Retroviruses

General Introduction to Retroviruses

Retroviruses

- Ubiquitous; found in all vertebrates
- Large, diverse family
- Includes HIV, FIV and FeLV

Definition and classification of retroviruses

- Common features- structure, composition and replication
- Distinctive life cycle: RNA-DNA-RNA
- Nucleic acid is RNA in virus, and DNA in infected cell

Transmission may be either:

- Horizontal- by infectious virus (exogenous virus) or vertical- by proviruses integrated in germ cells (endogenous virus)
- Can transmit either as free viral particle or (for some retroviruses) through cell-cell contact

Retrovirus Overview

Enveloped virus with lipid bilayer and viral spike glycoproteins.

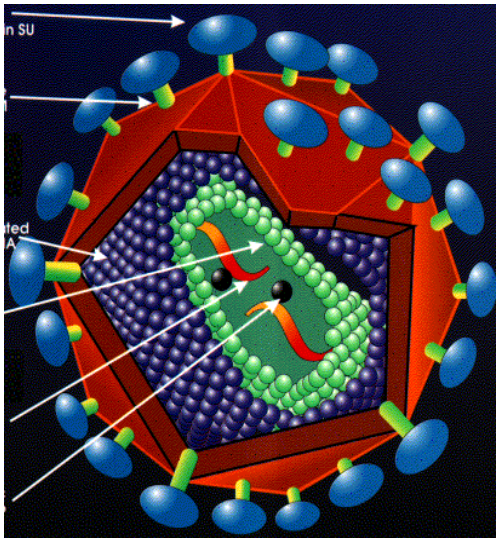
Have outer matrix protein and inner core capsid containing viral genome.

Genome: Two copies of single stranded positive-stranded RNA (8-10kb).

All retroviruses contain gag, pol and env genes.

Simple - only gag, pol, env

Complex - additional genes involved in replication.



Reverse transcriptase to generate DNA

Viral genes are integrated into host genome.

Progeny virus produced using host cell transcriptional and translational machinery.

