Retroviruses

Virology Lec. For M.Sc. Students

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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

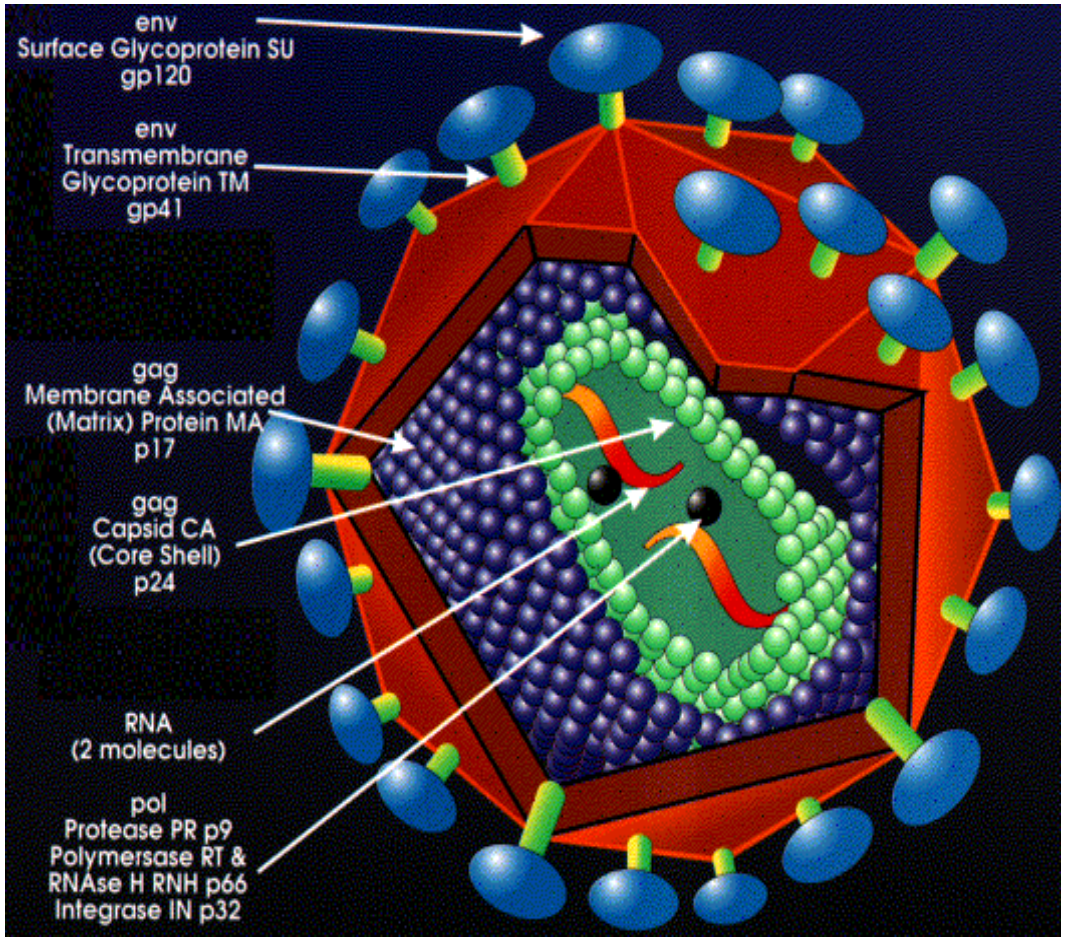
Transmission may be either:

- 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

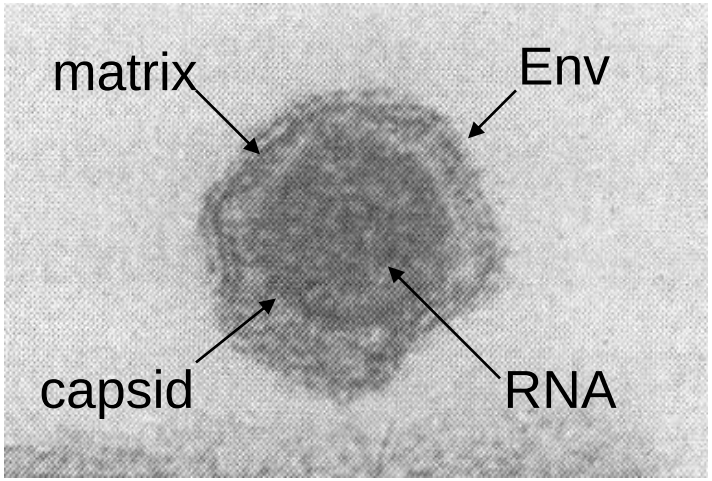
Retrovirus structure

- **Retrovirus virions are 80-120 nm in diameter, have spherical morphology, a phospholipid envelope with knobs**
- **Contain around 2000 molecules of nucleocapsid (NC) protein that bind to the two copies of (+) strand RNA genome**
- **Retroviral ribonucleoproteins are encased within a protein shell built from the capsid protein to form an internal core, which can have different shapes and has a conical shape in HIV**

