

Lentiviruses I (HIV-1, Pathogenesis)

lenti = slow (Latin)

Coffin, J.M. Science 267:483, 1995; Wain-Hobson, S. Nature 387:123, 1997; Finzi & Siliciano, Cell 93:665, 1998

Properties of Lentiviruses	
Family	Retroviridae
Major human	HIV-1, HIV-2
Size	80-130 nm
Capsid symmetry	Icosahedral
Envelope	Yes
Genome	Diploid linear + sense ssRNA; 10 kb
Genome replicated	Nucleus
Virus assembly	Cytoplasm
Common features	Slow disease
Diseases	AIDS; neurologic; arthritis; pneumonia

Brief list of lentiviruses

Virus	Abbrev.	Natural Host	Disease
<u>Primate Lentiviruses (5 groups):</u>			
A.	Human immunodeficiency virus, type 1	HIV-1 SIV _{cpz}	Human Chimpanzee AIDS None
B.	Human immunodeficiency virus, type 2 Simian immunodeficiency virus, smm/mac	HIV-2 SIV _{smm/mac}	Human Mangabey AIDS None
C.	Simian immunodeficiency virus, agm	SIV _{agm}	African Green monkey None
D.	Simian immunodeficiency virus, mnd Simian immunodeficiency	SIV _{mnd} SIV _{l'hoest}	Mandrill L'Hoest None None

	virus, I'hoest			
E.	Simian immunodeficiency virus, syk	SIV _{syk}	Sykes' monkey	None
F.	Simian immunodeficiency virus, col	SIV _{col}	Guezara colobus monkey	None
Non—primate lentiviruses (selected):				
	Visna-Maedi virus	VMV	Sheep	Pneumonia, wasting Encephalitis, arthritis
	Bovine immunodeficiency virus	BIV	Cattle	None
	Feline immunodeficiency virus	FIV	Cats	Immunodeficiency, wasting, encephalitis
	Caprine arthritis-encephalitis virus	CAEV	Goats	Anemia, wasting
	Equine infectious anemia virus	EIAV	Horses	Arthritis, encephalitis

All are genetically related more closely to each other than to oncoviruses

All have similar genetic structure: gag/pol/env plus accessory genes (trans-activators)

Visna-Maedi virus

The **prototype lentiviruses** are viruses which cause “**slow virus**” **infections of sheep**. These viruses were first described in the 1950s by Bjorn Sigurdsson who studied *maedi/visna*, a progressive infection of Icelandic sheep. **Maedi/Visna** is a pneumoencephalitic disease complex, with an incubation time of roughly 2 years. It is caused by a single virus, that is spread by a respiratory spread in adults, and via milk to lambs. Maedi is Icelandic for “labored breathing”, which refers to the chronic pneumonia that occurs in affected animals. This is the most common form of disease. Visna is Icelandic for “paralysis and wasting”, and refers to the progressive CNS degeneration in affected animals.

As a group, lentiviruses have a number of common features that are instructive with respect to HIV. In terms of their biology and genetic composition, key properties of lentiviruses are:

1. **Lentiviruses can infect non-dividing cells.** Unlike oncoretroviruses, lentiviruses can efficiently infect terminally-differentiated, non-dividing cells such as macrophages. Tissue resident macrophages, such as brain macrophages (or

- microglia) may be important for development of specific aspects of virally-induced disease.
2. **Lentivirus genomes encode regulatory proteins.** In addition to structural (*gag*, *env*) and enzymatic (*pol*) proteins, which are found in oncoretroviruses, lentiviruses also encode regulatory proteins. Most notable among these are Tat-like and Rev-like proteins which, respectively, regulate viral transcription and viral RNA transport.

In terms of the pathogenesis of lentivirus infections, some key properties are as follows:

