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. (Normal range: 500-1000/ μL)

WORLD ESTIMATES OF THE AIDS PANDEMIC 2015 (latest complete dataset)

People living with HIV in 2015

Total	36.7 million [34.0–39.8 million]
Children under 15 years	1.8 million [1.5-2.0 million] (an almost 50% drop in 2 years!)

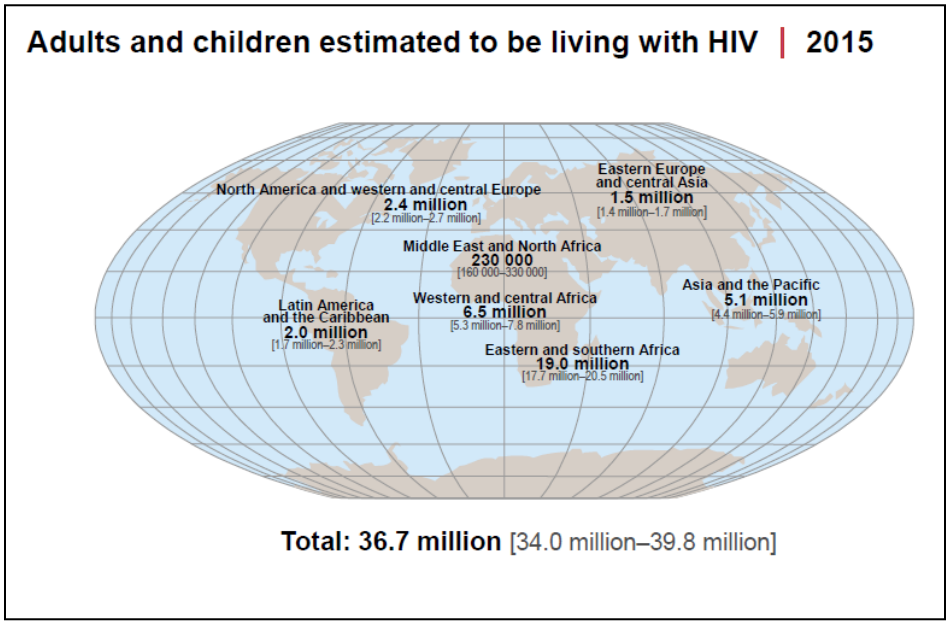
People newly infected with HIV in 2015

Total	2.1 million [1.8–2.4 million] (this is a 33% decrease from 2001)
Children under 15 years	240 000 [210 000–280 000]

AIDS deaths in 2015

Total	1.1 million [0.94–1.3 million]
Children under 15 years	110 000 [84 000–130 000]

World Health Organization/UNAIDS/HIV Department

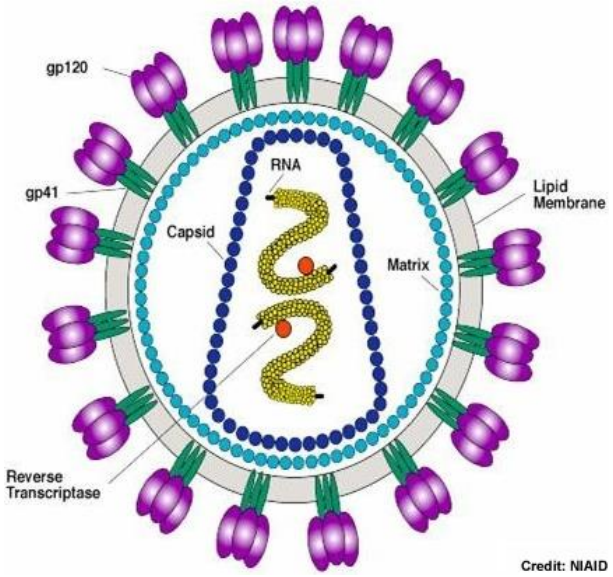


INCIDENCE AND PREVALENCE. First described in 1981, by 2004 AIDS was the 5th leading cause of death in the world, the 4th in developing countries. So far, 40 million [34-46] have died.