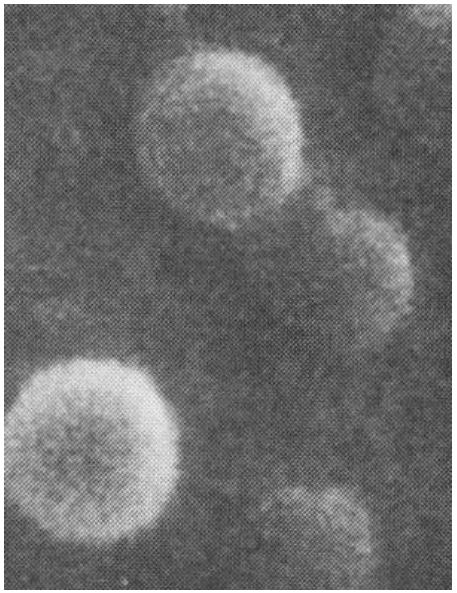
Retroviruses



Transmission EM



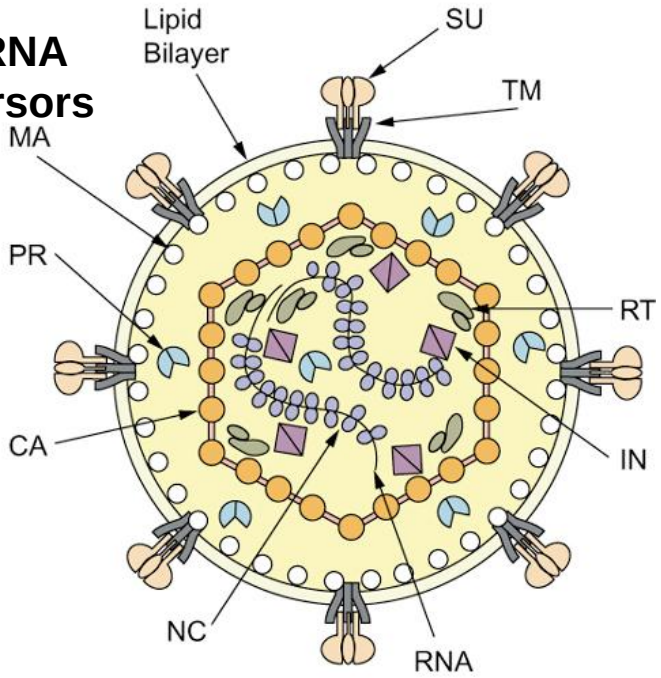
Scanning EM



3D representation of HIV virion:
http://www.mcld.co.uk/hiv/?q=3D%20HIV

Retroviral Structural genes

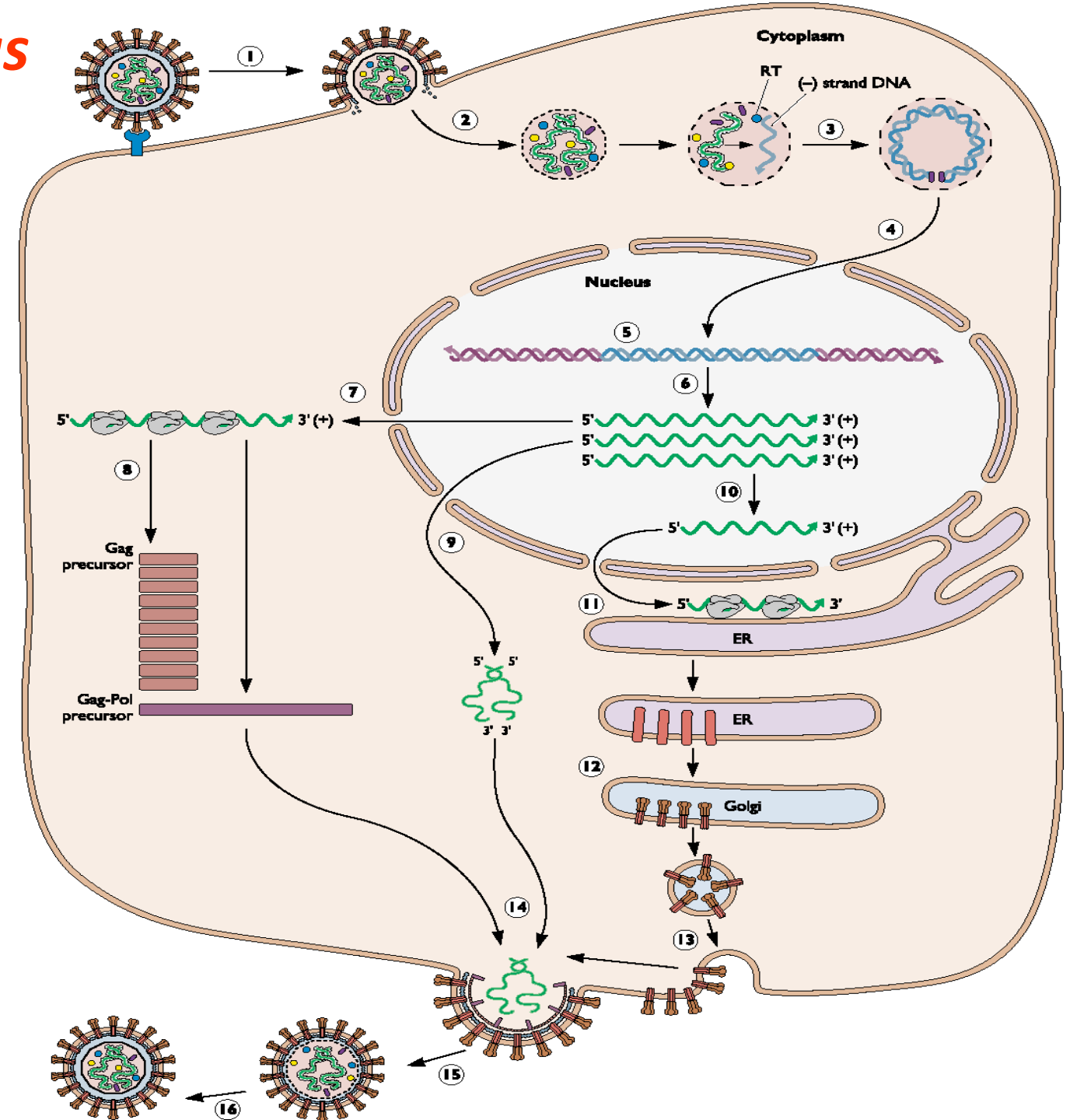
Gene	**Proteins**	**Function**
gag = group specific antigen (internal structural proteins)		
	matrix (MA),	**binds envelope, organization**
	capsid (CA),	**protects genome and enzymes**
	nucleocapsid (NC)	**chaperones RNA, buds**
pol = polymerase enzymes		
	reverse transcriptase +	**RNA to DNA**
	RNAase H (RT)	**degrades template RNA**
	protease (PR)	**maturation of precursors**
	integrase (IN)	**provirus integration**
env = envelope proteins		
	surface glycoprotein (SU)	**receptor binding**
	transmembrane protein (TM)	**virus-cell fusion**