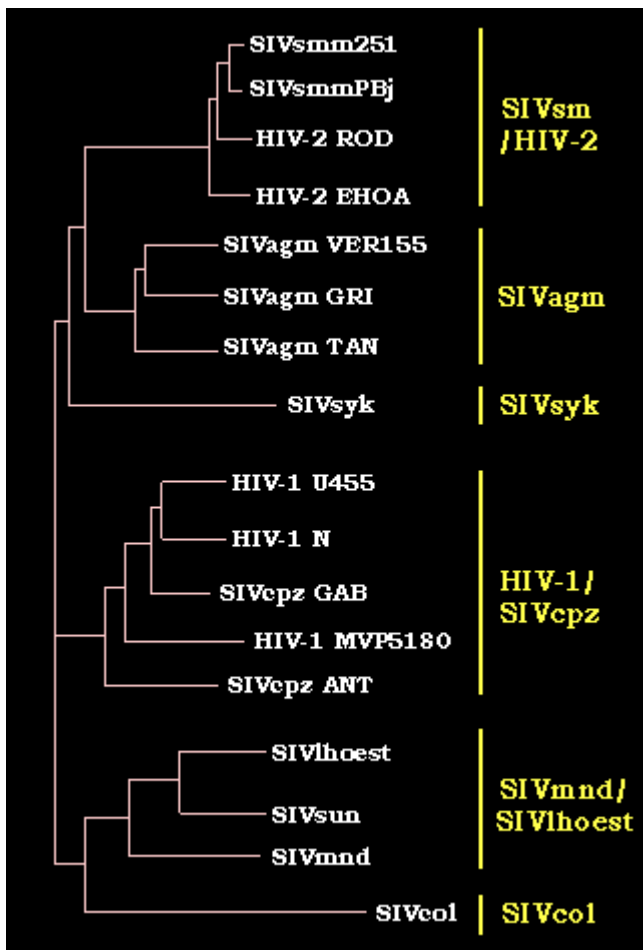
1. **Lentiviruses persist lifelong.** This is a function both of their ability to **integrate into the host chromosome and of their ability to evade host immunity**. This ability to evade host immunity may be related both to the high mutation rates of these viruses, and to their ability to infect immune cells (macrophages, and in the case of HIV, T-cells).
2. **Lentiviruses have high mutation rates.** Lentiviruses replicate, mutate and undergo selection by host immune responses.
3. **Infection proceeds through at least three stages.**
 1. Initial (acute) lentivirus infection is associated with rapid viral replication and dissemination, which is often accompanied by a transient period of disease.
 2. This is followed by a latent period, during which the virus is brought under immune control and no disease occurs.
 3. High levels of viral replication then resume at some later time, resulting in disease.
4. **There is variation in disease presentation.** Disease may present in the form of tumors or other infections (due to virally-induced immunodeficiency), as well as encephalitis, wasting, pneumonia and arthritis. In many cases, the disease lesions that are produced are the result of immunopathology (for example, Maedi is the result of lymphocytic infiltration of the lungs and Visna is the result of an immune response against virally-infected oligodendrocytes).
5. **There is variation in the time to disease onset.** Various cofactors influence the time which must elapse before disease occurs, including:
 1. The genetic background of the host (some strains of sheep, such as Icelandic sheep, are more susceptible to Visna-Maedi Virus)
 2. The age of the host (young hosts are generally more susceptible to rapid-onset disease)
 3. Stress (disease relapses in horses infected with Equine Infectious Anemia Virus are associated with overwork)
 4. Viral factors (some viral strains may be more or less virulent than others)

Discovery of human lentiviruses

- AIDS was first recognized as a new disease in 1981, with a report of 5 cases of *Pneumocystis carinii* pneumonia (PCP) in 5 previously healthy men in the Los Angeles area. At the time, even a single case of PCP was highly unusual and a cluster of several cases signaled that something new and unusual was taking place.
- In 1983, Françoise Barre-Sinoussi, Claude Chermann and Luc Montagnier at the Pasteur Institute isolated a retrovirus from the lymph node cells of a patient with lymphadenopathy and termed their virus lymphadenopathy-associated virus (LAV). The next year, Robert Gallo's group at the NIH confirmed and extended this finding, linking this virus to the immunodeficiency syndrome, AIDS.
- In 1986, a second HIV was isolated from West Africa (HIV-2).

Primate Lentiviruses: Phylogenetic Relationships

Courgnaud et al. J. Virol. 75:857, 2001



Note that 6 subgroups of primate lentiviruses exist: HIV-1/SIV_{cpz}; SIV_{mnd}; SIV_{agm}; SIV_{syk}; SIV_{col}; HIV-2/SIV_{sm}; in addition, several subgroups

of HIV-1 exist

Origins of human lentiviruses

Phylogenetic studies of primate lentiviruses (as shown above) provide compelling evidence that HIV-1 is closely related to a virus that naturally infects chimpanzees (SIV_{cpz}), while HIV-2 is closely related to a virus that naturally infects sooty mangabey monkeys (SIV_{smm}). It is believed that zoonotic (or trans-species) transfer of these simian immunodeficiency viruses resulted in the emergence of HIV-1 and HIV-2 -- an argument that is strengthened by the fact that, for example, sooty mangabeys are resident in the same region of West Africa in which HIV-2 is most prevalent. Thus, HIV-1 and HIV-2 represent **emerging infections**.

The key events in successful cross-species transmission are (1) initial infection and (2) facile transmission in the new host. Thus, HIV-1 evolved following both one or more initial infection events, following by subsequent human-to-human transmission events that resulted in widespread dissemination of the virus. The latter events most likely included changes such as urbanization, rapid mass transit and new trade opportunities.

In the case of HIV-1 group M viruses (for majority), it is currently believed that these viruses first entered human populations in the early **1930s** (Korber et al. Science 288:1789, 2000). The viruses diversified into well recognized, and distinct subtypes (or clades) by the 1950s. These findings argue against the possibility that HIV-1 entered human populations iatrogenically through SIV_{cpz} contamination of oral polio vaccines (OPV) used in Central Africa between 1957 and 1960 (*The River, by Edward Hopper: Little, Brown, 1999*).

The OPV vaccine was propagated using primary monkey kidney epithelial cells, as was standard practice at the time. The “**vaccine hypothesis**” for the origin of HIV-1 proposes that SIV_{cpz}-contaminated chimpanzee kidney epithelial cells may also have been used to propagate the OPV vaccine, and that this resulted in the spread of SIV_{cpz} to humans. However, recent PCR-based testing of remaining vaccine stocks failed to reveal the presence of SIV_{cpz} within the stocks.

