



Retroviruses (HTLV and HIV/AIDS)

Medical Microbiology; PTM 4310 (MBChB)

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RETROVIRUSES

- Enveloped, diploid, + sense- single stranded RNA viruses
- Encode reverse transcriptase
 - Converts RNA genome into double stranded DNA which is integrated into host genome
- Types:
 - Oncoviruses: produce tumors (leukemias, lymphomas, sarcomas)
 - HTLV-1 and 2
 - Lentiviruses: slow viruses (HIV)
 - HIV-1 and 2
- Transmission by intimate contact (blood/body fluids)
 - Highly susceptible to high surface tension



RETROVIRUSES

○ Oncoviruses:

- Do not kill host cell
 - Continue to produce new virus indefinitely
- Transduce/activate growth-promoting genes (oncogenes)
 - Ability to cause malignancies
- Examples: HTLV 1 and 2

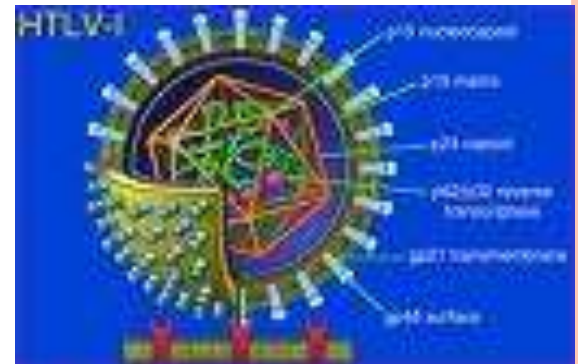
○ Lentiviruses

- Can persist for a long time in a host cell without killing it (latent state)
- Become highly cytolytic/cytopathic when cell is subject to certain stimuli
- Examples: Visna virus in sheep (causes neurological disease in sheep), HIV-1 and 2
 - HIV: Destruction of CD4⁺ T-lymphocytes

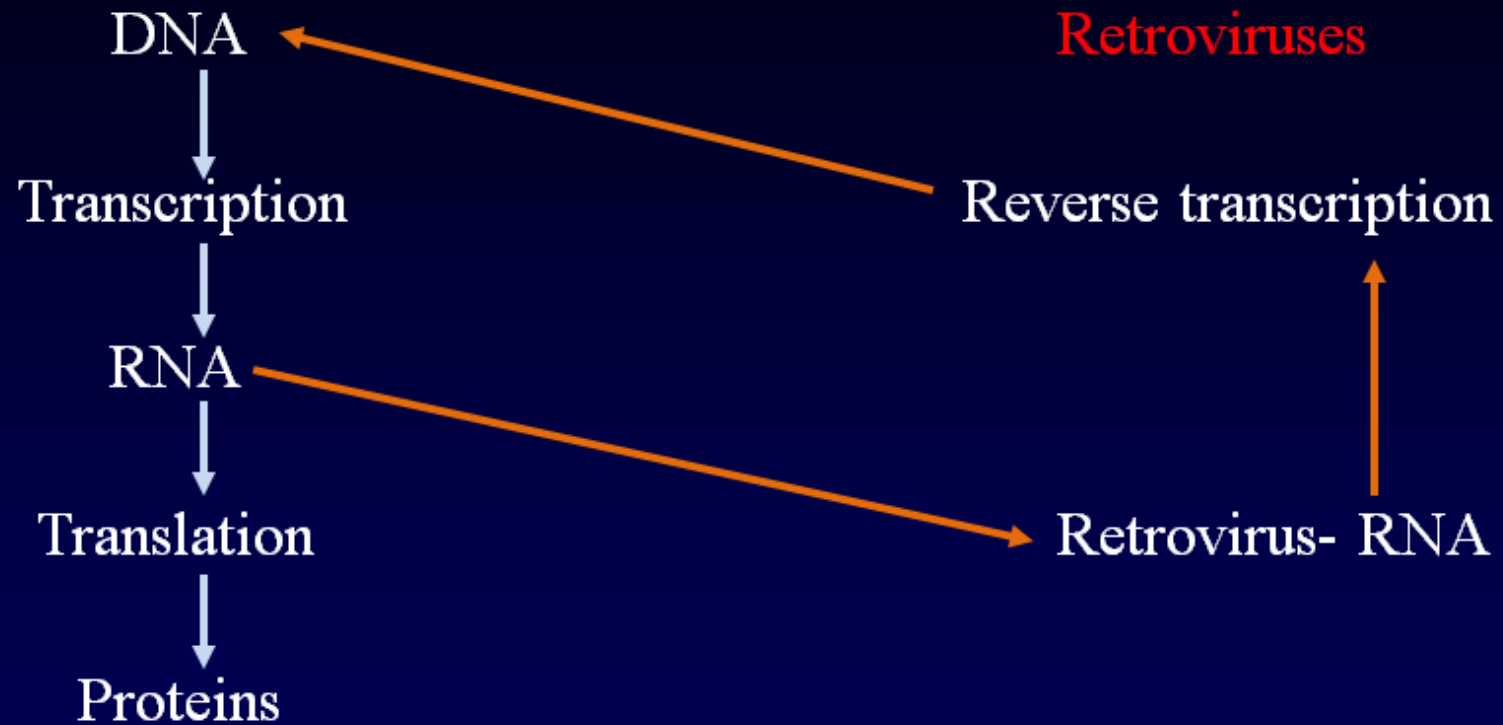


STRUCTURE

- Virion contains two single stranded RNA molecules
- Envelop is required during budding
 - Contains two viral glycoproteins
 - Surface glycoprotein for adsorption
 - Transmembrane for fusion of viral and host cell membrane
 - Provide direct cell-cell transmission



