

ACQUIRED IMMUNE DEFICIENCY SYNDROME

DEFINITION. The definition of this illness kept changing as we learned more about its course and causes. Originally it was ‘Any occurrence of an opportunistic infection or Kaposi’s sarcoma in a patient *without* a previous history of, or apparent cause for, immune deficiency.’ Now the overall diagnosis is made by detecting infection with HIV-1, the AIDS virus. People are ‘seropositive’ if they have antibody to HIV, which is the most common way in which infection is first detected; once they get symptoms of opportunistic infections or Kaposi’s sarcoma, or their Th (CD4⁺) cells fall below 200/μL of blood, it’s AIDS.

WORLD ESTIMATES OF THE AIDS PANDEMIC, DECEMBER 2008

People living with HIV in 2008

Total	33.4 million [31.1–35.8 million]
Adults	31.3 million [229.2–33.6 million]
Women	15.7 million [14.2–117.2 million]
Children under 15 years	2.1 million [1.2–2.9 million]

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People newly infected with HIV in 2008

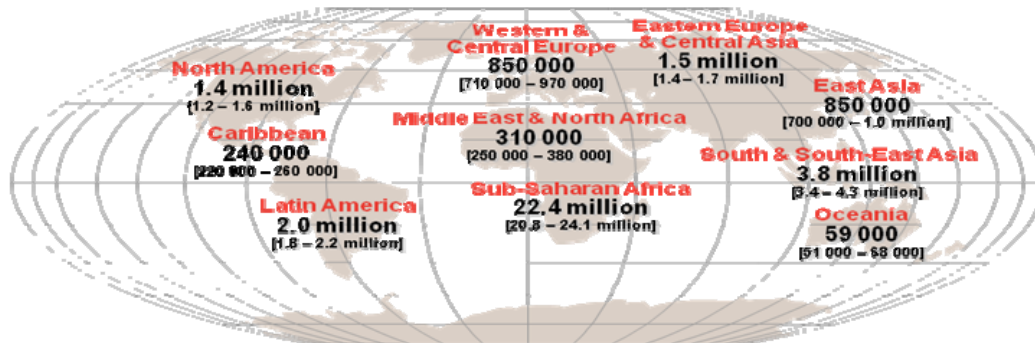
Total	2.7 million [2.4–3.0 million]
Adults	2.3 million [2.0–2.5 million]
Children under 15 years	430 000 [240 000–610 000]

AIDS deaths in 2008

Total	2.0 million [1.7–2.4 million]
Adults	1.7 million [1.4–2.1 million]
Children under 15 years	280 000 [150 000–41 000]

World Health Organization/UNAIDS [most recent available data]

Every minute of the day, 4 people get HIV, and 4 die of AIDS. Most are in sub-Saharan Africa.



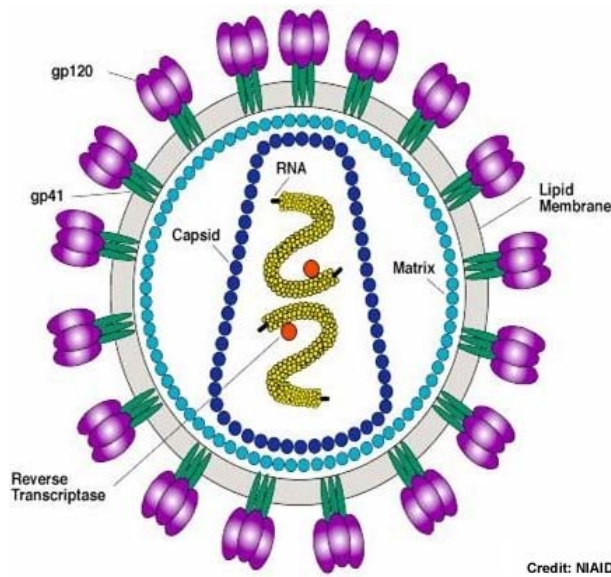
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INCIDENCE AND PREVALENCE. By 2004 AIDS was the 5th leading cause of death in the world, the 4th in developing countries. So far, 28 million have died.