In the USA, the CDC counted 1,160,000 cumulative cases of AIDS by the end of 2012, of whom about 800,000 had died. With new treatments, death rates have fallen remarkably: 38,000 died in 1996, 15,500 in 2010. However, ►about 1,200,000 people are living with HIV in the United States, and about 16% of them don't know it. There are 50,000 new cases a year. OraQuick, a home test for HIV antibody, was approved in May 2012. It produces only 1:5000 false positives, but 1:12 false negatives, so it should be useful but not definitive for reducing that 16%.

There are about 11,200 people living with HIV/AIDS in Colorado (2014 figure). Incidence in Colorado has been falling since 1993; there were about 400 new cases in Colorado in 2011, and 150 deaths. About 25% of new cases were first diagnosed when they already had AIDS; that number must come way down.

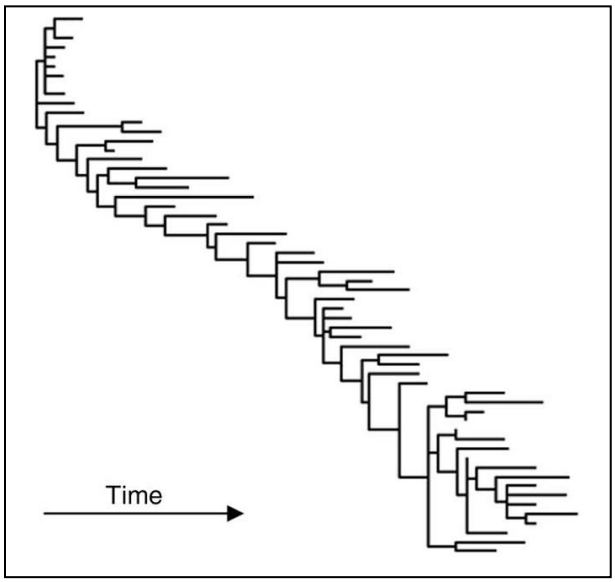
CAUSE. AIDS is caused by a virus called **HIV-1**, for Human Immunodeficiency Virus. HIV-2 has been isolated in West Africa, but has not gone global. HIV is a nontransforming retrovirus, that is, an RNA virus that carries no oncogene, and reproduces itself by copying its RNA into DNA by means of its own 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, and so the group are referred to as **lentiviruses**.



It is most closely related to a Simian Immunodeficiency Virus, SIV. HIV-1 evolved recently from SIV, probably around 1920 near Leopoldville (now Kinshasa) in Zaire (now the Democratic Republic of Congo). 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 earlier blood and tissue samples from D.R. Congo. Thus HIV is a new virus, which has jumped from simian to human and not yet adapted to its new host (similar in that respect to Ebola and Marburg viruses). Recent data suggest that the virus's virulence may be declining in at least some parts of Africa.

Seroepidemiology indicates that HIV 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 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 long been replaced by 'risky behaviors.'

HIV-1 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)

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 drug abuse *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. A male circumcision campaign has decreased transmission of HIV remarkably in South Africa.

In the years before the first HIV drug (1981-1987) 95% of all infected people died of tumors or opportunistic infections. A few were identified as **"long-term survivors"** (LTS). Of these, some are homozygous for a 32-base pair deletion in the gene for a chemokine receptor, CCR5 (they were $CCR5^{\Delta 32}$). As you'll see below, CCR5 is an HIV coreceptor. People with two $CCR5^{\Delta 32}$ 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. The mutated allele occurs at a 10% frequency in Caucasians, but is very rare in other populations. A different group of LTS were **"elite controllers."** They became infected but did not progress to AIDS. Two-thirds of them have the HLA-B57 allele. They make effective CTL to HIV peptides presented in HLA-B57. There is a good correlation between HIV-specific CTL numbers and prognosis.