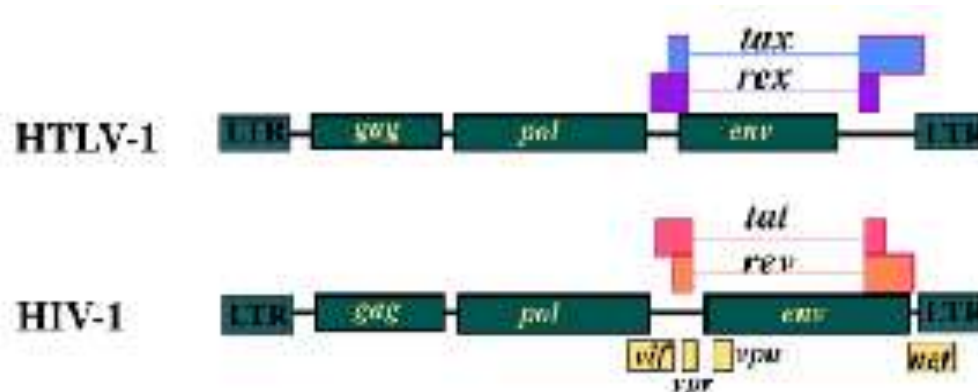
WHY RETROVIRUSES



Central dogma of Molecular Biology

FAMILY RETROVIRIDAE

Enveloped, single-stranded RNA viruses coding gag (group specific antigen), pol (polymerase), env (envelope) genes



lentiviruses

HIV-1,-2

SIV, FIV

visna (sheep)

oncoviruses

HTLV-1,-2

spumaviruses

SFV and other

foamy viruses



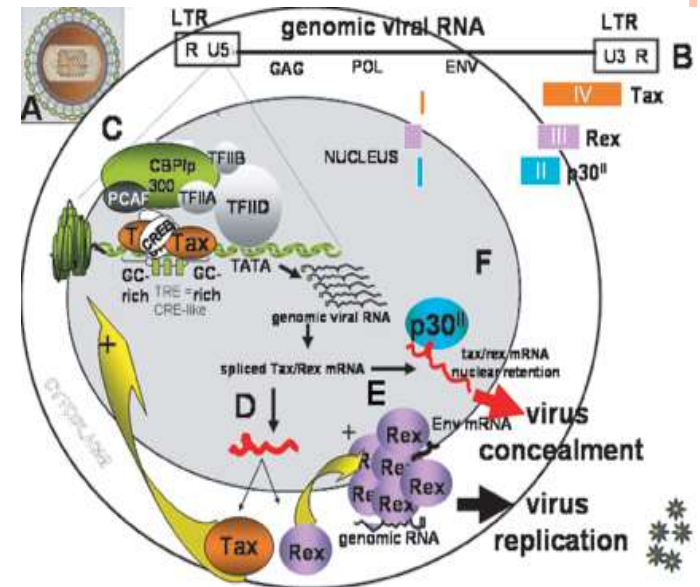
FAMILY RETROVIRIDAE, LENTIVIRIDAE

- Includes viruses responsible for a variety of neurological and immunological diseases, but not directly implicated in malignancies
- Prototypes were 'slow' (hence lenti-) such as visna
- Large genomes (~ 9-10 kb)
- Complex organization of genes in addition to gag, pol, env
- Able to replicate continuously in their host cells despite a competent host immune response



VIRAL REPLICATION

- Reverse transcriptase copies RNA into double stranded DNA
- DNA is integrated and replicated as provirus with host genome
- Provirus has its own promoter and signal that control transcription by host RNA polymerase
- Genomic RNA and spliced mRNAs are both produced
 - Later encode envelop glycoproteins and regulatory proteins



TRANSFORMATION BY RETROVIRUSES

- Noncytotoxic virus carrying cellular oncogenes can produce persistent transformation
- Integration of provirus adjacent to protooncogenes can activate/transduce them
 - Insertion mutagenesis
 - Enhancer or promoter elements
 - Long latent periods, integration is random. Hot spots?
- Production of tax protein, which activates expression cellular transforming gene(s) (protooncogenes)
 - HTLV-1



HUMAN T-LYMPHOMA VIRUS-1 (HTLV-1)

- First human virus to be associated with a malignancy
- Isolated in 1980 from patient with cutaneous Adult T-cell lymphoma (ATL) in the US
 - First described in Japan in 1977
- Wide spectrum of disease:
 - HTLV-1 associated Myelopathy/tropical spastic paraparesis (HAM/TSP)
 - Uveitis
 - Infective dermatitis in children
 - Other inflammatory and immune mediated conditions
 - Polymyositis, arthropathy, sjögren's syndrome and facial nerve palsy



VIROLOGY

- HTLV-1 is an enveloped, diploid single stranded RNA, type C virus
 - Family: Retroviridae
 - Subfamily: Oncovirus
- Tropism: T-lymphotropic
 - Cause T-cell proliferation
 - Causes persistent infection
- Receptor: Glucose Transporter 1 Protein (GLUT1) and Heparin Proteoglycan
- Replication: Reverse transcription to DNA which is integrated into the host genome. Replicates with host genome as provirus.