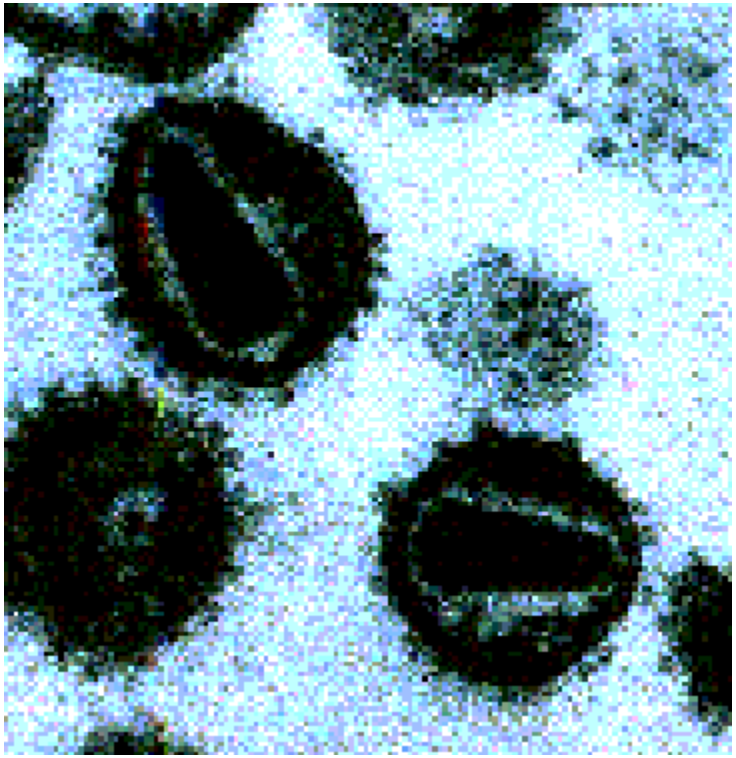
Retrovirus Classification

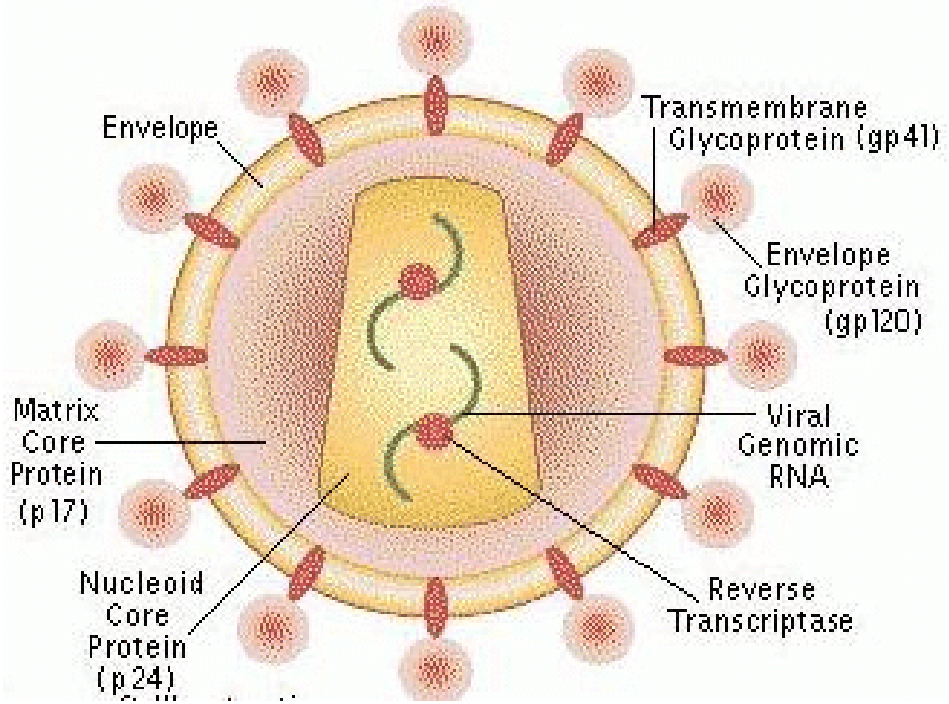
Genus	Example	Genome
Alpharetrovirus	Avian leukemia virus	Simple
Betaretrovirus	Mouse mammary tumor virus	Simple
Gammaretrovirus	Murine leukemia virus Feline leukemia virus Xenotropic murine leukemia-related virus	Simple
Deltaretrovirus	Human T-cell leukemia virus	Complex
Epsilonretrovirus	Wall-eyed sarcoma virus	Complex
Lentivirus	HIV, SIV, FIV	Complex
Spumavirus	Human foamy virus	Complex
Metavirus	Yeast TY-3	
Errantvirus	Drosophila melanogaster Gypsy	

Human Immunodeficiency Virus

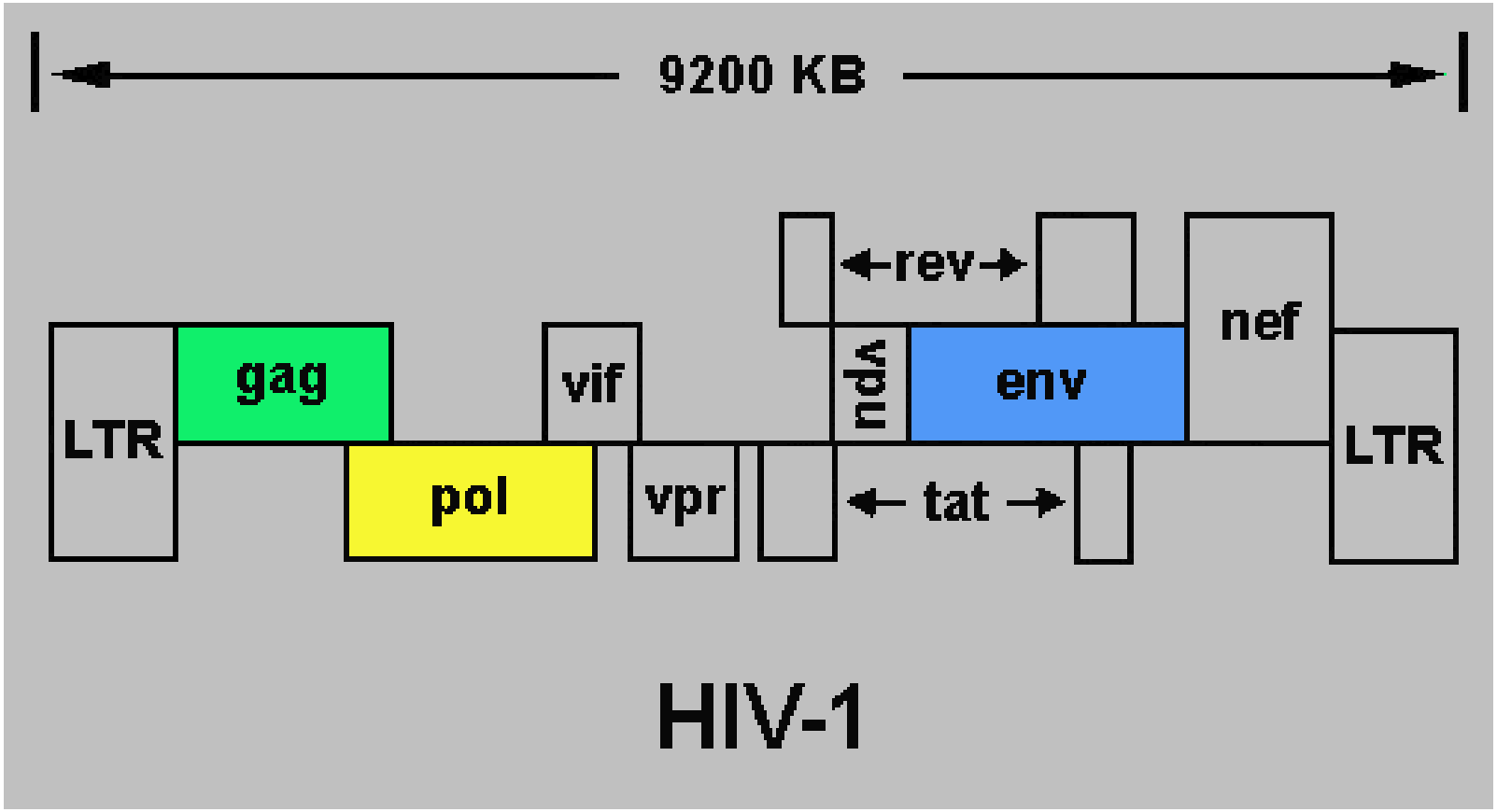
- HIV-1 may have spread to humans over 100 years ago
- The likely source was primate-to-human transmission through bites or blood exposure, with chimpanzees being the most likely candidate for HIV-1 transmission to humans.
- HIV was identified as the causitive organism of AIDS by Dr. Luc Montagnier in France and by Dr. Robert Gallo in the United States
- Acquired Immunodeficiency syndrome first described in 1981
- HIV-1 isolated in 1984, and HIV-2 in 1986
- Belong to the lentivirus subfamily of the retroviridae
- Enveloped, +ve ss RNA virus (two copies), 120nm in diameter
- HIV-2 shares 40% nucleotide homology with HIV-1
- Genome consists of 9200 nucleotides (HIV-1):
- gag core proteins - p17 (Matrix), and p24 (Capsid), NC (P7)
- pol - p16 (protease), p31 (integrase/endonuclease), RT, RNaseH
- env - gp160 (gp120:outer membrane part, gp41: transmembrane part)
- Other regulatory genes ie. tat, rev, vif, nef, vpr and vpu