Over 10,000 children have been reported as HIV+ in the USA, mostly of mothers who were at high risk for acquiring HIV infection (drug abuse, sex work).

PATHOGENESIS. ►After a single exposure, infected people develop high blood virus levels ($>10^5$ copies/mL) that peak at about 6 weeks. There is a loss of CD4 cells in the gut mucosa, and an associated increase in gut permeability. HIV spreads systemically. Antibody to HIV peaks by 9 weeks, whereupon virus levels fall sharply, but not to zero. This new level is the patient's "set point" and it seems to reflect the abilities of their immune system, rather than those of the virus. The mean incubation period (infection to AIDS) estimated from transfusion-acquired HIV infection, 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-SIGN[1]. 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 DNA moves into the nucleus. Helped by a viral integrase, it is then inserted into a random 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. By using all three reading frames, the small HIV genome (9749 bases) encodes 9 genes: the *gag*, *pol*, and *env* genes that all retroviruses have, and 6 others that regulate latency and virulence. By alternative RNA splicing, and protease-mediated cleavage of 3 large precursor proteins (HIV makes its own protease) it can make 16 polypeptides.

> **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 anymore?

HIV-infected T cells may die rapidly; become persistent virus-producers; or enter latency. In the first case, 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/gp41 is made early, and it becomes inserted into the 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 could be part of the reason 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). When the immune system can no longer cope, opportunistic infections take hold.

During the long seropositive period, ►the major site of HIV persistence is memory Tfh cells in the lymph nodes. They are able to suppress viral replication but not eliminate the virus DNA from their nuclei. If such a cell chances to get activated by its correct antigen, it will develop into a clone of virus-producing cells. This disruption of Tfh function leads to, or is accompanied by, a gradual dysregulation of B cells, which early on can be hyperactivated, and later become exhausted so that antibody production begins to decline.

[1] 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 a million people in the United States. After the acute infection, there is a phase of clinical latency—viral infection without symptoms—that may last years. Good therapy can keep the next stages from developing. Next would be the development of a minor opportunistic infection (OI) like *Candida albicans* (a yeast) of the mouth, esophagus, or rectum. There may be fevers, night sweats, weight loss and fatigue. With the appearance of major opportunistic infections (including TB) or malignancy (commonly Kaposi sarcoma, less commonly Burkitt lymphoma or other lymphoma), or an absolute CD4 count below 200, the full-blown AIDS picture is present. This progression, as we said, is with treatment no longer inevitable.

Because the brain also has cells that can be infected by HIV, including macrophages and microglia, there is a not-uncommon late 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. ►In fact, TB is the leading cause of death in people infected with HIV. High-grade, extracellular bacterial pathogens are less of a problem, possibly because the ability to make Tfh-independent antibody responses to capsular polysaccharides is preserved.

Kaposi sarcoma is a tumor of the endothelial cells lining lymphatics. It is caused by KSHV (Kaposi sarcoma herpesvirus,) also called 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 serum, whose antibodies must bind to the correct viral proteins (gp120, gp41) for the test to be considered a true positive. In richer countries, once the diagnosis is made, the virus is sequenced to see what drugs it will be susceptible to. ►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. 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. But so far, in spite of the occasional news story, only 3 people are known to have *remained* virus-free without continuous treatment; two treated in infancy, and the "Berlin patient."

ASK YOURSELF: Do I want to look the Berlin patient up?

The antibodies that patients typically make are obviously not protective. Though they bind to the virus, they do not block attachment to and infection of Th cells. There *are* neutralizing epitopes on the virus, but they are shielded by carbohydrate and not readily available to B cells. Usually, if a patient does make neutralizing antibody, the virus rapidly mutates and escapes. But broadly-neutralizing antibodies *are* possible; see below.

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. AZT, the first antiretroviral drug, was licensed in 1987.