GENOME

HTLV-1



○ Structural genes:

- *Gag* : Core proteins, p19, p24, protease
- *Pol*: RNA dependent DNA polymerase (Reverse transcriptase), protease,
- *Env*: transmembrane protein gp21 and surface gp46

○ Regulatory genes (Regulate replication and viral protein expression)

- *Tax*: activate LTRs, U3 region for transcription of provirus
- *Rex*
- Long Terminal Repeats (LTRs)



EPIDEMIOLOGY

○ Geographical distribution

□ Endemic in

- Southern Japan
- The Caribbean islands
- Parts of Africa
- Middle East
- South America
- Pacific Melanesian Islands
- Papua New Guinea



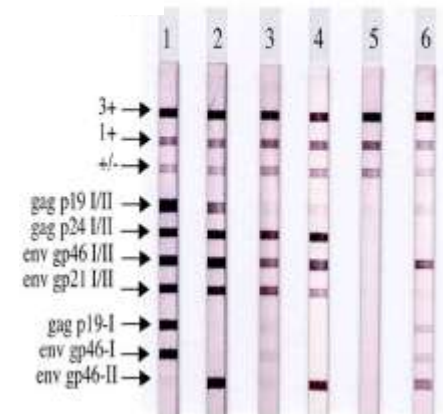
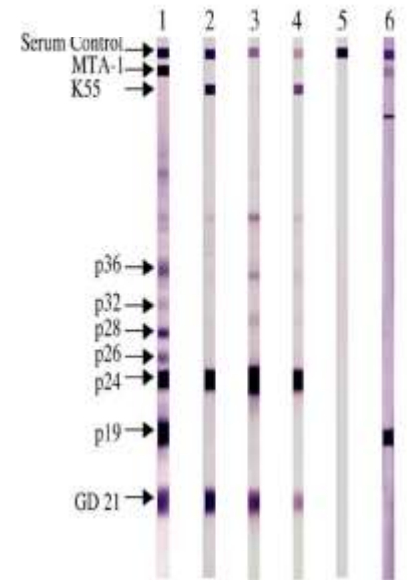
TRANSMISSION

- Transfusion
 - Screening in Japan, USA
- Mother to child
 - Breast milk
- Sexual contact



DIAGNOSIS

- ELISA:
 - Antibodies to core, envelop and tax proteins
- Western Blotting
 - For confirmation
- PCR
 - Detect HTLV in tumor tissue
 - Distinguish HTLV 1 Vs 2



PATHOGENESIS

- Strong evidence implicating Transcription transactivation protein, tax as a critical oncoprotein of HTLV-1
- Proviral deletions in ATL/L are common but the *tax* gene is generally conserved
- Tax is capable of transforming Rat 1 fibroblasts
 - Tax is a pluripotent transcriptional activator that does not independently bind DNA but rather enhances the activity of cellular transcription factors and chromatin modeling determinants
 - Activates viral promoters via CREB-binding protein
 - Activates NF κ B by binding to IKK and by enhancing phosphorylation of MAPKK.
 - NF κ B activation is critical for immortalization in culture and tumorigenesis in tax transformed mice
 - NF κ B enhances expression of proteins that promote cell proliferation and angiogenesis and resistance to apoptosis



PATHOGENESIS

- Secondary genetic/Epigenetic events
 - Tax protein not usually present in all ATL/L tissues
 - Only 10% of those infected through breast milk develop ATL/L, and later in life
- DNA methylation
 - Can modulate oncogenesis
 - Hypermethylation of DNA sequences
 - Down regulation of Kruppel-like factor 4 gene (cell cycle regulator), early growth response gene 3, regulator of fas-L expression resulting in resistance to induction of apoptosis



PATHOGENESIS

- The virus activates Th1 cells and results in proliferation of Th1 cells and overproduction of Th1 related cytokines (mainly IFN-gamma and TNF-alpha).
- Feedback mechanisms of these cytokines cause a suppression of the Th2 lymphocytes and a reduction of Th2 cytokine production (mainly IL-4, IL-5, IL-10 and IL-13).
- The end result is a reduction in the ability of the infected host to mount an adequate immune response to invading organisms that require a predominantly Th2 dependant response (these include parasitic infections and production of mucosal and humoral antibodies).



TAX

- Transcriptional transactivator protein is the critical oncoprotein of HTLV-1.
- Proviral deletions in ATLL patients are common, but the tax gene is generally conserved.
- Tax is capable of transforming Rat 1 fibroblasts.
- Tax expression in a Herpesvirus samirii or retrovirus vector results in CD4+ cell immortalization.
-
- Tax expression in transgenic mice results in various neoplasms, including lymphoma.