Finally, it should be noted that at least 3 separate monkey-to-human transfers have probably occurred, giving rise to the 3 genetically distinct HIV-1 groups - groups M (for “majority”), N (“non-M/non-O”) and O (for “outlier”). These groups are genetically distinct and have unique geographic distributions, with M being responsible for the global HIV-1 epidemic. Group O is represented by fewer strains and is localized to Cameroon, Gabon and Equatorial Guinea; group N was discovered only very recently and is represented by very few strains, all (to date) from Cameroon.

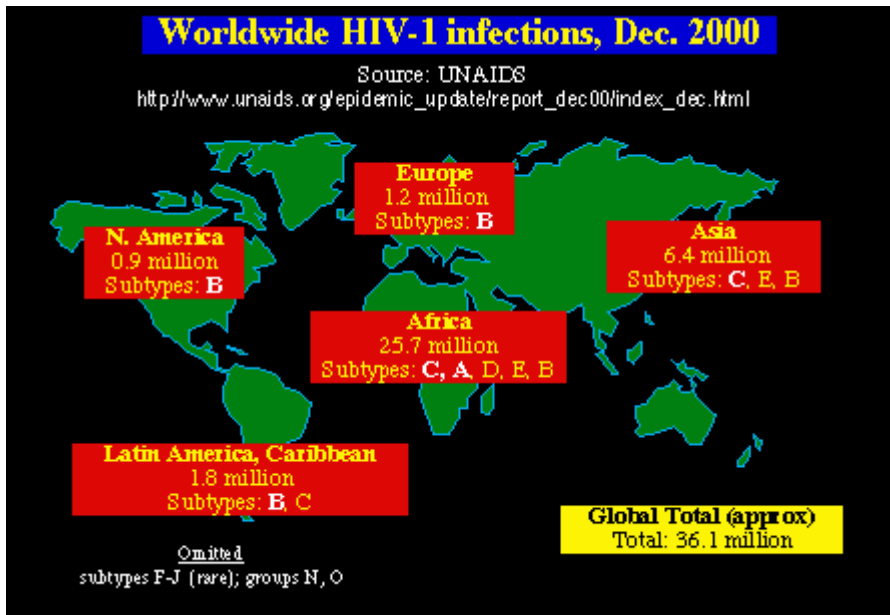
HIV-2

Of the two viruses associated with human AIDS, HIV-1 is distributed throughout the world, while HIV-2 remains largely restricted to West Africa. Both viruses appear to be spread by a similar route (principally, by sexual transmission but also via infected blood and body fluids). Some important facts about HIV-2:

- There is no HIV-2 pandemic -- that is, there is no global epidemic of HIV-2. In fact, HIV-2 is still mostly restricted to West Africa (eg, Cameroon, Ivory Coast, Senegal). This suggests that HIV-2 may not be transmitted as efficiently as HIV-1, although it is not clear why.
- HIV-2 may be less pathogenic than HIV-1. When compared to HIV-1, persons can be infected with HIV-2 for much longer periods without developing disease.
- There is evidence that HIV-2 infected persons may be at a decreased risk for acquiring HIV-1 infection. This suggests that prior HIV-2 infection may protect against HIV-1 infection. This finding has important implications for the design of HIV-1 vaccines, perhaps based on “attenuated” or weakened viral strains.

Epidemiology of HIV-1 infection

The global epidemic (or pandemic) of HIV-1 infection is growing most rapidly in the nations of South-East Asia and in the subcontinent of India, although the largest number of HIV-1 infected persons is still in sub-Saharan Africa. Recent data suggest that over 36 million adults and children are infected by HIV-1 (*UNAIDS estimates published in Jan 2001, based on data as of the end of 2000*). Both in the United States and worldwide, infection is disproportionately common among minorities and among the poor.



The global distribution of HIV-1 infection is shown above. In addition, the major HIV-1 subtypes which are found in particular regions of the world are denoted (bold lettering indicates the predominant subtype in the indicated region). It can be seen that subtype B is most prevalent in the Americas and in Europe, while subtype C viruses are the most common viruses globally (with subtype A being the next most prevalent). What this highlights is that there are regional epidemics of genetically distinct viruses, which may have important implications for vaccination. For example, it is not clear that vaccines based on HIV-1 subtype B (which are presently the focus of most of the ongoing vaccine development efforts) will be the best choice for protecting against HIV-1 infection in sub-Saharan Africa, where subtype “C” and subtype “A” viruses predominate.