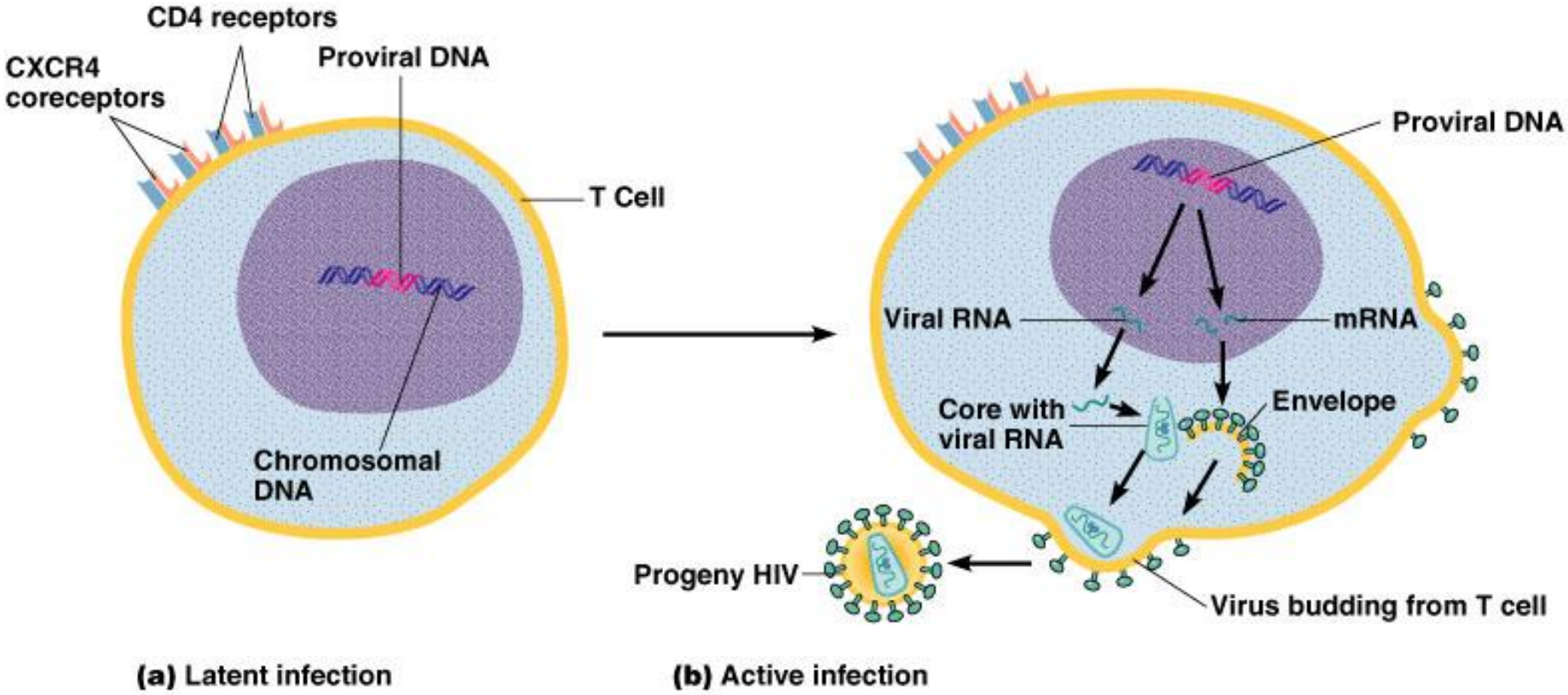
Retrovirus replication cycle

1. **Attachment of the virion to a specific cell surface receptor**
2. **Penetration of the virion core into the cell**
3. **Reverse transcription within the core structure to copy the genome RNA into DNA**
4. **Transit of the DNA to the nucleus**
5. **Integration of the viral DNA into random sites in cellular DNA to form the provirus**
6. **Synthesis of viral RNA by cellular RNA polymerase II using the integrated provirus as a template**
7. **Processing of the transcripts to genome and mRNAs**
8. **Synthesis of virion proteins**
9. **Assembly and budding of virions**
10. **Proteolytic processing of capsid proteins**

Retrovirus life cycle:



Latent vs. active infection



In latent infection- retroviral genome is present but is not transcribing viral genome or mRNA for structural proteins.

Oncogenic retroviruses

- **Cancer is a genetic disease- oncogenesis consists of the processes that result in growth of cells in which mutations have accumulated**
- **Viruses are a contributing factor in about 20% of all human cancers**
- **Growth properties and morphologies of cultured cells could be changed upon infection with certain viruses- cells become transformed**
- **Cells become immortal in an early step in oncogenesis-they continue to grow and divide even though the body has sufficient numbers of these cells**
- **They lose contact inhibition and the need to adhere to a surface**
- **They look different, more rounded**

Oncogenic viruses

- **Oncogenesis is the result of genetic changes that alter the expression or function of proteins that play critical roles in the control of cell growth and division**

- **Oncogenic viruses cause cancer by inducing changes that affect cell growth and division**

- **Cancer arises from a combination of dominant gain of function mutations in proto-oncogenes and recessive loss of function mutations in tumor suppressor genes**

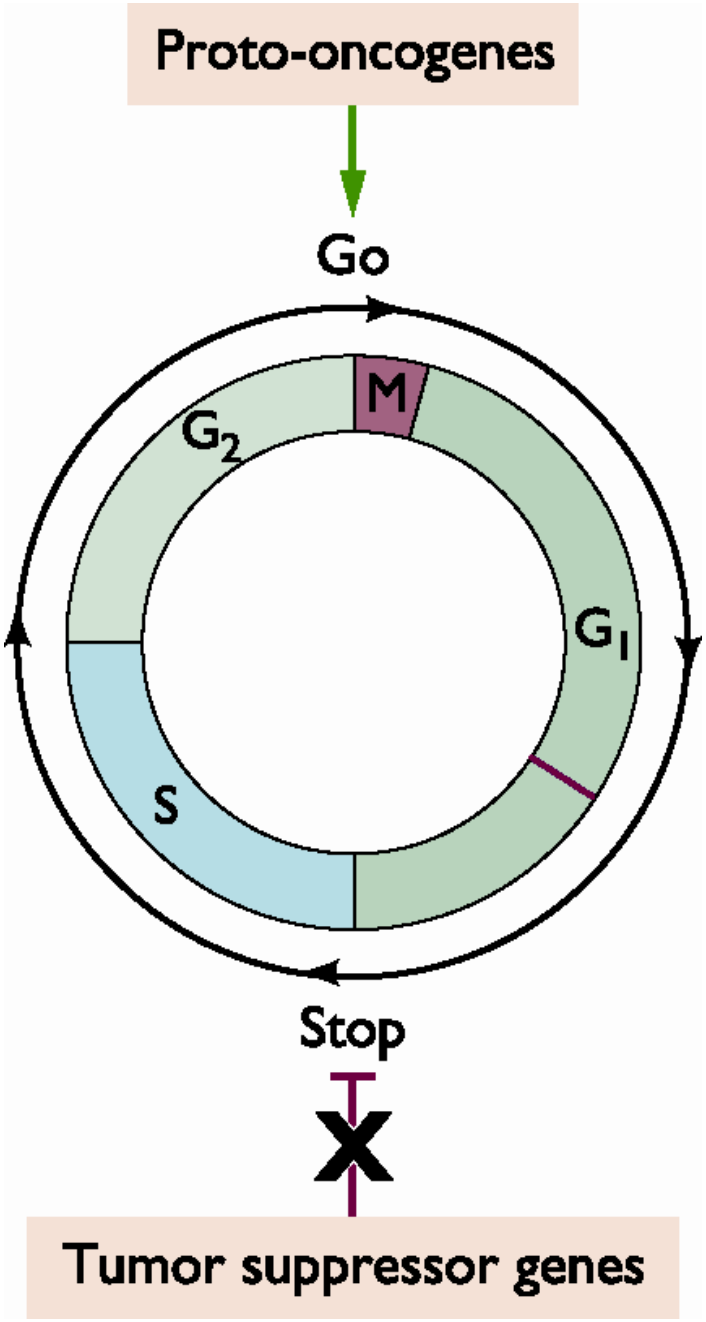


Table 16.2 Oncogenic viruses and cancer

Families	Associated cancers
RNA viruses	
Flaviviridae	
Hepatitis C virus	Hepatocellular carcinoma
Retroviridae	Hematopoietic cancers, sarcomas, and carcinomas
DNA viruses	
Hepadnaviridae	Hepatocellular carcinoma
Papovaviridae	
Papillomaviruses	Papillomas and carcinomas
Polyomaviruses	Various solid tumors
Adenoviridae	Various solid tumors
Herpesviridae	Lymphomas, carcinomas, and sarcomas
Poxviridae	Myxomas and fibromas