TAX (SUFFICIENT AND NECESSARY?)

- The HTLV-1 Tax protein is required for the virus to transform cells; however, *tax transcripts are detected in only ~40% of all ATLs.*
- Analyses of HTLV-1 proviruses and *tax transcripts in ATL cells revealed three ways in which cells can silence Tax expression:*
 - the accumulation of nonsense mutations, insertions and deletions in *tax*;
 - silencing viral transcription by DNA methylation of the provirus;
 - and the deletion of the proviral 5'LTR.
- Genetic changes in *tax are seen in ~10% of ATLs*, DNA methylation in another 15% of ATLs, and 5'LTR deletions are found in a further 27% of ATL cases. The last change is especially prevalent in aggressive forms of ATL.



CELLULAR FUNCTIONS OF TAX

- ***Cell-cycle progression:***
- Levels of G1 D cyclins are increased.
- Tax activates cyclin-dependent kinases (CDKs) leading to the hyper-phosphorylation of retinoblastoma (RB) protein. The phosphorylation and/or degradation of RB frees the E2F1 transcription factor, accelerating cell-cycle transition from G1 to S.
- *Tax transcriptionally represses* CDK inhibitors (CKIs)



WHY DO ATL CELLS SILENCE TAX EXPRESSION?

- The currently accepted view is that Tax is needed early after infection to initiate transformation, but is not required later to maintain the transformed phenotype of ATL cells.
- As Tax is the main target of the host's CTLs, cells that shut down Tax expression (using one of the three genetic or epigenetic means described above) have an advantage in evading immunosurveillance and are preferentially selected for *in vivo during disease progression*.



CHEMO-ANTIRETROVIRAL THERAPY

- Combination chemotherapy regimens with median survival of 1 year, with a 5-year survival rate of less than 5%.
- Combination of interferon-alpha and zidovudine
- Relapses occur in most individuals when treatment is discontinued
- An infusional regimen, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin, is currently being evaluated in combination with interferon-alpha and combivir based of its activity in refractory lymphomas and in HIV-associated lymphomas.



STEM CELL TRANSPLANTATION

- Autologous stem cell transplantation is generally ineffective
- Allogeneic SCT for ATLL remains unclear
 - 16 patients underwent allogeneic SCT, a reduced-intensity conditioning regimen with fludarabine, busulfan, and rabbit antithymocyte globulin was well tolerated.
 - Proviral load became undetectable in 8 patients, but remission rates are not yet available.
 - A graft-versus-ATLL effect was found with this procedure.
- In a study of ATLL patients who obtained complete remission after non-myeloablative allogeneic SCT from HLA-identical siblings, CD8+ cytotoxic T lymphocytes directed against the HLA-A2 restricted dominant Tax epitope were found after, but not before, transplantation. Cytotoxic T lymphocytes to HTLV-1 Env epitopes may also result in ATLL cytotoxicity



ANTIBODIES

- Anti-Tac antibody, which recognizes the interleukin-2 receptor, has been shown to have therapeutic efficacy for ATLL cells in patients and in a murine xenograft model.
- A recent study showed that antitumor activity depended on Fc receptor-mediated clearance.
- Improved activity is seen with radioimmunoconjugates, such as Yttrium90-labeled anti-Tac. In one study of 18 ATLL patients given doses of 5 to 15 mCi, there were 7 partial and 2 complete remissions.
- A monoclonal antibody to human transferrin receptor, constitutively expressed at high levels on ATLL cells, blocked ATLL cell growth in culture.
- Similarly, anti-CD2 antibody has activity in the scid mouse model of ATLL.
- Anti-CD52, anti-CD40, anti-Tax antibodies .



NOVEL THERAPIES

- Arsenic trioxide has been shown to synergize with interferon- α in inducing growth arrest and apoptosis of ATLL cells in culture
- Bortezomib blocks I κ B degradation, inhibits NF κ B activity in HTLV-1 immortalized cells and Tax transgenic tumor cell lines in culture in murine transplant models
- Retinoids and angiogenesis inhibitors have also been explored for anti-ATLL activity



PREVENTION

- Counseling infected individuals on sexual and breast feeding practices is the only proven method to reduce HTLV-I related disease.
- The genomic stability of HTLV-I and minimal strain variation between isolates are encouraging the success of similar vaccines against bovine and feline leukaemia retroviruses.
- Candidate vaccines should elicit an immunogenic response to *env proteins, which are critical* for infectivity, and a target for the induction of neutralising antibodies, and *gag proteins, which stimulate cell-mediated* immunity.
- Synthetic peptides and recombinant proteins are being used to characterise immunodominant epitopes of HTLV-I.