Pathogenesis of HIV infection

Clinical Features of HIV infection

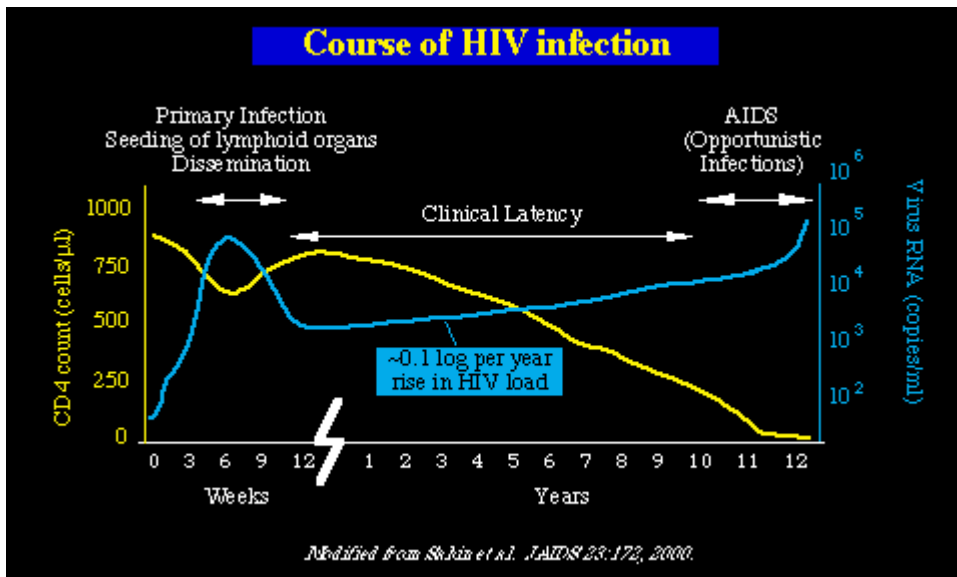
HIV infection can be divided into distinct stages:

- **Acute Primary Infection Syndrome.** Primary infection can be asymptomatic, or it may associate with an influenza-like illness with fevers, malaise, diarrhea and neurologic symptoms such as headache. This illness usually lasts 2 to 3 weeks, with full recovery.

- **Asymptomatic Infection.** This refers to the asymptomatic carrier state that follows initial infection. It typically lasts for many years, with a gradual decline in the number of circulating CD4+ T cells. In a minority of cases, infection does not proceed beyond this asymptomatic phase and CD4 counts remain stable (these persons are known as long-term survivors or *long-term nonprogressors*).
- **Symptomatic HIV infection and AIDS.** Symptoms that are related to HIV infection ultimately begin to develop. AIDS typically occurs about 10 to 12 years after initial HIV-1 infection and is defined by more serious AIDS-defining illnesses and/or by a decline in the circulating CD4 count to below 200 cells per microliter. *Note that future efforts to better define parameters of disease progression in HIV infected persons will very likely focus both on viral load (RNA copies per microliter of plasma) and on CD4 counts.*

Examples of AIDS-defining illnesses include the following:

- Infections: *Pneumocystis carinii* pneumonia, *Mycobacterial tuberculosis*, esophageal candidiasis, toxoplasmosis of the brain, CMV retinitis
- Cancers: cervical cancer, Kaposi's sarcoma, various B-cell lymphomas linked to EBV
- HIV-related encephalopathy, HIV-related wasting syndrome, lymphoid interstitial pneumonia (kids)



Pathogenesis of AIDS

- **Primary infection:** Once a person has become infected with HIV-1, a *primary* or *acute* viral infection results within a few weeks. This *primary* infection represents the immune system's first encounter with HIV. While the immune response learns to deal with HIV, the virus is able to replicate to very high levels for a period of several weeks. In addition, the number of CD4+ T cells in the blood drops. During the acute phase, the viral doubling time is 10 h, and the peak of viremia occurs at 21 days after infection; in addition, the mean basic reproductive number is 19.3 (i.e., each virus-positive cell infects 20 new cells). *See: Little et al. J. Exp. Med. 190:841, 1999).*
- **Asymptomatic phase:** Within a few weeks, a specific immune response to HIV is mounted, and viral replication is greatly reduced -- thereby lowering the virus load, and allowing the number of CD4+ T cells to rebound to near-normal levels. During this stage of the disease, virus load continues to slowly but inexorably increase in most patients (HIV RNA levels rise by roughly 0.1 log₁₀ per year). Thus, the infection never reaches a true steady state or a completely stable equilibrium. Nonetheless, the steady state of HIV infection can be regarded as approximating an equilibrium state, in that the levels of virus production and virus elimination are very nearly identical. Likewise, the numbers of CD4 cells that are killed are very nearly identical to the numbers of new CD4 cells that are generated to replace them. It has been estimated that roughly 10 billion (10¹⁰) viral particles are produced and **roughly one billion (10⁹) CD4+ T lymphocytes are killed each day** (*Perelson et al. Science 271:1582, 1996).*
- **AIDS:** Eventually, the CD4 count drops below a level that is compatible with effective immune function, and disease progresses, culminating in death.