In the USA, the CDC counted 988,376 cumulative cases of AIDS by the end of 2008, of whom 550,394 had died. With new treatments, death rates have fallen remarkably: 38,000 died in 1996, 14,000 in 2006. However, ► about 1, 180,000 people are living with HIV in the United States, and about 20% of them don't know it. A new assay for seropositivity that can distinguish recent from established infection, and a sophisticated statistical analysis, indicate that incidence may be underestimated by as much as 40%.¹

There have been about 10,000 cases in Colorado. Incidence in Colorado has been falling since 1993; there were about 420 new cases in Colorado in 2009, and 105 deaths.

CAUSE. AIDS is caused by a virus, originally called HTLV-III, LAV, or ARV, and now generally called **HIV-1**, for Human Immunodeficiency Virus. HIV-2 has been isolated in West Africa. HIV is a nontransforming retrovirus, that is, an RNA virus that carries no oncogene, and reproduces itself by copying its RNA into DNA by means of its enzyme, reverse transcriptase. It is similar to visna virus of sheep, equine infectious anemia virus, and the feline immunodeficiency virus, all of which cause slow, ultimately fatal illnesses.



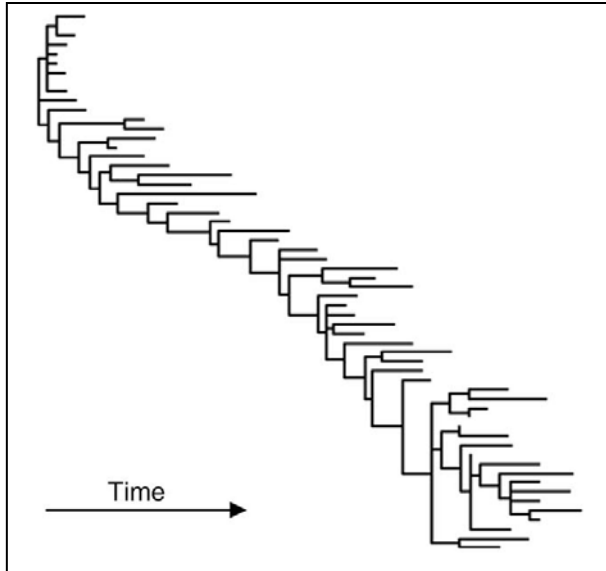
It is most closely related to a Simian Immunodeficiency Virus, SIV. It is thought that HIV-1 evolved recently from SIV, perhaps as recently as the 1930s and possibly in Zaire (now the Democratic Republic of Congo). We can try to determine when it originated by checking banks of stored human sera for antibodies. The first sera in the USA with antibody to HIV-1 are found in 1978; in Africa, some sera from 1959 are positive, and HIV-1 sequences have been cloned from a blood sample of 1959 from D.R. Congo. Thus this seems to be a relatively new virus, that has perhaps jumped from simian to human and not yet adapted to its new host (similar in that respect to Ebola and Marburg viruses).

Seroepidemiology indicates that this disease moved to the Caribbean in the mid-60's (perhaps brought there by Cuban soldiers returning from Angola) and to Europe a bit later. The epidemic in the USA, which started in New York, Los Angeles, and San Francisco, was probably brought in by men who had vacationed in Haiti. There was a very high incidence of AIDS among recent Haitian immigrants and refugees, which made people think originally that Haitians comprised some particular risk group, but this is now known to be untrue; risk groups have now been

¹ H. Irene Hall et al. (2008) Estimation of HIV Incidence in the United States. *JAMA*. 300(5):520-529.

replaced by 'risky behaviors'. By 2011, it is likely that AIDS has been detected in every country in the world...

This is the most antigenically variable pathogenic virus we have encountered. Reverse transcriptase is a highly error-prone enzyme, without proofreading capability. It makes a mistake about once in 100,000 base replications, so infected people have many variants in their body.



Variant HIV isolated from the blood of a single individual. Each branchpoint represents a new RNA sequence mutation.