HIV particles





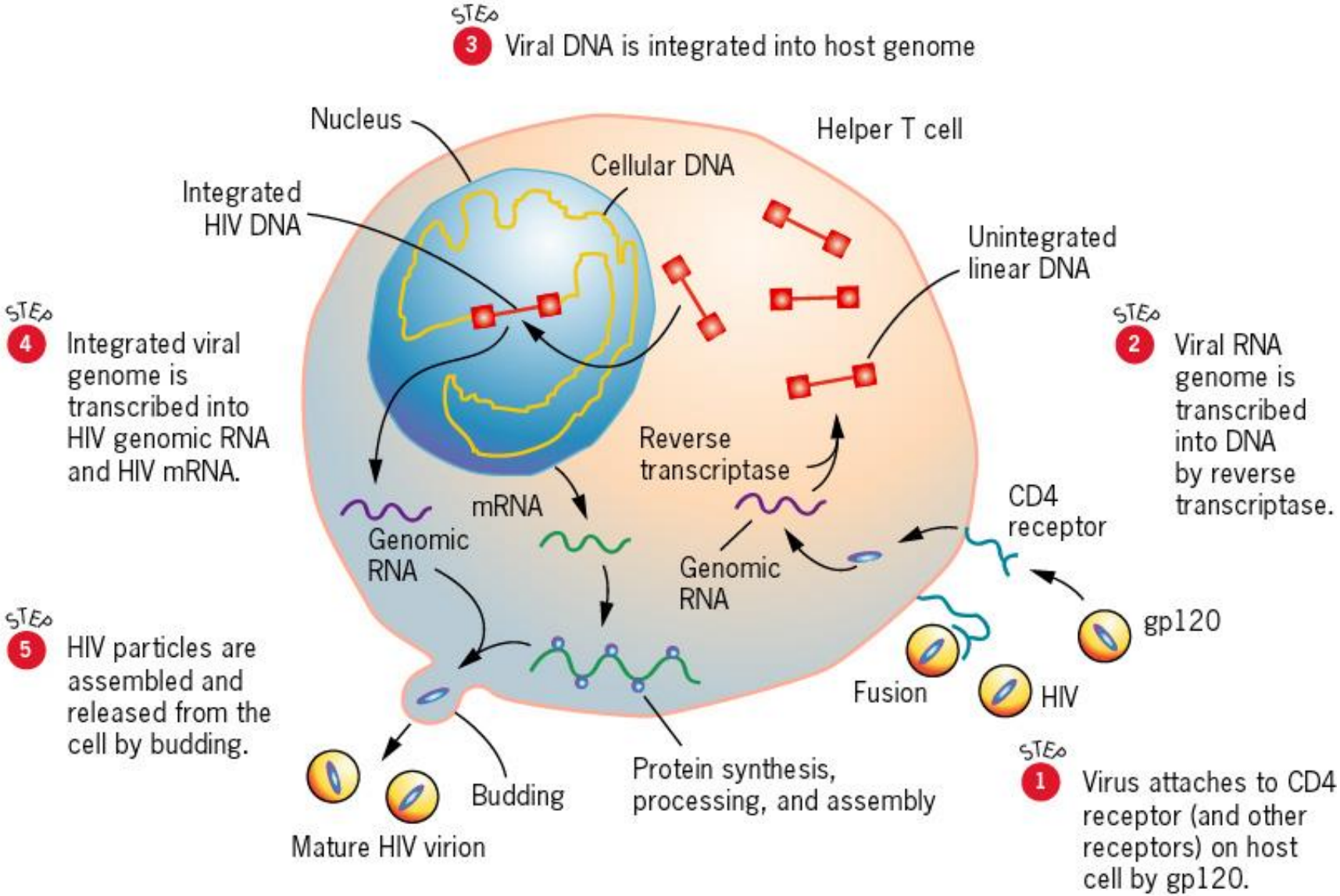
HIV Genome



Replication

- The virus attaches to and enters human cells, especially cells known as CD4 T-cells, macrophages, and dendritic cells
- The first step of infection is the binding of gp120 to the CD4 receptor of the cell, which is followed by penetration and uncoating.
- The RNA genome is then reverse transcribed into a DNA provirus which is integrated into the cell genome.
- HIV may remain quiescent (latent) in the genome or may be actively transcribed, causing the virus to replicate
- This is followed by the synthesis and maturation of virus progeny.
- HIV is a prolific virus and is able to create trillions of copies of itself in a short period of time
- There are two types of HIV: HIV-1 and HIV-2. HIV-1 is the primary cause of HIV infection and AIDS in the world. HIV-2 is less common and less easily transmitted

HIV life cycle



Epidemiology

- In the United States, surveillance statistics show there are approximately 1.1 million people living with HIV infection, but 18% are unaware that they are infected.
- Approximately 50,000 people get infected with HIV each year
- Of the 47,500 new infections in 2010, 63% were in men who had sex with men, 25% were acquired through heterosexual contact, and 8% were from injection drug use.
- HIV infection has hit the African-American community harder than other groups, with 44% of new infections occurring in this population, compared to 31% in whites and 21% in Hispanics.
- Over 600,000 Americans have died of HIV infection since the epidemic began.
- Worldwide, there are approximately 34 million people living with HIV, and there are 2.5 million new cases each year.
- Since HIV infection was recognized, there have been 30 million deaths as a result of HIV infection

Mode of transmission and risk factors

1. Sexual transmission - male homosexuals and constitute the largest risk group in N. America and Western Europe. In developing countries, heterosexual spread constitute the most important means of transmission. Oral sex is less common but is still possible.
2. Blood/blood products - IV drug abusers represent the second largest AIDS patient groups in the US and Europe.
3. Vertical transmission - the transmission rate from mother to the newborn varies from around 15% in Western Europe to up to 50% in Africa. Vertical transmission may occur transplacentally route, perinatally during the birth process, or postnatally through breast milk.

- Other less common modes of transmission include accidental needle stick or sharps injuries in health-care workers and splashes of contaminated material onto mucous membranes or nonintact skin
- HIV infection from a transfusion is less than one in 1.5 million.
- Casual contact (shaking hands, sneezing, coughing, sharing drinking glasses, sharing a toilet, exposure to saliva from social kissing, etc.) does not pose a threat for HIV infection

HIV Staging

- The CDC uses a staging system based on how much damage has been done to the immune system:
  - **Stage 1** disease is the earliest phase. Stage 1 has no unusual infections or cancers or other conditions that would be associated with AIDS. In other words, stage 1 disease has no "AIDS-defining conditions". Although blood tests are positive for HIV, the CD4 cell count is at least 500 cells per microliter of blood (or >29% of all lymphocytes).
  - **Stage 2** disease occurs when the CD4 count is between 200-499 cells per microliter (14%-28% of all lymphocytes), but again there are no AIDS-defining conditions present.
  - **Stage 3** disease is synonymous with AIDS and is the most severe stage. There are two ways of diagnosing stage 3 disease: either by CD4 counts below 200 cells per microliter (<14% of lymphocytes) or through documentation of an AIDS-defining condition