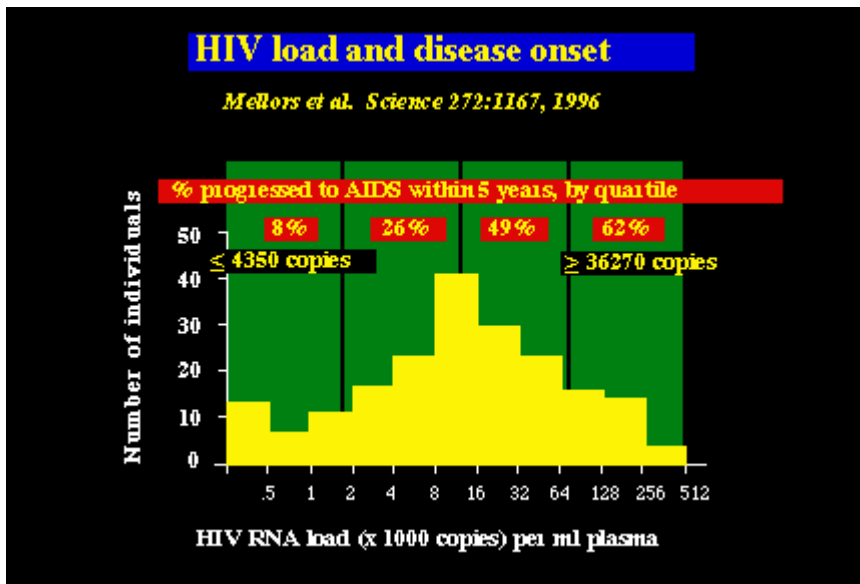
A model for pathogenesis: “*Its the virus, stupid*”.

The very dynamic, active, state of HIV-1 replication goes on for 10+ years, during which time the body has to continue to replace all the T cells that are being killed. This constant requirement to replace billions of T cells per day is believed to gradually wear out the body's ability to produce these cells.

Note that alternative theories to explain the pathogenesis of AIDS also exist, including the idea that the continuous evolution of the HIV-1 genome eventually overwhelms the immune system's ability to respond (“immune overload”, proposed by Martin Nowak and others). However, as Eric Pelletier and Simon Wain-Hobson have noted, “*it would be mistaken, a little perverse perhaps, to insist that some genetic change in HIV-1 is required for the onset of AIDS, discounting the effects of five to 15 years of persistent viral replication*” (*J. NIH Res. 8:45, Sept. 1996).*

High virus load is associated with rapid progression to disease.

As might be expected, on the basis of the above model for HIV-1 pathogenesis, high levels of HIV-1 replication (ie, high virus loads) are associated with rapid disease progression, as shown below:



Mellors and colleagues measured the amount of HIV RNA in the blood (plasma) of a large group of asymptomatic HIV infected persons. They then divided up their cohort of HIV+ persons into 4 groups or quartiles, on the basis of how much virus was in their blood, and examined the rate at which these individuals progressed to AIDS. They found that 62% of persons with high virus loads (> 36,270 copies) developed AIDS within 5 years, while only 8% of persons with low virus loads (< 4,350 copies) developed AIDS within this time period (see above).

Long term survivors

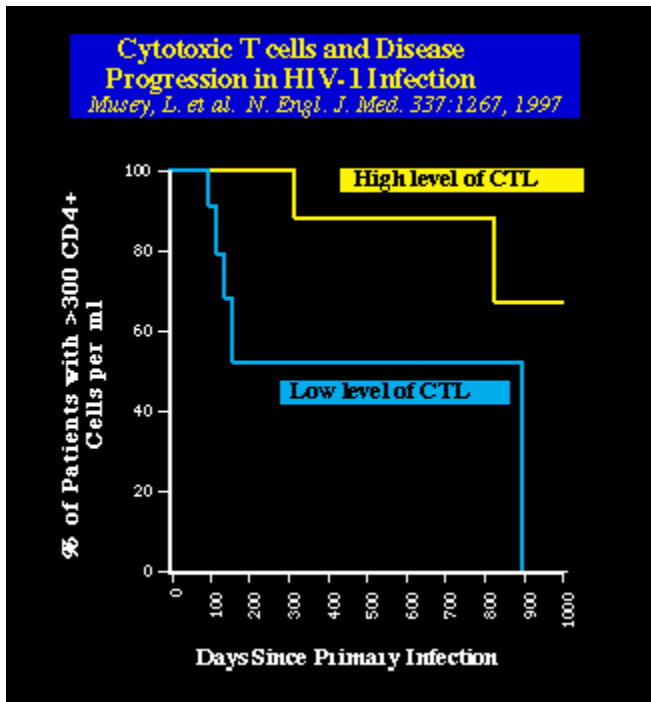
Despite the grim picture of what happens to most people after they become infected by HIV, there are also a significant number of HIV-infected persons who never become sick, never experience a decline in their CD4 counts and never develop high loads of viral RNA. These **long-term survivors** make up perhaps 5% of all people infected by HIV. The reason(s) that they never become sick are uncertain, but may include host and viral factors, such as the presence of mutations which confer resistance to HIV (e.g., the CCR5Δ32 mutations) and infection by naturally attenuated strains (e.g., Nef deleted viruses).

Viral diversity

The enormous genetic diversity of HIV-1 can be traced to the **unprecedented extent of viral replication**, and to the error-prone nature of that replication. It has estimated that the average HIV-1 generation time *in vivo* (defined as the time from release of a viral particle until it infects another cells and causes release of a new generation of virions) is 2.6 days (*Perelson et al. Science 271:1582, 1996*). This means that **HIV-1 replicates at a rate of about 140 cycles per year** -- continually generating new mutations.