HTLV-II

- HTLV-II was first isolated from a patient with T-cell variant of hairy-cell leukemia
- HTLV-II is able to transform primary lymphocytes in vitro and persist in infected individuals or experimental animals, but it is much less pathogenic than HTLV-I and has not been clearly linked to any disease
- Prevalence of both HTLV-I and HTLV-II is associated with IV injection use



HTLV-I/ HTLV-II

- HTLV-I infection occurs in clusters in certain geographic locations around the world. It is endemic in Southern Japan (15-30%), Caribbean (3-6%), Papua New Guinea and some parts of Africa
- Among different ethnic groups, HTLV-I prevalence is highest among African Americans, and HTLV-I and HTLV-II both have a high prevalence among native/indigenous populations of North America, South America, Australia and the Caribbean
- Transmission of HTLV-I is believed to occur from mother to child via breastfeeding; by sexual contact; and through exposure to contaminated blood, either through blood transfusion or sharing of contaminated needles



HTLV-I HTLV-II

- HTLV-I and II share most properties such as density, morphology, a 70S RNA, reverse transcriptase and structural proteins with all other replication-competent C-type retrovirus
- HTLV-I and II belong to the oncornavirus subfamily of retroviruses
- ssRNA enveloped virus
- Two copies of the RNA genome with 3 major genes:
 - gag (structural proteins)
 - pol (reverse transcriptase)
 - env (envelope glycoproteins)

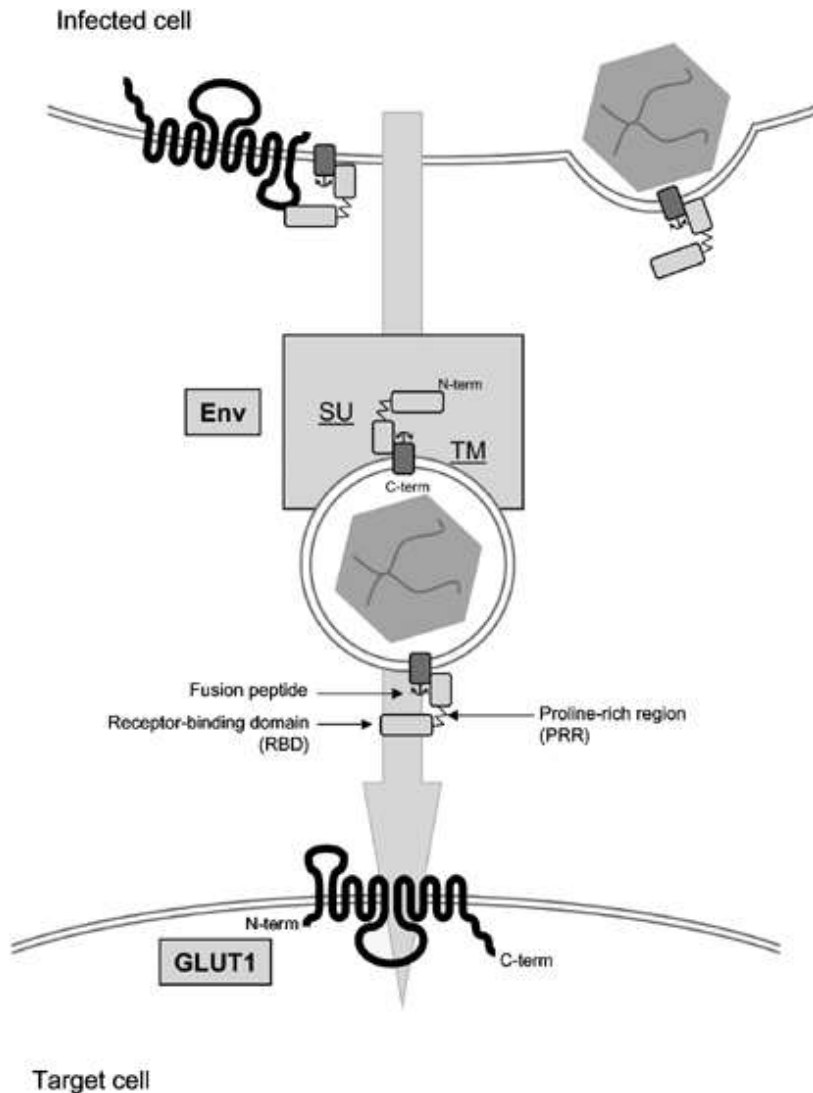


HTLV-I HTLV-II

- HTLV-I and HTLV-II use different receptor complexes to enter T cells
- CD4+ T cells are the primary target of HTLV-I
- CD8+ T cells are the primary target of HTLV-II
- Transfection studies show that overexpression of GLUT-1 in CD4+ T cells increases HTLV-II entry, while expression of HSPGs on CD8+ T cells increases entry of HTLV-I
- Glucose transporter-1 (GLUT-1) functions as a receptor for HTLV-I
- In primary CD4+ T cells, heparan sulfate proteoglycans (HSPGs) are required for efficient entry of HTLV-I