Oncogenic retroviruses are classified into two groups:

1) Transducing oncogenic retroviruses:

- highly carcinogenic, cause malignancies in 100% of the infected animals in a matter of days

- cause cancer because their genomes contain transduced cellular genes that become oncogenes

-virally transduced versions of cellular genes are called v-oncogenes, their cellular counterparts are called c-oncogenes or proto-oncogenes

2) Nontransducing oncogenic retroviruses:

-less carcinogenic

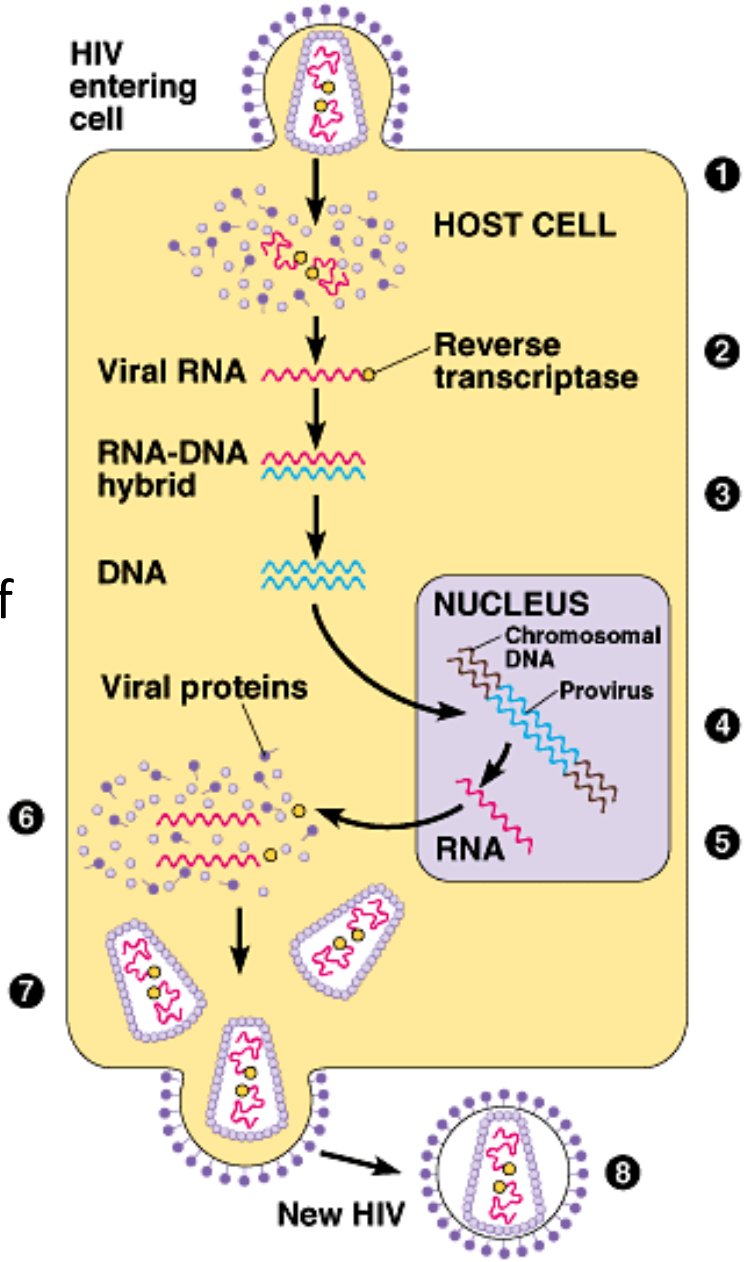
-do not encode cell derived oncogenes

-activate transcription of proto-oncogenes by integration of the provirus close to these genes in the host genome