Importance of antiviral immunity

Antiviral immune responses, and antigen-specific **cytotoxic T cells** (CTL) in particular have long been considered to be critical for the control of the initial phase of viral replication, and for the ultimate outcome of HIV-1 infection in the infected person. Consistent with this contention, a study by Musey and coworkers found that the level of early CTL responses against HIV-1 was strongly correlated with protection from subsequent disease progression, as defined by a decline in CD4+ T cell counts (see below).



Viral Dynamics of HIV-1 Infection

One of the areas that has seen the greatest expansion of knowledge in the last several years is our understanding of the dynamics of HIV-1 infection. This has become possible because of the advent of highly sensitive methods for the direct measurement of HIV-1 RNA in blood and in tissues, as well as the availability of antiretroviral drugs which essentially allow one to completely shutoff all new virus infection, at least for a time. Thus, one can measure how fast virus levels fall over time, in a situation in which the only virus being produced must be coming from reservoirs that existed at the time when antiviral therapy was started.

Highly sensitive assays for viral RNA.

Assays for quantitation of viral RNA in plasma include:

- Reverse transcription (RT) polymerase chain reaction (PCR) amplification (**RT-PCR**). This assay can be made quantitative through the use of internal controls (quantitative competitive, or QC, RT-PCR). RT-PCR is probably the most widely used assay for quantitation of HIV-1 RNA.
- Branched DNA (**bdNA**) analysis. This procedure does not involve the amplification of viral nucleic acids, but instead relies on a super-sensitive method to probe for the presence of viral RNA, using a highly branched and labeled DNA probe.
- Nucleic acid sequence-based amplification (**NASBA**). This method involves the amplification of HIV RNA using a bacterial RNA polymerase, in combination with a reverse transcriptase.

“Steady-state” viral replication and the infected cell pool.

As noted above, HIV-1 replication during the asymptomatic phase of infection is characterized by a very high and reasonably constant level of viral replication. An important consideration during this time is the size and composition of the infected cell pool.

In a normal, HIV-negative, individual, the vast majority of T cells at any given time are in a resting state, with roughly equivalent numbers of resting and memory cells. In HIV-infected persons, the proportion of activated T cells rises -- although these cells are still very much in the minority. The proportion of CD4+ T cells that are HIV-positive at any given time has been estimated (*Chun et al. Nature 387:183, 1997; Wain-Hobson, S. Nature 387:123, 1997*) as follows:

Cell pool	Number of cells
Total body lymphocytes	10 ¹² cells <i>(98% are in lymphoid tissue, not blood)</i>
CD4+ T cells containing HIV DNA (unintegrated)	approx. 0.5% (~10 ⁹ cells)
CD4+ T cells containing integrated, latent HIV DNA	approx. 0.05% (~10 ⁸ cells)
CD4+ T cell actively making virus	approx. 0.005% (<10 ⁷ cells)

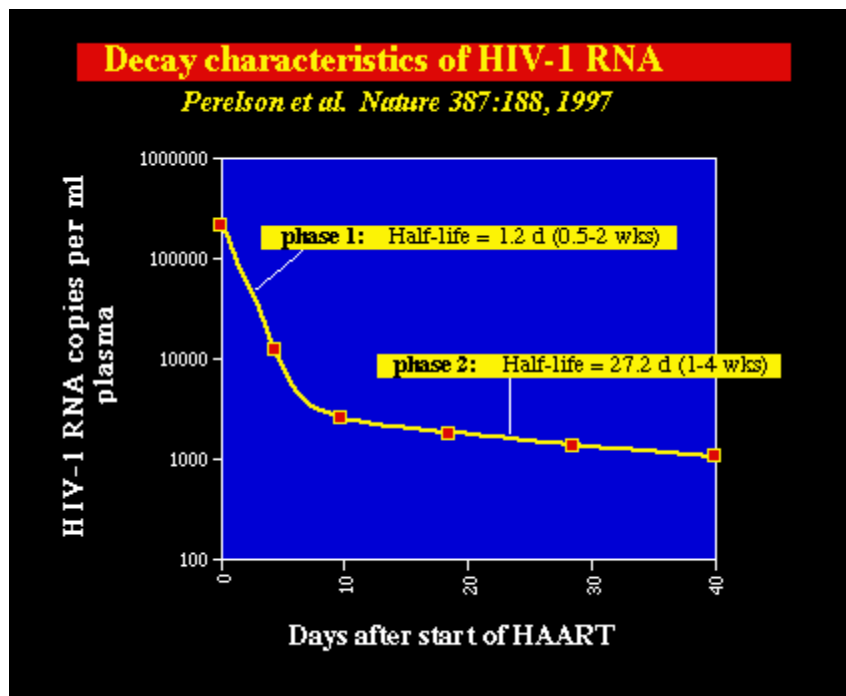
The most important point of this work is the fact that the **total number of cells which are actively making HIV-1 is quite low (<10⁷ cells)**; these cells make roughly 10³-10⁴ virions each. The small size of the active viral reservoir is somewhat encouraging from a therapeutic standpoint, but it is important to note that there is a **considerably larger pool of latently infected cells** which are capable of making virus upon appropriate stimulation. This pool of cells is a major cause for concern, since these cells will be unaffected by current antiviral therapies. The question therefore arises: How long do

HIV-1 infected cells live? In particular, is there a long-lived reservoir of infection in the body?