HTLV-I HTLV-II

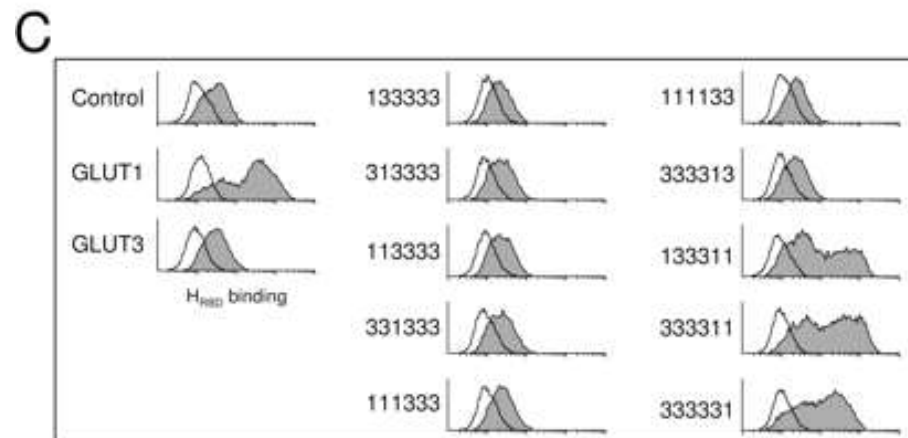
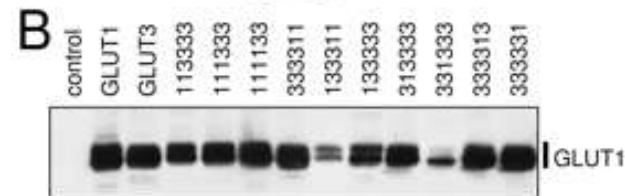
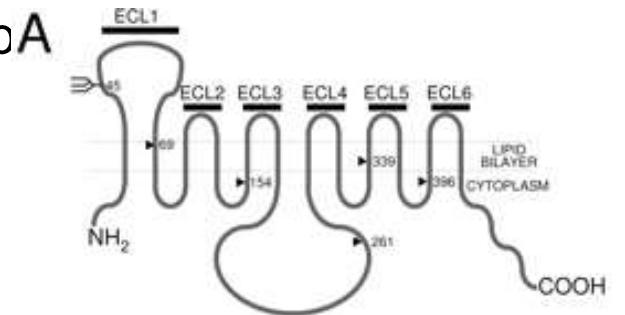


- HTLV-1 and HTLV-2 differ in their T-cell entry requirements and differences in the in vitro cellular tropism for transformation and in vivo pathobiology of these viruses may reflect different interactions between their Env proteins and molecules on CD4+ and CD8+ T cells involved in entry

Cell-to-cell transmission of HTLV. HTLV particles bud from GLUT1-positive infected cells in areas of the cell membrane enriched for the viral envelope glycoprotein (Env). HTLV particles bind to GLUT1 on target cells via the Env receptor-binding domain (RBD) located in the SU. Binding to GLUT1 is most likely followed by conformational changes that involve the proline-rich region (PRR), between the SU RBD and carboxy terminus (C-term), leading to the unmasking of the fusion peptide located in the Env transmembrane component (TM) and penetration of the viral core in the cytoplasm