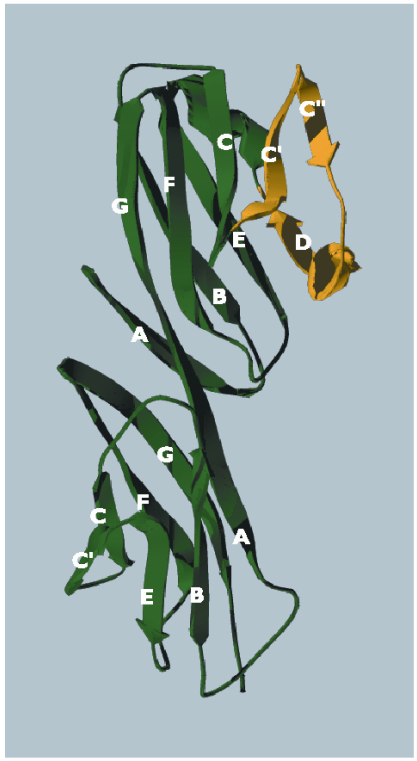
HIV

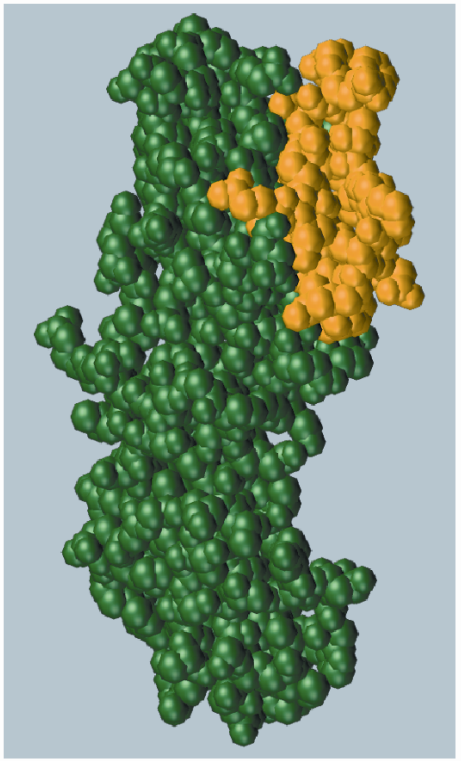
HIV infection

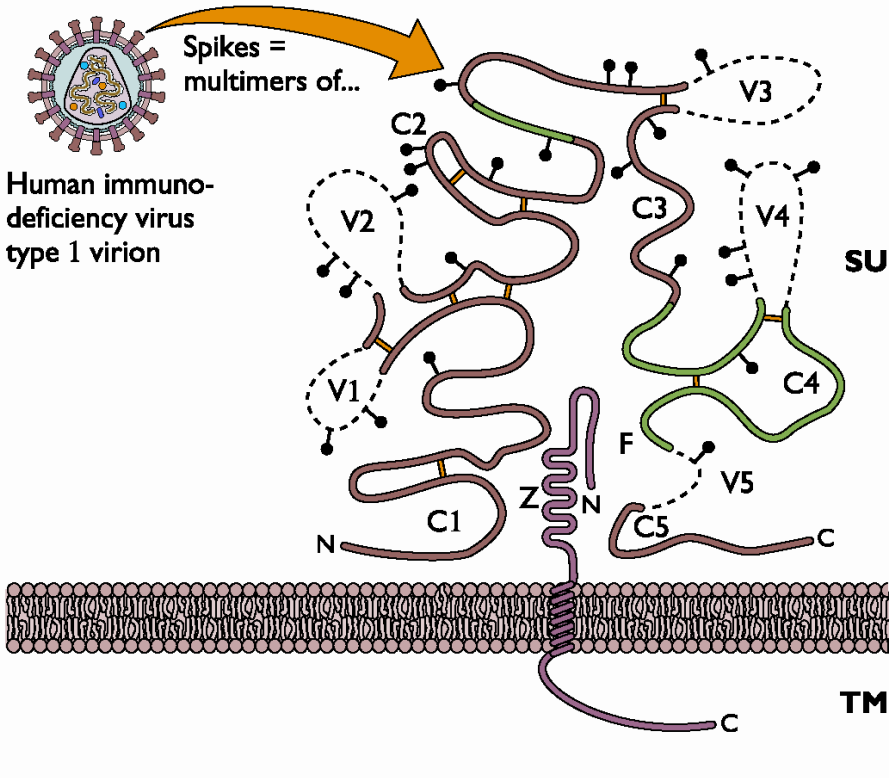
- HIV enters host cell
  - macrophage & CD4 WBCs
    - cell-surface receptor
  - reverse transcriptase synthesizes double stranded DNA from viral RNA
    - high mutation rate
- Transcription produces more copies of viral RNA
  - translated into viral proteins
  - proteins & vRNA self-assemble into virus particles
  - released from cell by "budding" or by lysis



HIV binding and entry

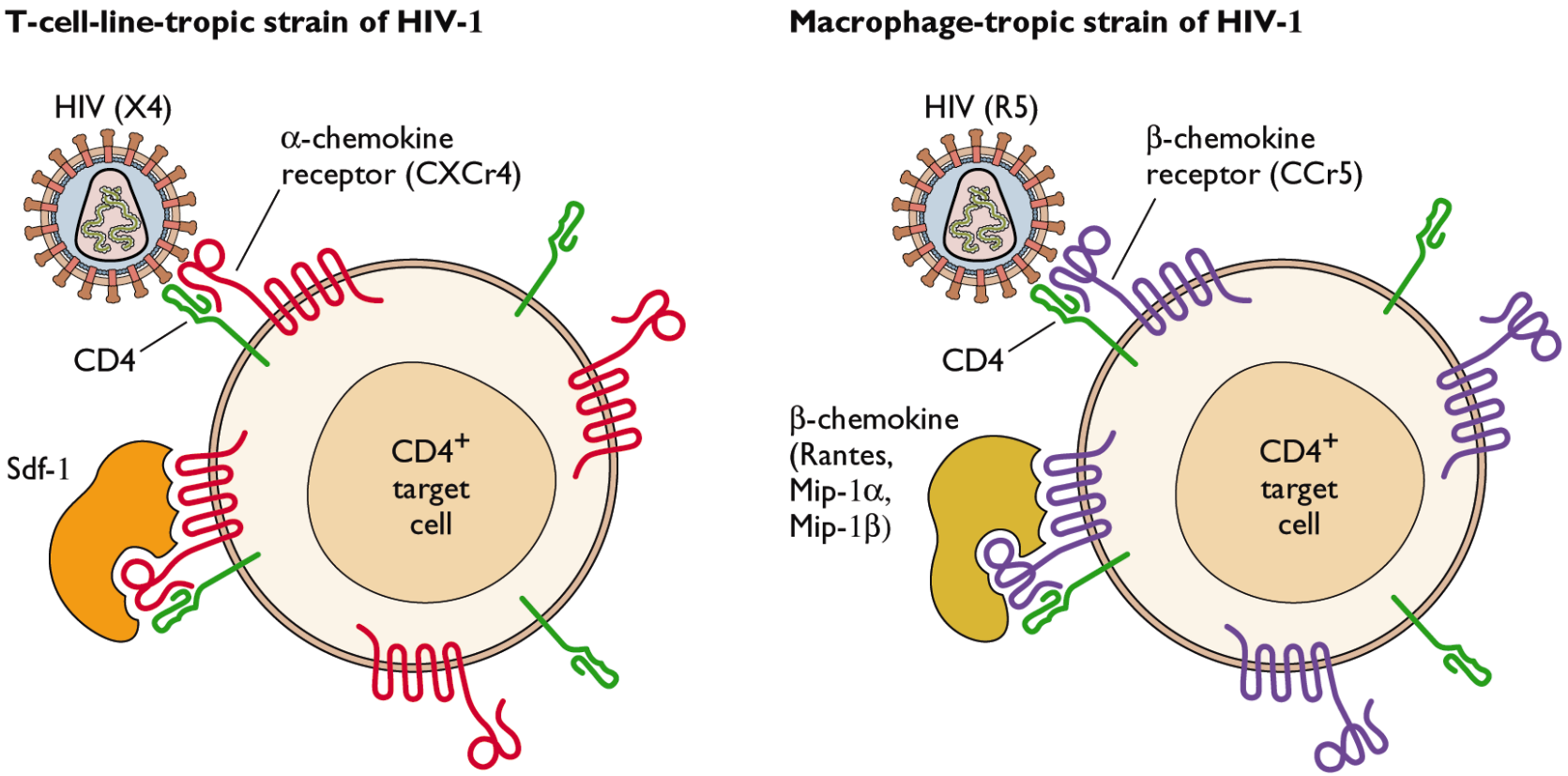






- **The gp120 has a specific domain that binds to the CD4 molecule present on susceptible cells**
- **Upon binding to CD4, the gp120 undergoes a conformational change that allows binding to specific subset of chemokine receptors on the cell surface, the CCr5 receptor and the CXCr4 receptor**

Coreceptors for macrophage and T-cell-tropic Strains of HIV



CXCr4 is the major coreceptor for T-cell-tropic strains

CCr5 is the major coreceptor for macrophage-tropic strains

HIV binding and entry

- **The use of each coreceptor corresponds to viruses with different biological properties and pathogenicity.**
- **Viruses isolated at the beginning of infection use the CCr5 co-receptor, which is the major coreceptor for macrophage-tropic strains, these viruses are not cytophatic**
- **In full-blown AIDS cases, new viral species appear with high level of replication, cytophatic effects and they use the coreceptor CXCr4, which is the major receptor for T-cell tropic strains**
- **There are also dual tropic viruses that can use both CXCr4 and CCr5 coreceptors or alternative chemokine coreceptors**
- **The fusion between the viral membrane and the cellular membrane involves a change in conformation of gp41, which enables it to insert into the cellular phospholipid bilayer**

