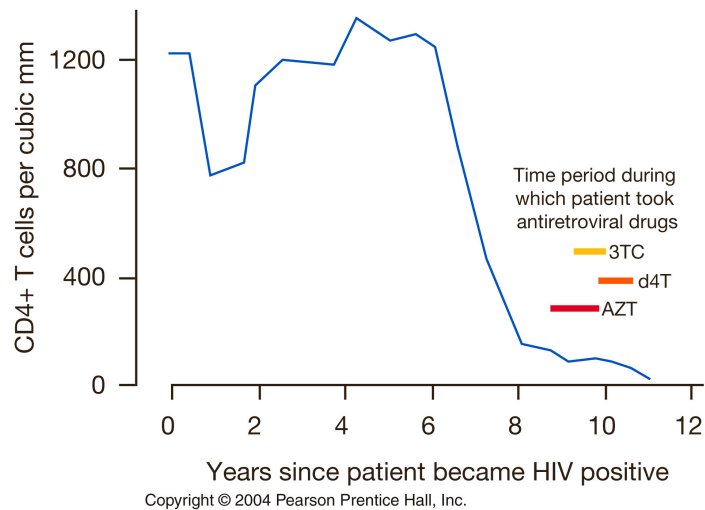
(a) Divergence from founder population



Why the decreased rate of evolution after year 7?

Did the HIV population stop evolving? Yes, but why?

(c) T cell counts



Notice that at the time that HIV diversity became static, the host CD4 T-cell count decreased dramatically.

No selection on the HIV → no evolution

The HIV that presented the epitopes that helped to evade the host immune system persisted and grew in population (no selection against it).

HIV evolution is short-sighted

Natural selection drives HIV evolution, but HIV evolution is exactly what kills the host.

Why would a virus kill its own host?

Evolution by natural selection does not look to the future...no "choice" is made.

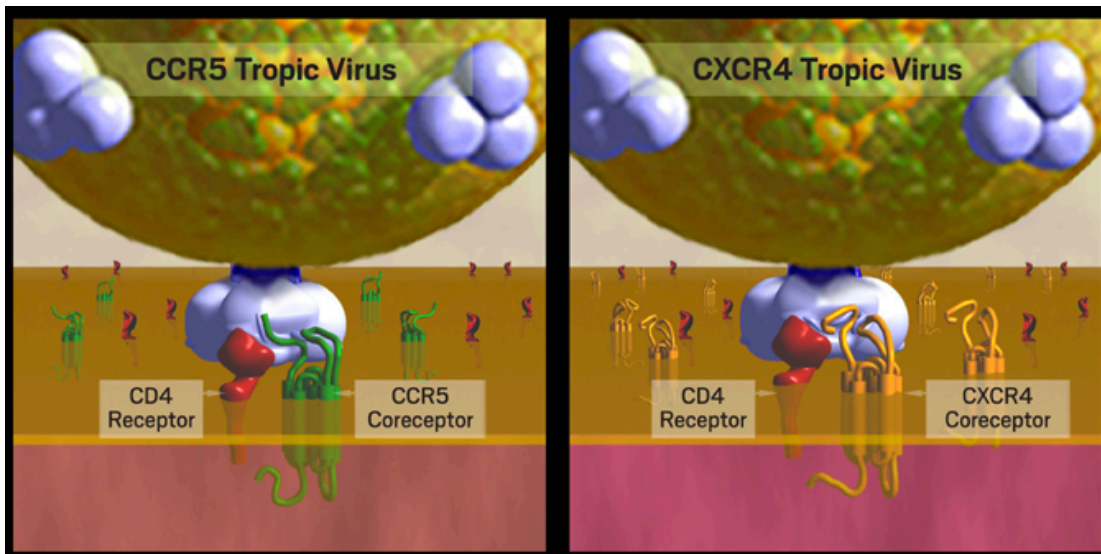
An example of this short-sightedness is illustrated by *co-receptor switching*.

The co-receptor is the second of two proteins (CD4 is the first) that HIV binds to when attaching to a host cell.

CCR5 early in an infection

CXCR4 in later stages

The ability to use an alternative co-receptor is the result of a mutation in gp120.



The mutation is unfavorable for HIV early in the infection process.

So, what drives the preference for the CXCR4 co-receptor in the later stages of the infection?

CCR5 and CXCR4 are found on different types of T-cells.

In the *Acute Phase* of the infection, CCR5 T-cells divide more rapidly than CXCR4 T-cells. **Why is this an important factor?**

In the *Chronic Phase* of the infection, CXCR4 T-cells divide more rapidly than CCR5 T-cells.

The evolution of co-receptor use in HIV is driven by natural selection based on

- (i) cells that can divide rapidly and allow HIV to quickly multiply, and
- (ii) mutations in gp120 that allow binding to either CCR5 or CXCR4.

The evolution of HIV to use CXCR4 is short-sided for two reasons.

1. For some reason, HIV produced in CXCR4 T-cells do transmit to new hosts very well.
2. HIV using CXCR4 T-cells hasten the collapse of the host immune system.

CXCR4 T-cells are used later in the infection process when the immune system is already weakened.

Therefore, one reason that we can consider HIV fatal is because of its short-sighted evolution within the host.

While evolution of HIV allows it to enjoy short-term success, in the long term, HIV drives its own demise by killing its host.