To address these questions, a number of investigators have examined viral decay kinetics in persons who have received highly active anti-retroviral therapy (HAART), for treatment of their HIV-1 infection (*HAART represents the use of multiple drugs in combination, and typically includes three drugs of which at least one is a protease inhibitor and one is a reverse-transcriptase inhibitor*).

Viral decay kinetics. Studies of viral decay kinetics in persons receiving HAART have revealed the existence of at least two distinct phases of viral decay:

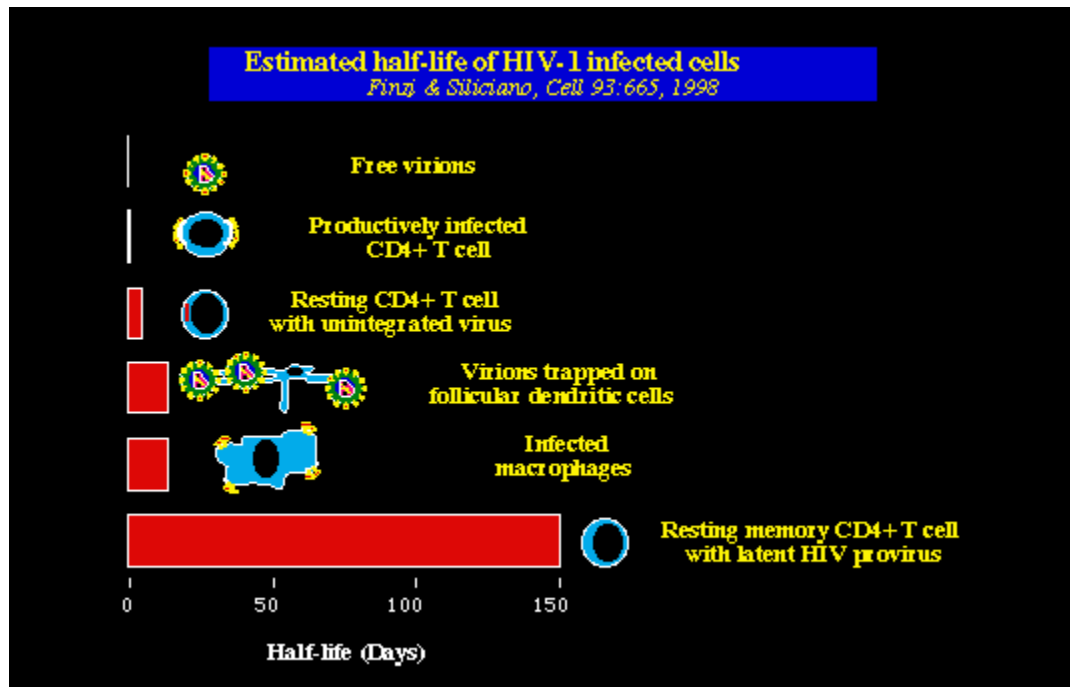
1. During the first phase, which is typically complete within 2 weeks following the initiation of HAART, virus loads fall by 100-fold (phase 1; see below). The characteristics of this initial phase of viral decay suggest that the **actively infected CD4+ T cell pool** is rather short-lived, and that its elimination therefore leads to a rapid drop in virus loads.
2. During the second phase, virus loads continue to decline slowly over time. This slower rate of decline (phase 2, below) reflects the turnover of a more long-lived viral reservoir or infected cell population. It has been suggested that this may be due to virus production by **infected macrophages**, which have a longer half-life in cell culture than virally-infected T cells.



Does a third reservoir of HIV-infected cells exist? On the basis of the predicted half-life of the second, more stable, population of HIV-1 producer cells, it is estimated that HAART treatment would need to be continued for 2-3 years, in order to completely eliminate HIV-1 from the body. However, this prediction is predicated on the assumption that there is no additional reservoir of actively replicating, or inducible, virus.

At present it remains unclear if such a compartment exists. However, it has been noted that **CD4+ memory T cells**, some of which may harbor **latent HIV-1** provirus, are extremely long-lived, with a half-life of 22 weeks or more. This has very serious implications with respect to the length of time that it may be necessary to maintain HAART treatment for HIV-infected persons, since it suggests that there may be a very stable reservoir of virus which could "re-ignite" the infection if therapy were to be discontinued too soon.

An additional consideration that has recently become apparent is the fact that virus replication continues even during HAART. This does not appear to be due to emergence of multiply-resistant virus strains, but rather it is thought to reflect **residual replication of wild-type virus during HAART.** This may occur because of incomplete penetration of antiviral drugs into all tissues and cells. Whatever the reason, this finding adds an additional complication in terms of the development of approaches aimed at eradicating HIV from virally-infected persons.