HIV Genome

Three major genes

- Gag gene codes for CA, MA and NC proteins
- Pol gene codes for reverse transcriptase, protease, integrase and ribonuclease.
- Env gene codes for TM and SU

HIV Replication

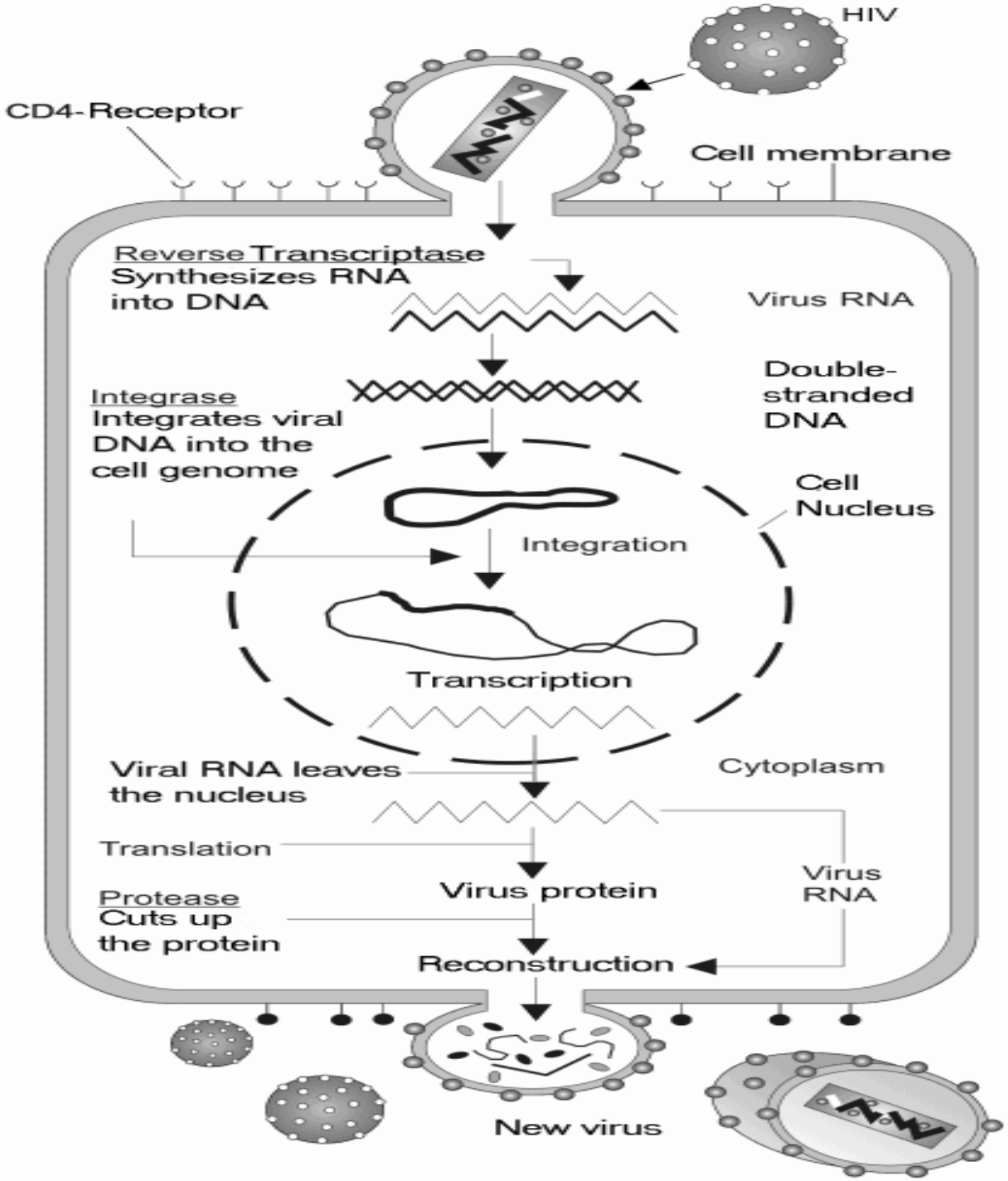
The first phase of viral entery, reverse transcription and integration into host genome is accomplished by viral proteins.

The second phase of synthesis and processing of viral genomes, mRNAs and structural proteins, uses host cell machinery.

- Attachment to specific cell surface receptor: gp120 binds CD4 molecule on the helper T cells, monocytes and dendritic cells
- Viral entery.
- Reverse transcription of viral RNA into DNA. The resulting double stranded DNA is called provirus.
- Integration of provirus into host cell DNA. The viral integrase cleaves the chromosomal DNA and covalently inserts the provirus. The insertion site is random.
- Transcription and translation of viral DNA sequences. The provirus is transcribed into a full length mRNA by the cell RNA polymerase II.
- Assembly and maturation of progeny virus.

Replication

- 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.
- This is followed by the synthesis and maturation of virus progeny.


HIV
CD4-Receptor
Cell membrane
Reverse Transcriptase
Synthesizes RNA
into DNA
Virus RNA
Double-
stranded
DNA
Integrase
Integrates viral
DNA into the
cell genome
Cell
Nucleus
Integration
Transcription
Viral RNA leaves
the nucleus
Cytoplasm
Translation
Virus protein
Virus
RNA
Protease
Cuts up
the protein
Reconstruction
New virus

HIV-1 Genotypes

- There are 3 HIV-1 genotypes; M (Main), O (Outlayer), and N (New)
- M group comprises of a large number subtypes and recombinant forms
  - Subtypes - (A, A2, B, C, D, F1, F2, G, H, J and K)
  - Recombinant forms - AE, AG, AB, DF, BC, CD
- O and N group subtypes not clearly defined, especially since there are so few N group isolates.
- As yet, different HIV-1 genotypes are not associated with different courses of disease nor response to antiviral therapy.
- However, certain subgroups may be difficult to detect by certain commercial assays.

Transmission of HIV

- Sexual contact: HIV is present in semen and vaginal secretions; either homoxesual or heterosexual contact
- Transfusions: whole blood, plasma, clotting factors or cellular fractions of blood.
- Contaminated needles: accidentally or sharing needles by drug users.
- Perinatal: Transplacental, during delivery or via breast milk.

Clinical Features

1. Seroconversion illness - seen in 10% of individuals a few weeks after exposure and coincides with seroconversion. Presents with an infectious mononucleosis like illness.
2. Incubation period - this is the period when the patient is completely asymptomatic and may vary from a few months to a more than 10 years. The median incubation period is 8-10 years.
3. AIDS-related complex or persistent generalized lymphadenopathy.
4. Full-blown AIDS.