(B.T. Grenfell et al., Unifying the epidemiological and evolutionary dynamics of pathogens. *Science* 303: 327-332, 2004)

RISK GROUPS AND RISKY BEHAVIORS. Who is at risk for AIDS? Risky behavior is whatever increases your chance of receiving an inoculum of HIV. It is sexually transmitted so frequent sex is risky if it involves partners who might have the virus themselves. Any lesion on or injury to mucous membranes increases risk. Injection of blood containing virus is highly risky, although much less so than with blood containing hepatitis virus. In over 3000 reports of accidental exposures of health care workers in the USA to HIV, only nine were documented to have become antibody-positive. We do not think that use of drugs *per se* is risky, nor use of sexual stimulants like amyl nitrite.

Heterosexual contacts now account for more than half of new cases worldwide, and more than half of those are women and girls.

Over 10,000 children have been reported as HIV+ in the USA, mostly of mothers whose behaviors put them at high risk for acquiring HIV infection (drug abusers, sex workers).

Sixty percent of all reported cases have died; 92% of those diagnosed before 1985 have died. The original impression, that most patients in whom AIDS was diagnosed would die of the disease, with a mean survival time of about 3.5 years, is now, with treatment, far too pessimistic. There is a link between HLA genotype and susceptibility; 65% of 'elite controllers,' who harbor HIV but retain normal immune function for many years, are HLA-B57. They make more, and more diverse, CTL against HIV peptides than people with other HLA alleles. New treatments have extended life expectancy remarkably. None of these treatments were available when the AIDS pandemic started.

PATHOGENESIS. After a single exposure, infected people develop high blood virus levels that peak at about 6 weeks, and then antibody to HIV by 9 weeks, whereupon virus levels fall, but not to zero. Untreated seropositive people develop AIDS symptoms at the rate of about 3% per year. The mean incubation period estimated from transfusion-acquired AIDS, where it could be most precisely timed, it was about 9.5 years without treatment.

When the virus enters the body, it may adhere to a lectin on dendritic cells called DC-SIGN². Taken up by this means it is not harmed, and thus uses the DC as a Trojan horse to get to the lymph nodes where the Th are. HIV binds by its envelope glycoprotein, gp120, to the CD4 molecule on the surface of Th cells. This induces a conformational change in gp120, which allows it to now bind a co-receptor, one of the chemokine receptors, **CCR5** or CXCR4. When a person is first infected, almost all the virus is CCR5-tropic. In turn, binding the chemokine receptor changes the conformation of the gp41 glycoprotein that is associated with gp120, exposing a very hydrophobic region that literally melts away the T cell's membrane, so the cell and virus fuse. The virus can thus inject its core into the cell, activate its reverse transcriptase and make a double-stranded DNA copy of its RNA. The complex moves into the nucleus. Helped by a viral integrase, the DNA is then inserted randomly into a break in the host cell's DNA as latent virus. We know little about how latency is regulated, or whether it is harmless to the cell. It may be that HIV goes latent in resting cells and replicates productively in activated ones. If true, this poses a real problem for therapy: it may be bad to try to stimulate the immune system. The virus has 6 genes in addition to the *gag*, *pol*, and *env* genes that all retroviruses have. These, it is thought, have some role in regulating latency. By alternative RNA splicing, and protease-mediated cleavage of a huge precursor protein (HIV makes its own protease) it can make close to thirty peptides. Note, CCR5, the reverse transcriptase, integrase, and the protease are thus targets for therapy.

ASK YOURSELF: With its horrendous mutation rate, HIV can easily evade the immune response. But how can it survive? Won't it rapidly make, say, a mutated protease that doesn't work any more?

About 10% of Caucasians have a mutant form of CCR5, with a 32-bp deletion. People with two CCR5^{Δ32} alleles do not express any surface CCR5; although they can be chronically infected with HIV they do not become ill. It seems probable that the infection in these people remains in DC and macrophages and does not affect helper T cells.