HTLV-I HTLV-II

The COOH-terminal domain of GLUT1 is responsible for HTLV Env bA

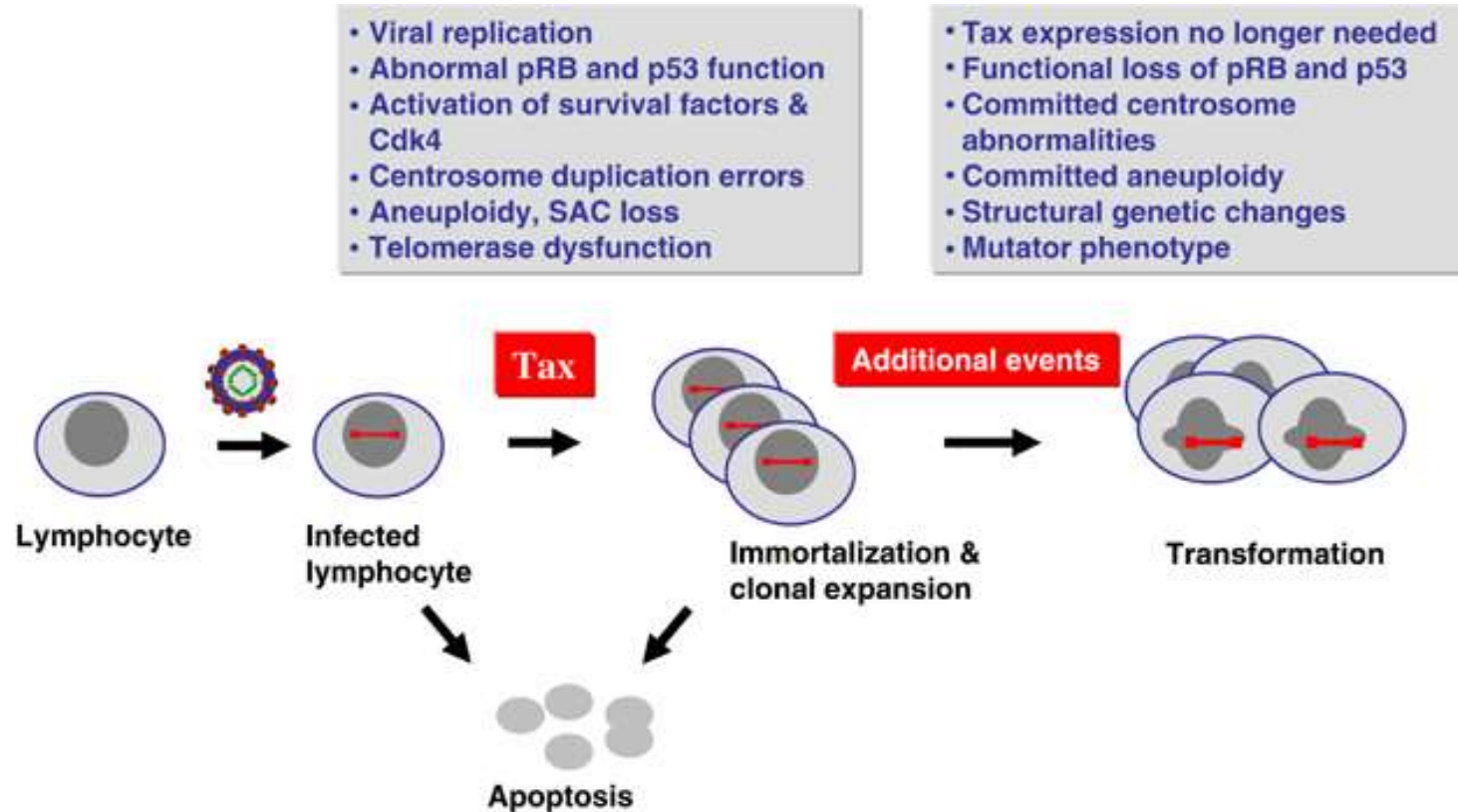


HTLV-I HTLV-II

- The HTLV Tax protein is crucial for viral replication and for initiating malignant transformation leading to the development of adult T-cell leukemia
- Tax has been shown to be oncogenic, since it transforms and immortalizes rodent fibroblasts and human T-lymphocytes
- Tax does not directly bind DNA, but associates through its N-terminus with the CREB protein which docks at the LTR's cyclic-AMP responsive (CRE)-motifs
- Tax also recruits transcriptional coactivators, CBP and p300
- A second major function of Tax is to activate NF-kappaB
- Recent data indicate that Tax activates the anaphase promoting complex/cyclosome (APC/C) ahead of schedule, causing premature degradation of cyclin A, cyclin B1, securin, and Skp2. Premature loss of these mitotic regulators is accompanied by mitotic aberrations and leads to rapid senescence and cell cycle arrest in HeLa and *S. cerevisiae* cells



HTLV-I HTLV-II



Progression of T-lymphocytes from immortalization and clonal expansion to transformation. Various changes in the cell during the different stages are listed in the shaded boxes. As shown, virus-infected cells can also go into apoptosis

HTLV-I HTLV-II

- Infection with HTLV is lifelong and there is no treatment for HTLV infection but 95% of people infected with HTLV never develop any HTLV related symptoms
- ATLL is usually treated with anti-cancer drugs, but recently transferring to anti-viral treatment after starting with anti-cancer treatment has been shown to improve the outcome.
- Diagnosis of HTLV infection is performed by:
 - ELISA
 - Western blot (WB)
 - Line immunoassay
 - PCR