Acute HIV Infection

- Transient symptomatic illness in 40-90%
- Usually mild but can be severe
- 2-6 weeks after infection
- Often not recognized by primary care clinicians
  - Symptoms non-specific
  - Often resembles influenza, mononucleosis
  - “Cold symptoms” absent
- Can be asymptomatic
- Duration: 14 days

Pathogenesis and clinical significance (1)

- Initial infection:
  - genital tract macrophages
  - HIV disseminates via blood
  - Dendritic cells in lymphoid tissue
  - CD4+ lymphocytes

- Acute phase viremia: several weeks after the initial infection, 1/3 – 2/3 of individuals experience an acute disease syndrome similar to infectious mononucleosis. Circulating antibody appears in 1 – 10 weeks after the initial infection (seroconversion).

- Latent period: lasts from months to many years (average 10 years). During this period, 90% of HIV proviruses are transcriptionally silent. Although there is continous loss of CD4+ cells in which HIV is replicating, active replacement through stem cell multiplication is occurring. The infection remains clinically asymptomatic as long as the immune system is functional.

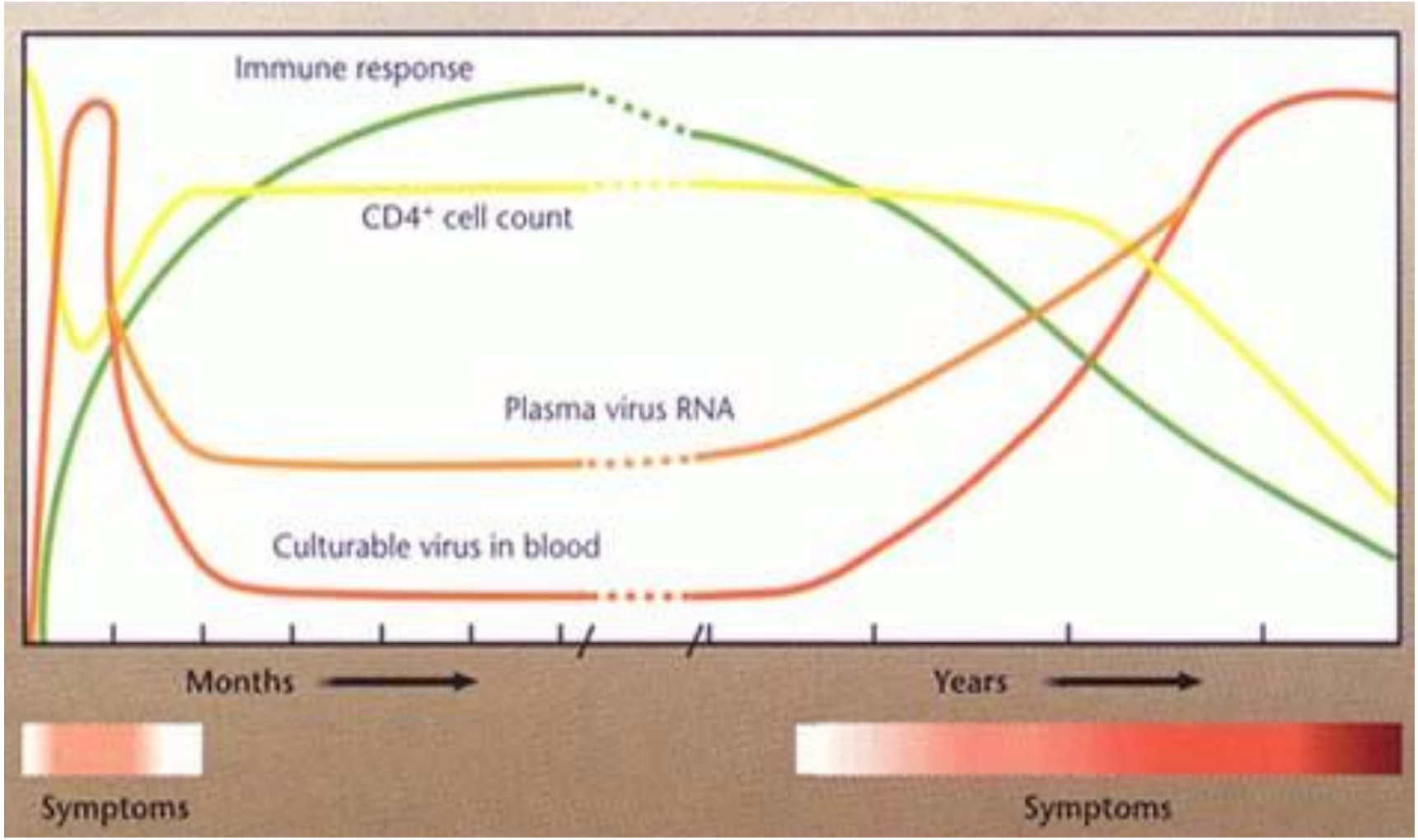
Pathogenesis and clinical significance (2)

- Clinical complications during the latent period: there are multiple non-specific conditions such as persistent generalized lymadenopathy, diarrhea, chronic fevers, night sweats and weight loss. The more common opportunistic infections such as herpes zoster and candidiasis may occur repeatedly during this period. The CD4+ cell count remains normal or gradually declines but is greater than 200 / ul. Progression from asymptomatic infection to AIDS is not sudden.

- AIDS: Coinfection with HHV-6 can transactivate transcription from the silent HIV provirus, increasing HIV replication. Any stimulation of an immune response causing activation of resting T cells also activates HIV replication. Appearance of HIV mutants with altered antigenic specificity which are not recognized by the existing humoral antibody or cytotoxic T lymphocytes; also contributes to progression with CD4+ count falling below 200 / ul and appearance of serious diseases and opportunistic infections.

Malignancies associated with AIDS

- Kaposi's sarcoma HHV-8
- Lymphomas EBV


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

Diagnosis of HIV Infection

- Viral antibodies
- Viral antigens
- Viral RNA/DNA
- Culture

Laboratory Findings
Acute HIV Infection

- Lymphopenia $\rightarrow$ lymphocytosis
- Atypical lymphocytes
- Transient CD4 decline

Diagnosis of Acute HIV Infection

- Recognition of clinical symptoms
  - No true constellation of signs/sympoms
  - Presence of any symptom(s)
  - History of activity associated with HIV risk
- Detectable plasma HIV RNA
  - Highly sensitive
  - False positive possible
- Detectable p24 Antigen
  - Less sensitive
  - False positive rare

Acute HIV Infection

- High virus levels (10^5-10^6 copies/mL)
- 2-9% of HIV-negative have false positive results
  - Usually associated with low RNA titers <10,000
- VL in new infections
  - Correlates with rate of CD4 decline
  - Prognostic indicator in early disease

Laboratory Diagnosis

Antigen / antibody detection

- ELISA, serum
- HIV-1 & -2 antibodies, HIV-1 (p24) antigen
- Screening of blood donors
- Western Blotting

PCR

- Viral RNA or DNA provirus
- Blood or tissue specimens
- Quantitative PCR (viral load): to determine disease stage and treatment follow up.