Note: Follicular dendritic cells (FDC) are specialized cells within lymphoid tissues, which trap antigens and present them to trafficking immune cells. They can also trap infectious HIV-1.

Vaccines

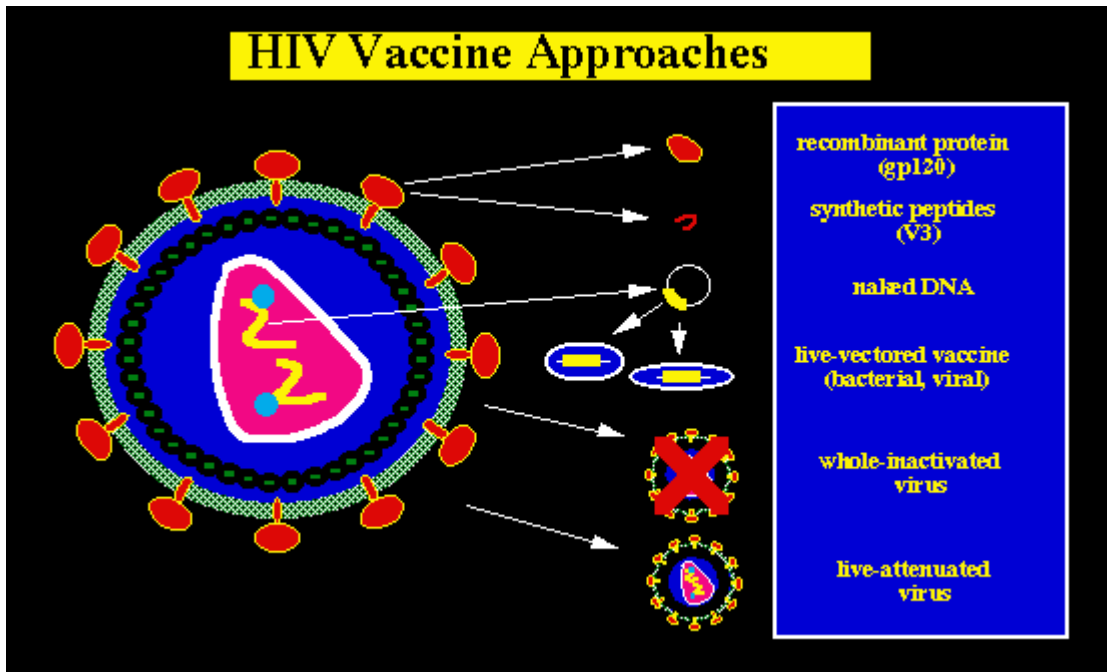
The primary current goal of vaccine efforts is to prevent HIV-1 infection (i.e., to engender “sterilizing” immunity). If this should prove unreasonably difficult, it may be necessary to adopt a secondary goal – the development of a vaccine that can elicit immune responses that reduce virus replication and thereby protect against disease. President Clinton has committed the U.S. to developing an HIV vaccine by the year 2007.

Current HIV vaccine approaches are shown below, and include the following:

1. **Recombinant proteins**, produced in *E. coli*, yeast or mammalian cells. Probably the most widely explored is recombinant soluble gp120, produced in mammalian cells. Soluble protein is reasonably good at eliciting an antibody response, but poor at generating a T-cell response.
2. **DNA vaccines**. Purified recombinant DNA expression vectors which encode HIV-1 proteins. These can be delivered into the skin via a "gene gun" (which delivers microscopic DNA-coated gold beads at high velocity), or via intramuscular or intradermal injection. Such vaccines appear reasonably good at eliciting T-cell responses, but poor at inducing antibodies.
3. **Live-vectored vaccines** (bacteria or viruses). These vaccines are capable of expressing recombinant HIV-1 antigens within infected cells. Examples that have already been tested include vaccinia virus or canarypox vectors which express HIV-1 genes. One new vector is the Venezuelan equine encephalitis virus (VEE), which has the ability to infect dendritic cells (the body's major antigen presenting cells). Typically, live-vectored vaccines are good at eliciting T-cell responses, but poor at inducing antibodies.

Each of the strategies listed above is presently being tested in humans, and some of these approaches have entered Phase III clinical trials -- meaning that small scale testing within high-risk populations is being conducted, so as to determine whether they may be effective at preventing HIV-1 infection. If promising results are obtained, large-scale efficacy trials (Phase IV) will be needed.

Perhaps the most promising vaccine candidates may be combination approaches. One such approach is the use of a DNA vaccine to "prime" the immune response, followed by a vaccinia-virus vector to "boost" that response. Other **prime-boost** strategies are also being evaluated.



A more high-risk approach to HIV vaccine development is the use of a **live-attenuated vaccine**. Several methods for genetically attenuating HIV have been identified. These include the deletion of multiple viral genes that are not required for *in vitro* replication, but which influence disease induction *in vivo* (such as *nef*, *vpr*, *vpu*). The concern here is that such a vaccine could one day cause disease. However, if other approaches prove unsuccessful, it may be necessary to re-consider such a vaccine -- particularly since the great majority of all viral vaccines that are presently in use represent live-attenuated viruses.