AIDS-defining conditions

- Candidiasis of bronchi, trachea, lungs, or esophagus
- Cervical cancer, invasive
- Disseminated or extrapulmonary coccidioidomycosis or *Cryptococcus*
- Chronic intestinal cryptosporidiosis or isosporiasis
- Cytomegalovirus disease of the retina or an unusual site (other than liver, spleen, or nodes)
- HIV encephalopathy
- Herpes simplex that does not heal or that occurs in the lungs or esophagus
- Histoplasmosis that is disseminated or extrapulmonary
- Kaposi's sarcoma
- Selected lymphomas including Burkitt's, or arising in the brain
- Disseminated or extrapulmonary *Mycobacterium avium-intracellulare* complex or *Mycobacterium kansasii*, or other species of mycobacterium
- *Mycobacterium tuberculosis* infection
- *Pneumocystis jirovecii* pneumonia
- Recurrent bacterial pneumonia
- Progressive multifocal leukoencephalopathy
- Recurrent or multiple bacterial infections
- Recurrent *Salmonella* septicemia

Clinical manifestations

- **Acute infection**: 2-4 weeks up to 3 months after infection with HIV, "the worst flu ever." It is called primary HIV infection (caused by the body's natural response to the HIV infection). Symptoms: fatigue, sore throat, enlarged lymph nodes, and loss of appetite. Orally: thrush or mouth sores. Fever, neck stiffness, headache, and rash may occur
- **Clinical latency**: (asymptomatic HIV infection or chronic HIV infection) During this phase, HIV reproduces at very low levels. No symptoms (last up to eight years or longer). Pts are contagious to others. Constitutional symptoms such as fatigue and other nonspecific symptoms develop.
- **AIDS**: CD4 cells <200 cells/cubic milliliter. (Normal CD4 500-1,600) Vulnerable to opportunistic infections. AIDS pts survive about 3 yrs without treatment. Once someone has a dangerous opportunistic infection, life expectancy falls to about one year.

Opportunistic Infections

Protozoal	*Pneumocystis jirovecii* (fungus), toxoplasmosis, crytosporidosis
Fungal	candidiasis, crytococcosis histoplasmosis, coccidiodomycosis
Bacterial	Mycobacterium avium complex, MTB atypical mycobacterial disease salmonella septicaemia multiple or recurrent pyogenic bacterial infection
Viral	CMV, HSV, VZV, JCV

Opportunistic Tumours

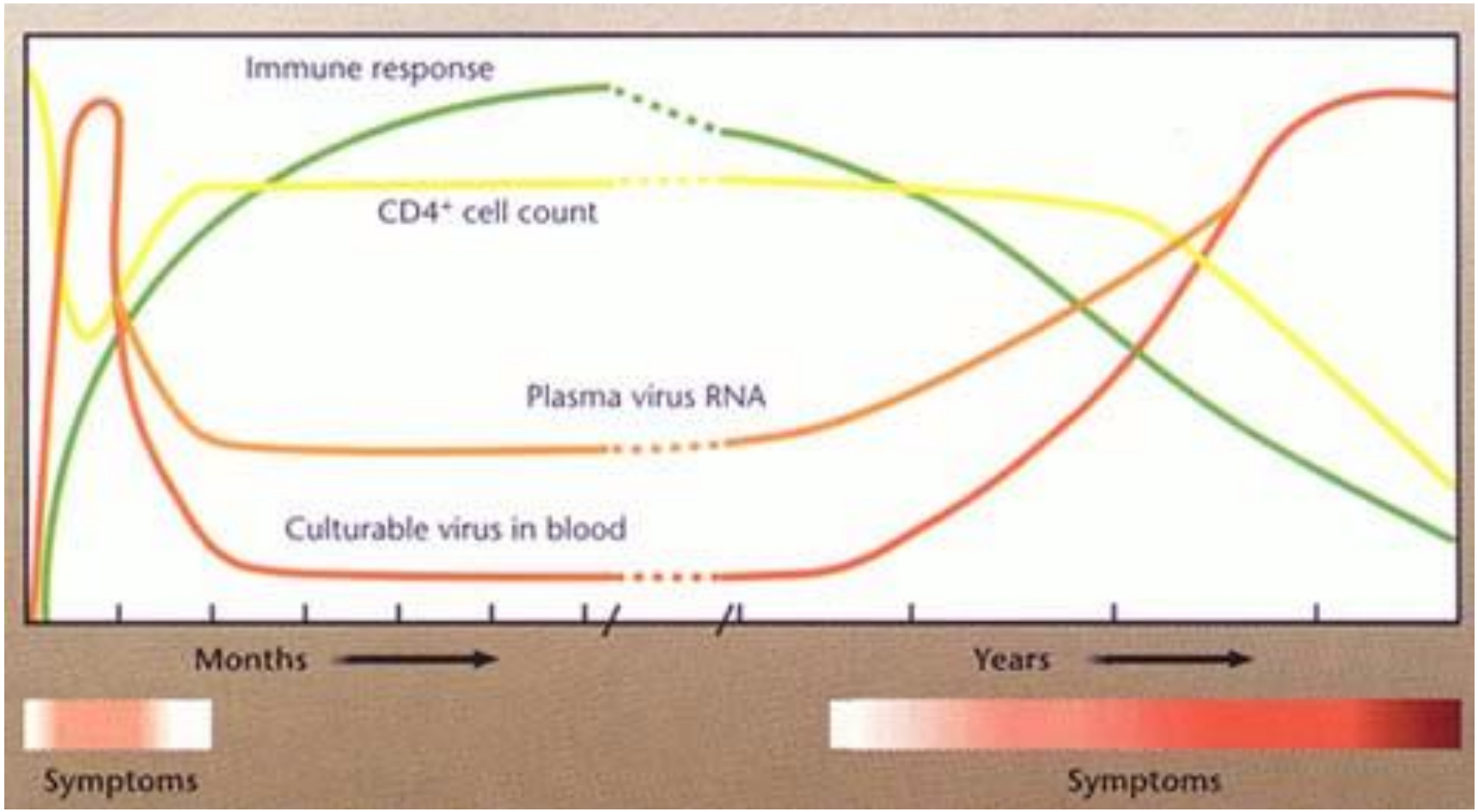
- The most frequent opportunistic tumour, Kaposi's sarcoma, is observed in 20% of patients with AIDS.
- KS is observed mostly in homosexuals and its relative incidence is declining. It is now associated with a human herpes virus 8 (HHV-8).
- Malignant lymphomas are also frequently seen in AIDS patients.