CLASSES OF DRUGS. Reverse transcriptase (RT) is unique to retroviruses, using their RNA template to create DNA, so it's a target. ►There are two classes of **RT inhibitors**: **nucleosides (NRTI)**, which are competitive inhibitors and chain-terminators; and **non-nucleoside (NNRTI)** 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 each of these classes of drugs together greatly lowers the odds of escape.

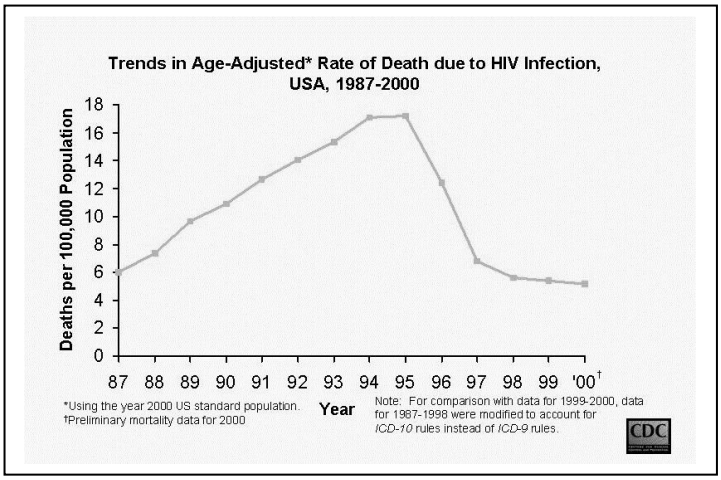
The gag, pol, and env proteins are made as a single gag-pol-env polyprotein which the virus cleaves using its own protease, which therefore is a drug target for **protease inhibitors**.

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**.

►Maraviroc (Selzentry®) is a small-molecule **CCR5 antagonist** that blocks viral entry into $CD4^+$ cells. It binds to the transmembrane portion of CCR5, causing 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.

►Standard antiretroviral therapy, or **ART**, combines two NRTIs and a third drug from a different class; in the typical first-line formulation that would be an NNRTI. These can all go in a single one-a-day pill, which increases compliance considerably. The cost of caring for an AIDS patient exceeds $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 US and other patent laws and prepared cheap 3-drug combination ART pills that are available in sub-Saharan Africa for about $100-$150/year. Nearly 50% of HIV+ people were receiving ART in 2017, an all-time high.



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 or high-*G* centrifugation on human blood, be sure to know the production of aerosols by your machines.

All HIV seropositive people should be treated with ART. Early treatment probably increases survival and definitely decreases transmission. 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.) Compliance was a problem; the most compliant subjects were protected much more effectively.

VACCINE PROBLEMS AND PROSPECTS. *Will 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.[2] 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.

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 many times recently that a small amount of the antibody about 20% of HIV-positive people make is ►broadly neutralizing (bnAb); it can block infection by almost all HIV strains and mutant forms. The most interesting are ones directed against the site on the gp120 that binds to CD4; this site can't mutate much, because if it did, the virus would no longer be infective. Getting everyone to make these antibodies in response to a practical vaccine is full of roadblocks, but some very aggressive work is pushing the enterprise forward[3].

[2] 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.
[3] Jardine, JG et al. (2015) Priming a broadly neutralizing antibody response to HIV-1 using a germline-targeting immunogen. Science 349: 156-161.

HIV vaccine development challenges:

1. HIV exhibits tremendous global genetic diversity.
2. Its immense mutational capacity allows evasion of both T and B cell immunity.
3. HIV goes latent in the host genome, from which it cannot be eliminated by conventional antiretroviral drugs.
4. There has been no known example of spontaneous immune clearance, to use as the basis for data-driven vaccine design.
5. Although bnAbs have been found, they are rare, only found in a subgroup, take years to develop, and are extensively hypermutated; no method exists now for induction of these Abs by immunization.
6. But a lot of smart people think that they can figure out a way to make these epitopes immunogenic in everybody.

* * *

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):
https://www.cdc.gov/hiv/basics/prevention.html

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

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.

12. Define and discuss "elite controllers."