As viruses bud en masse from the infected cell, they tear so many holes in the membrane that the cell dies. In the early, pre-AIDS stage of the disease, the clearance rate of virus and the replacement rate of CD4 cells are incredible: it has been estimated that the entire population of virus is replaced daily, and CD4 cells every 3 days. A very significant behavior of the virus is this: when the virus is replicating, gp120 is made early, and it becomes inserted into the infected cell's plasma membrane. This allows fusion of the infected cell to nearby uninfected CD4 cells, and a syncytium forms. In this way the virus can spread without an extracellular phase. This is one reason why the antibody patients make seems to be useless. With time, CD4 cells are gradually lost; it looks like simple exhaustion of the ability to make more of them. This is commonly expressed as a falling blood CD4/CD8 ratio (the normal ratio is from ~1.5 to 3). An accelerating fall in this ratio, or an absolute CD4 count below 400/μL, are poor prognostic signs. When the immune system can no longer cope, opportunistic infections take hold.

² Stands for Dendritic cell (DC)-specific intercellular adhesion molecule 3 (ICAM-3) grabbing nonintegrin; surely the most annoying acronym in all immunology?

SYNDROMES. The most common condition is to be seropositive without symptoms, as are about 1,000,000 people in the United States. The next syndrome is usually Chronic Lymphadenopathy Syndrome, which is defined as enlargement of at least two groups of nodes, not inguinal (groin), for three months. The patient is otherwise asymptomatic. This phase, when it ends, usually does so with the development of a minor opportunistic infection (OI) like *Candida albicans* (a yeast) of the mouth, esophagus, or rectum. Then the condition has escalated to AIDS Related Complex. ARC is often accompanied by fevers, night sweats, weight loss (in Africa it is called ‘slim sickness’) and fatigue. With the appearance of major opportunistic infections (including TB) or malignancy (commonly Kaposi’s sarcoma, less commonly Burkitt’s lymphoma or other lymphoma), or an absolute CD4 count below 200, the full-blown AIDS picture is present. This progression, which took on average 9 years, is with treatment no longer inevitable.

Because the brain also has cells that can be infected by HIV, including macrophages and glia, there is an AIDS dementia complex which is terribly distressing for patients and family. It is probably the consequence of toxic cytokine release by virus-activated phagocytes.

The infections seen in AIDS are primarily of types that require T cell-mediated immunity, as might be expected given the virus’ primary target. We see viral infection, including cytomegalovirus, hepatitis and especially herpes simplex and zoster. We see fungi, especially *Candida albicans* and *Pneumocystis jirovecii*. Protozoan infections, such as *Toxoplasma*, *Cryptosporidium* (which causes a sometimes-fatal diarrhea), and *Isospora* are very serious. Infections with opportunistic intracellular bacteria—usually *Mycobacterium avium* complex or MAC, and more and more commonly, *M. tuberculosis*—are frequent. High-grade, extracellular bacterial pathogens are less of a problem, possibly because the ability to make T-independent antibody responses to capsular polysaccharides is preserved.

Kaposi’s sarcoma, a tumor of the endothelial cells lining lymphatics, is more common in homosexual males with AIDS than in others, though the reason is not known. It is initiated by a newly identified virus, called KSHV (Kaposi’s sarcoma herpesvirus) or HHV8 (human herpesvirus 8).

DIAGNOSIS. The patient will often have made the diagnosis. The most common test is for antibody to HIV. Antibody is measured by an ELISA which has a certain false-positive rate, so a positive ELISA must be confirmed by Western Blot analysis, in which standardized viral protein preparations are separated by electrophoresis, blotted and fixed to nitrocellulose, and then ‘stained’ with the patient’s antibodies, which must bind to the correct viral proteins (gp120, gp41) for the test to be considered a true positive. Very small amounts of the virus RNA itself can now be detected by the polymerase chain reaction (PCR), and this is very useful for following therapy; it is mostly available in wealthier areas, which have a better success rate in AIDS treatment because they can do this sort of test. In patients who can be gotten down to about 50 viral particles/mL of blood and kept that low, disease progression seems to be halted.

The antibody that patients make is obviously not protective. Many scientists are beginning to think that most people with HIV infection have a Th2/Tfh-dominated helper T cell response. Perhaps the way HIV loads into DC has something to do with this, polarizing the DC so that it favors Th2/Tfh over Th1. If patients made more Th1 they might stimulate more CTL, and do better. There is evidence that HIV positive patients who make good CTL against HIV antigens have an improved prognosis.