Other Manifestations

- It is now recognised that HIV-infected patients may develop a number of manifestations that are not explained by opportunistic infections or tumours.
- The most frequent neurological disorder is AIDS encephalopathy which is seen in two thirds of cases.
- Other manifestations include characteristic skin eruptions and persistent diarrhoea.

HIV Pathogenesis

- The profound immunosuppression seen in AIDS is due to the depletion of T4 helper lymphocytes. (500-1600/mm^3)
- Acute infection:
  - In the immediate period following exposure, HIV is present at a high level in the blood (as detected by HIV Antigen and HIV-RNA assays).
  - Rapid fall in the CD4 count→ viral set point (low level)→ CD4 count begins to increase (CD4 cells killed by HIV are replaced efficiently), but not to pre-infection levels. Virus suppression but not elimination.
- Clinical latency:
  - Undetectable viral load and a healthy CD4 cell count without the use of medication for a time
  - Toward the middle and end of this period, the viral load begins to rise and the CD4 cell count begins to drop
- Eventually, the immune system succumbs and AIDS develop when killed CD4 cells can no longer be replaced (witnessed by high HIV-RNA, HIV-antigen, and low CD4 counts < 200/mm^3).


Immune response
CD4+ cell count
Plasma virus RNA
Culturable virus in blood
Months
Years
Symptoms
Symptoms