Enzyme Immunoassay

Enzyme-Linked Immunosorbent Assay (EIA, ELISA)

- Primary HIV antibody screening test
- Serum plasma, dried blood spots, oral fluids, urine
- HIV-1/2, HIV-1, HIV-2
- High degree sensitivity and specificity
- Repeatedly reactive: confirmatory testing

Negative Antibody Test Results

- HIV negative
- Recent infection: too early for seroconversion
- CDC: follow-up testing at 6 weeks, 12 weeks, 6 months

Confirmation Process

- Non-negative screenings should be confirmed
  - Western Blot (WB)
  - Immunofluorescent Antibody Assay (IFA)
- Higher specificity than EIA
- Interpretation can be subjective

Window Period

- Time delay from infection to positive EIA
  - Average: 10-22 days
  - Most seroconvert within six months

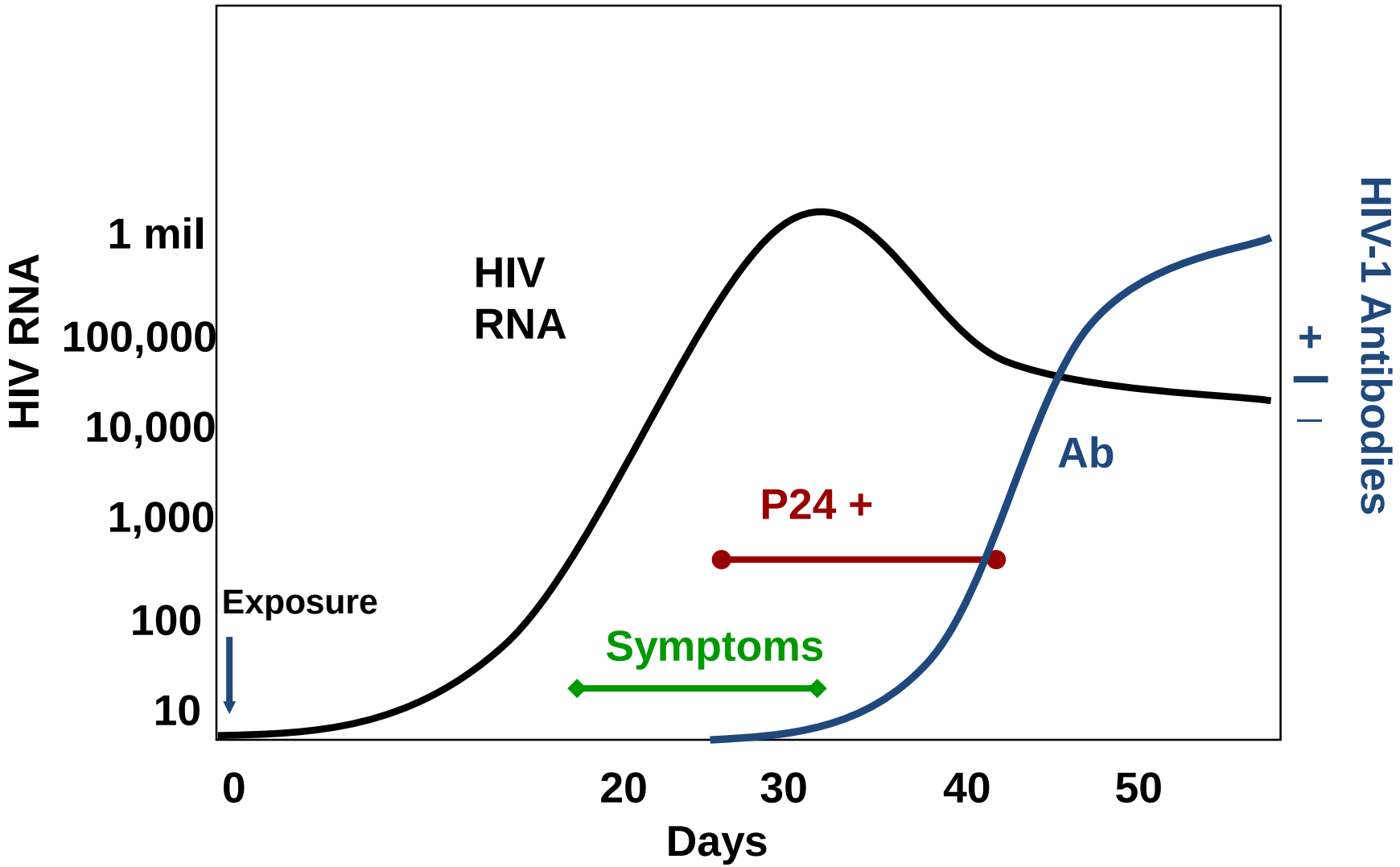
HIV-1 vs HIV-2

- HIV-1: Most cases
- Group M: predominant strain world-wide
  - Subtypes (clades): A to K, N, O
  - Clade B
    - US and Europe
    - 98% of HIV-1 in US
  - Clade C: Southeast Asia
  - N (“new”): 1998
  - Group O: West Africa
- Recombination between viruses of different clades becoming more common

HIV-2

- Primarily found in West Africa
- Causes immune deficiency due to depletion of CD4 cells
- 5-8 fold less efficient transmission compared to HIV-1
- Associated with lower viral load
- Slower rate of CD4 decline and clinical progression
- Negative Ab tests in 20-30% depending on EIA assay
- WB: not well standardized nor FDA approved

Typical Course of Primary HIV



Viral Detection

- p24 Antigen
- HIV-1 DNA PCR
  - Most sensitive: able to detect 1-10 copies of proviral DNA
  - S/S: 99% / 98%
- HIV-1 RNA (RT-PCR, bDNA)
  - S/S: 95-98%
- Viral culture of PBMC: expensive, labor intensive, reliability variable

Treatment

- Anti-retroviral drugs
  - Reverse transcriptase inhibitors
  - Protease inhibitors

- Multidrug therapy
  - RT has no proofreading activity, resulting in production of many errors during viral DNA synthesis which leads to mutations in all HIV genes and accumulation of mutant viral strains. In presence of an antiviral drug, there is strong selection for mutations that confer resistance to that drug. Use multidrug therapy

- Early therapy
  - Viral load is a prognostic indicator of the rate of progression to AIDS. Infection should be treated as aggressively and as early as possible to minimize initial spread of the virus and give a lower chance for mutants to arise.

Highly active antiretroviral therapy (HAART)

Nucleoside analog reverse transcriptase inhibitors

- Act by serving as a chain terminator
  - Zidovudine (AZT)
  - Didanosine (ddi)
  - Lamivudine (3TC)

Non-nucleoside reverse transcriptase inhibitors

- Act by targeting the enzyme itself
  - Efavirenz
  - Delaviridine
  - Nevirapine

Protease inhibitors

- Interfere with the processing of polyproteins in the budding virion, resulting in non-infectious particle.
  - Ritonavir
  - Amprenavir
  - Indinavir
  - Lopinavir

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.