TREATMENT. When AIDS was first identified there were no antiretroviral drugs, and many people thought that there never would be any, because viruses use our human metabolic pathways to do their evil deeds, and anything that killed a virus would be very likely to kill us, too. Fortunately (one of the few good things to come out of the AIDS pandemic) they were wrong. Viruses may be parasites but they're not Mini-Me, and they do have vulnerabilities.

CLASSES OF DRUGS. Reverse transcriptase (RT) is unique to retroviruses, using an RNA template to create DNA, so it's a target. There are two classes of **RT inhibitors**: **nucleosides**, which are competitive inhibitors and chain-terminators; and **non-nucleoside** inhibitors, which bind a hydrophobic pocket on the enzyme that changes the conformation, and thus the activity, of the catalytic site. Because escape from inhibition due to mutation is so common, using one each of these classes of drugs together greatly lowers the odds of escape.

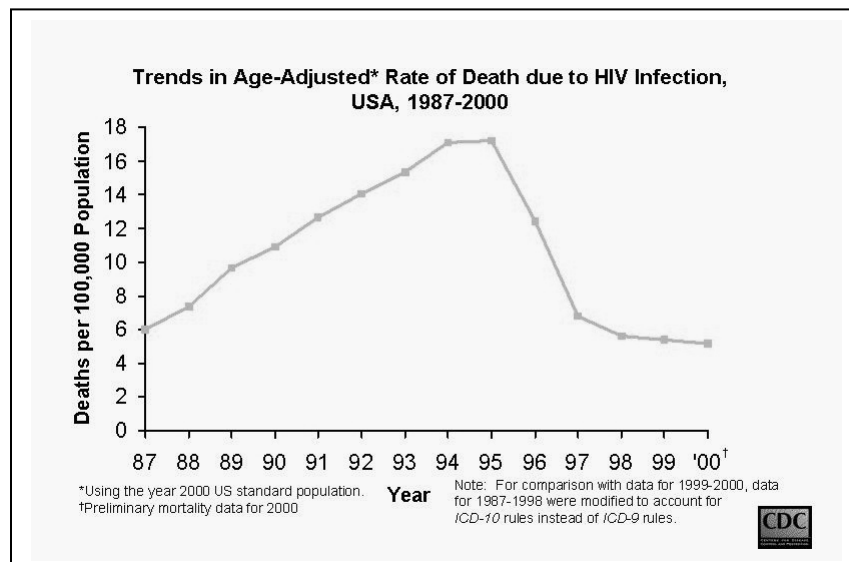
The gag, pol, and env proteins are made as a single gag-pol-env polyprotein which the virus cleaves using its own protease, which therefore has become a drug target for **protease inhibitors**.

Enfuvirtide (Fuzeon®) binds to part of gp41 so that it cannot change conformation to fuse the viral membrane with the helper cell's. It is a small peptide **fusion inhibitor**.

Newly approved in 2007 is maraviroc (Selzentry®), a small-molecule **CCR5 antagonist** that blocks viral entry into CD4⁺ cells. It binds to the transmembrane portion of CCR5 which causes changes in the conformation of the external receptor so that it no longer engages gp120.

When the viral DNA copy reaches the nucleus, a viral integrase function, part of the RT complex, inserts it randomly into the cell's DNA. Raltegravir, an **integrase inhibitor**, which received FDA advisory panel approval in 2007, blocks that step, and has been shown to be effective in patients with RT inhibitor-resistant strains of HIV.

The standard antiretroviral therapy, or **ART**, combines a protease inhibitor and two reverse transcriptase inhibitors. The cost of caring for an AIDS patient approaches \$25,000/year in the USA, which is of course greater than the health budgets of most of the world's villages. The ethical and practical problems surrounding trials and prices of new drugs, especially in the Third World, are formidable. However, several generic pharmaceutical companies around the world have defied USA and other patent laws and prepared cheap 3-drug combination ART pills that are available in sub-Saharan Africa for about \$100-\$150/year. Less than 7 million of the 34 million HIV+ people get them.



Treatment works.