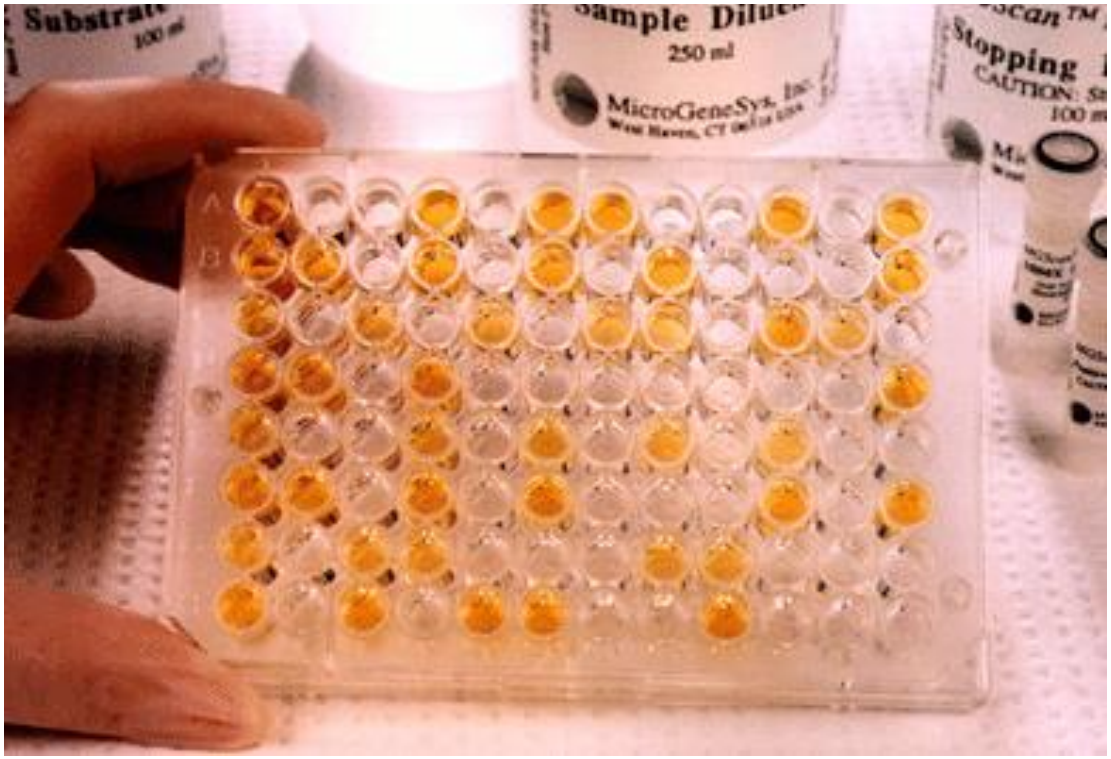
Laboratory Diagnosis

- Serology is the usual method for diagnosing HIV infection. Serological tests can be divided into screening and confirmatory assays. Screening assays should be as sensitive whereas confirmatory assays should be as specific as possible.
- Screening assays - ELISA are the most frequently used screening assays. The sensitivity and specificity of the presently available commercial systems now approaches 100% but false positive and negative reactions occur. Some assays have problems in detecting HIV-1 subtype O.
- Confirmatory assays - Western blot is regarded as the gold standard for serological diagnosis. However, its sensitivity is lower than screening ELISAs. Line immunoassays incorporate various HIV antigens on nitrocellulose strips. The interpretation of results is similar to Western blot it is more sensitive and specific.

- New fourth-generation tests combine viral detection and antibody detection. Viral detection is done by testing for a component of the virus known as p24 antigen
- Viral load is measured by testing the amount of viral RNA in the blood

ELISA for HIV antibody

It normally takes 4-6 weeks before HIV-antibody appears following exposure



Microplate ELISA for HIV antibody: coloured wells indicate reactivity

Western blot for HIV antibody

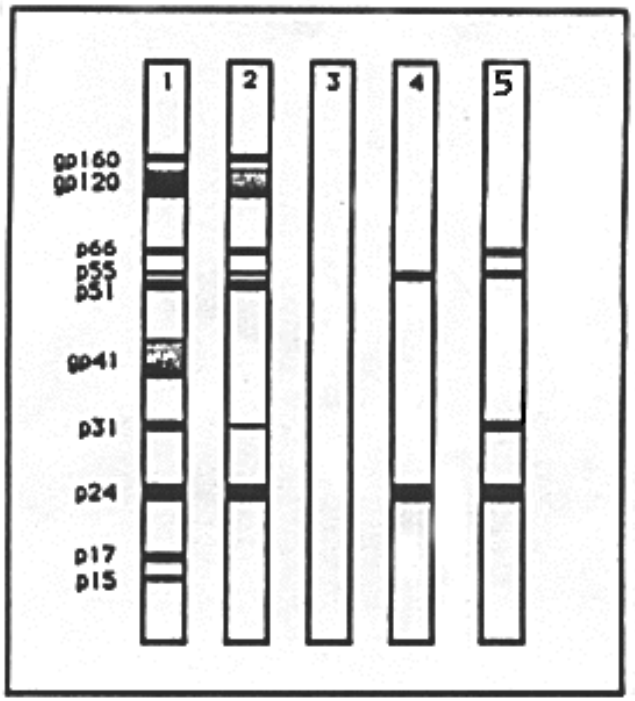


Figure:

Examples of reactions by an HIV-1 Western blot:

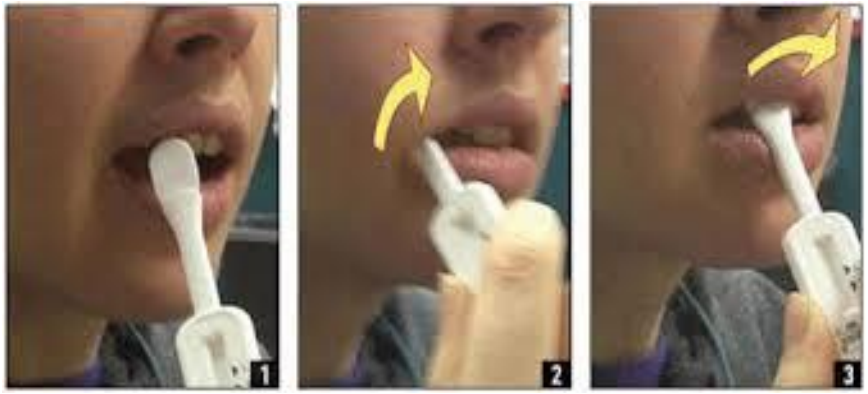
1. Positive control (strong)
2. Positive control (weak)
3. Negative control
4. Indeterminate profile
5. Indeterminate profile (highly suggestive)

- There are different criteria for the interpretation of HIV Western blot results e.g. CDC, WHO, American Red Cross.
- The most important antibodies are those against the envelope glycoproteins gp120, gp160, and gp41
- p24 antibody is usually present but may be absent in the later stages of HIV infection

Other diagnostic tests

- Rapid HIV tests (such as Clearview) were developed that could provide results during the initial visit

- self-test or home-test, the person buys a kit (for example, OraQuick), swabs the inside of their cheek, places the swab in the supplied fluid, and reads the results in a test window



Prognostic tests

Once a diagnosis of HIV infection had been made, it is important to monitor the patient at regularly for signs of disease progression and response to antiviral chemotherapy.

HIV viral load - HIV viral load in serum may be measured by assays which detect HIV-RNA e.g. RT-PCR. HIV viral load has now been established as having good prognostic value, and in monitoring response to antiviral chemotherapy.

HIV Antigen tests - they were widely used as prognostic assays. It was soon apparent that detection of HIV p24 antigen was not as good as serial CD4 counts. The use of HIV p24 antigen assays for prognosis has now been superseded by HIV-RNA assays.

Treatment

- Zidovudine (AZT) was the first anti-viral agent shown to have beneficial effect against HIV infection. However, after prolonged use, AZT-resistant strains rapidly appears which limits the effect of AZT.
- Combination therapy has now been shown to be effective, especially for trials involving multiple agents including protease inhibitors. (HAART - highly active anti-retroviral therapy)
- The rationale for this approach is that by combining drugs that are synergistic, non-cross-resistant and no overlapping toxicity, it may be possible to reduce toxicity, improve efficacy and prevent resistance from arising.

Anti-Retroviral Agents

- Nucleoside analogue reverse transcriptase inhibitors e.g. AZT, ddI, lamivudine
- Non-nucleoside analoque reverse transcriptase, inhibitors e.g. Nevirapine
- Protease Inhibitors e.g. Indinavir, Ritonavir
- Fusion inhibitors e.g. Fuzeon (IM only)
- Integrase inhibitors impair the ability of the transcribed viral DNA to insert into the human genome
- HAART (highly active anti-retroviral therapy) regimens normally comprise 2 nucleoside reverse transcriptase inhibitors and a protease inhibitor. e.g. AZT, lamivudine and indinavir. Since the use of HAART, mortality from HIV has declined dramatically in the developed world.

Prognosis of an HIV infection

- Without treatment, HIV infection progresses to AIDS in approximately 10 years, with death following within three years after onset of AIDS.
- With appropriate treatment, a 20-year-old with HIV infection can expect to live to reach 71 years of age.
- This dramatic increase in life expectancy emphasizes the need for early diagnosis and treatment.
- There are some factors that decrease life expectancy, including use of illicit drugs and the coexistence of other conditions like chronic hepatitis

Prevention

- The risk of contracting HIV increases with the number of sexual partners. A change in the lifestyle would obviously reduce the risk. Using condoms with every sexual encounter reduces the risk of infection.
- The spread of HIV through blood transfusion and blood products had virtually been eliminated since the introduction of blood donor screening in many countries.
- AZT had been shown to be effective in preventing transmission of HIV from the mother to the fetus. The incidence of HIV infection in the baby was reduced by two-thirds.
- The management of health care workers exposed to HIV through inoculation accidents is controversial. Anti-viral prophylaxis had been shown to be of some benefit but it is uncertain what is the optimal regimen.
- Items that may be contaminated with blood, such as razors or toothbrushes, should not be shared
- Vaccines are being developed at present but progress is hampered by the high variability of HIV. Since 1987, more than 30 HIV candidate vaccines have been tested in approximately 60 Phase I/II trails, involving more than 10,000 healthy volunteers. A phase III trial involving a recombinant gp120 of HIV subtype B was reported in Feb 2005 to be ineffective in preventing HIV infection.