PREVENTION. Safe sex, safe addictions. You don't get AIDS from casual contacts. Male circumcision is very effective and a growing practice in parts of Africa. Condoms work if they are used, and stay intact. Spermicides don't, but an anti-HIV drug (tenofovir) incorporated in a barrier gel had partial effectiveness in a South African trial in 2010. Prophylactic ART protects the non-infected member of a couple, and ART therapy to pregnant HIV+ mothers protects the fetus. The virus is not hardy, and common disinfectants (alcohol, Clorox) kill it readily. Health care practitioners should use hepatitis precautions. If you do flow cytometry on human blood, be sure you understand the production of aerosols by your machine.

In November 2010 a trial of a two- reverse transcriptase inhibitor pill found that highly sexually-active men had a 44% decrease in HIV infection compared to placebo (both groups were instructed in other prevention strategies.)

VACCINE PROBLEMS AND PROSPECTS. *Only a vaccine will ever be able to put an end to this terrible worldwide epidemic.* The concept of a vaccine is exciting, and it was recently shown that an SIV vaccine will protect chimpanzees against SIV infection. Suppose I developed a candidate HIV vaccine. What problems do you think I'd have in testing it and getting it generally available? Although there was initial excitement, a large vaccine trial fizzled (May 2003); it produced good antibody responses but did not, overall, decrease infection rates. Why? Because we need a vaccine that can preferentially stimulate Th1 cells and CTL; the current vaccines seem to be best at inducing antibody responses, and antibody doesn't protect very well (otherwise seropositive people wouldn't get sick). The key epitope on HIV seems to be well-concealed within the gp120/gp41 complex and almost invisible to B cells. However, it has been shown several times recently that a small amount of the antibody some people make is neutralizing; analysis of the recognized epitopes, it is hoped, could lead to a new 'designer' vaccine. Merck has provided a proof-of-concept epitope made this way.³ This is encouraging, because in 2007 Merck reluctantly had to close the 4-year 'Step' trial of an adenovirus-based vaccine with 3 HIV genes engineered in, which was just plain was not protecting high-risk people from infection. In 2009, a large trial (called RV144) in Thailand reported significant protection for the first time, though the effect was disappointingly modest.

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HIV may be the closest thing to a perfect virus we have encountered. It is variable, it destroys the patient's defenses, it can hide, and it allows time for spread before it kills. Fortunately, it is not highly infectious. It presents an incredibly difficult intellectual and moral challenge to scientists, physicians, and people. Will we ever be able to cure it? Or prevent it?

INTERESTING SOURCES:

NIH's very complete AIDS launch site (parallel site in Spanish):
<http://aidsinfo.nih.gov/>

CDC's AIDS/HIV Prevention page (lots of good links):
<http://www.cdc.gov/hiv/dhap.htm>

ACT-UP New York. 'We advise and inform. We demonstrate. Silence = Death.'
<http://www.actupny.org/>

³ Bianchi E, Joyce JG, Miller MD, et al. (2010) Vaccination with peptide mimetics of the gp41 prehairpin fusion intermediate yields neutralizing antisera against HIV-1 isolates. Proc Natl Acad Sci U S A 107:10655-10660.

Learning objectives for Immunology of AIDS

1. Explain the difference between 'HIV-seropositive' and 'AIDS'.
2. Name the virus that causes AIDS, and its classification.
3. Discuss the origin of the AIDS virus and the origins of the current epidemic.
4. Identify the approximate number of cases in the U.S. and in the world, and discuss the rate of change in incidence.
5. Discuss the pathogenesis of AIDS, including target cell types, mode of entry of the virus into a cell, mode of exit, latency versus productive infection.
6. Distinguish between the roles of Th1 and Th2/Tfh in the progression of HIV infections.
7. Discuss the types of infections seen in AIDS patients, and provide an immunological basis for this spectrum.
8. Discuss possible reasons for which the total number of CD4 cells in AIDS patients decline.
9. Discuss reasons for the apparent ineffectiveness of antibody in HIV infection.
10. Describe the laboratory diagnosis of AIDS.
11. Discuss the prospects and problems of AIDS vaccine development.