HTLV III/ HIV-1

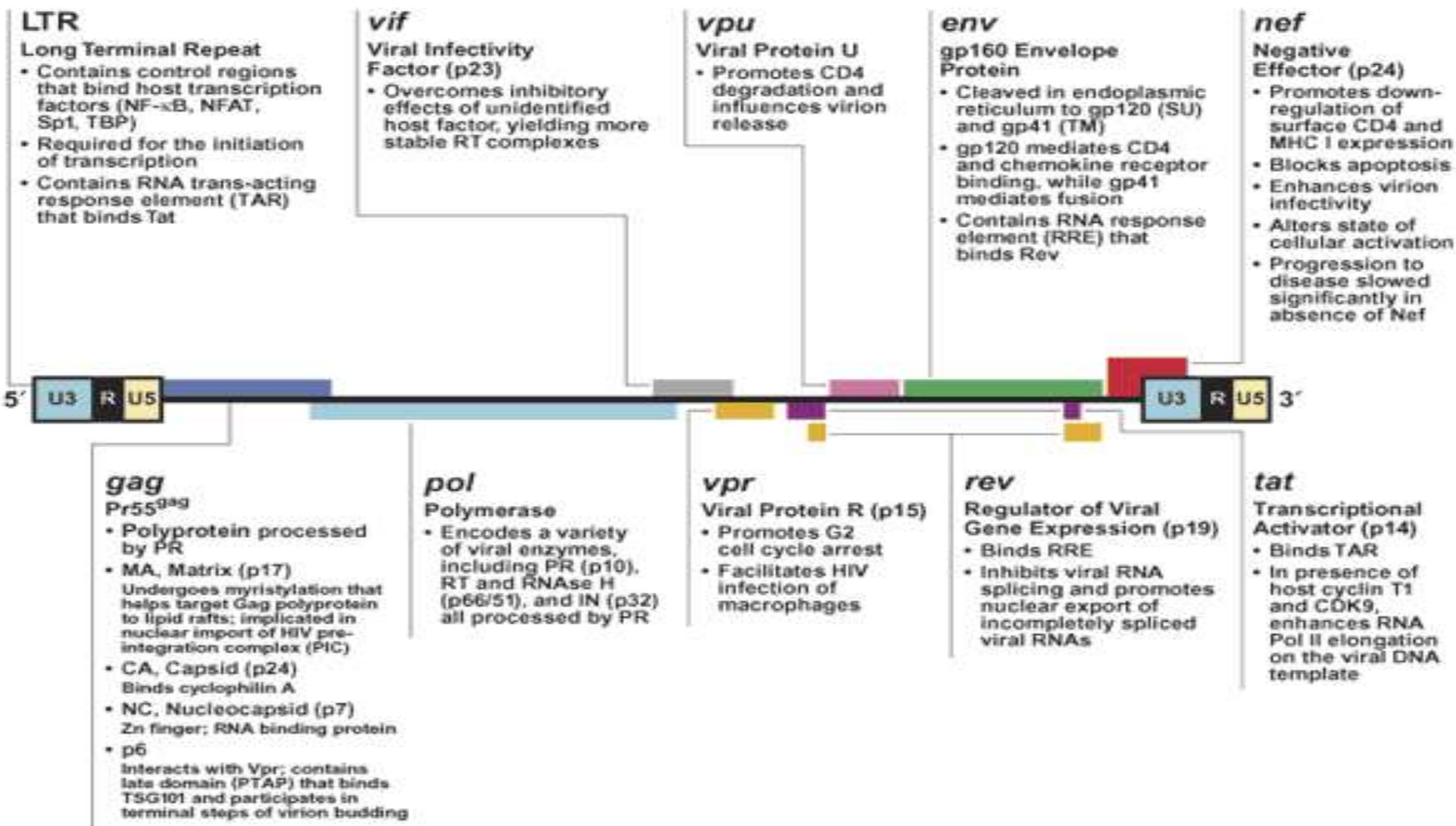
- Enveloped, diploid + sense single stranded RNA lentivirus
- Encode reverse transcriptase
 - Converts RNA genome into double stranded DNA which is integrated into host genome



DNA replication → transcription → proteins → assembly

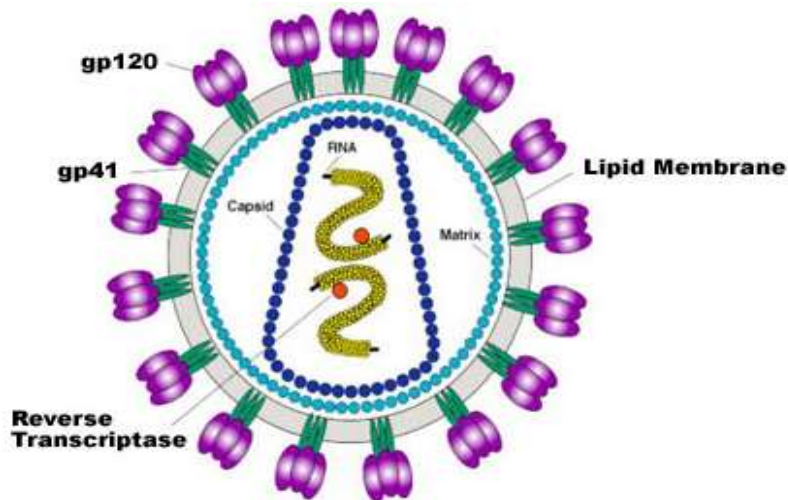


HIV-1 GENOME

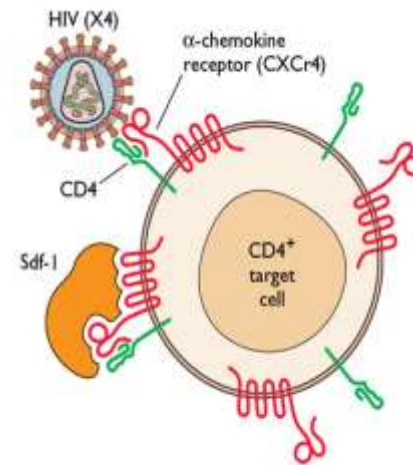


HIV-1 BINDING AND ENTRY

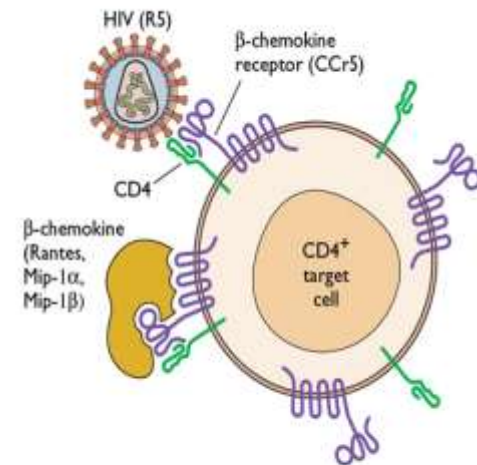
- HIV-1 attaches via gp120/gp41 to the CD4 surface receptor and chemokine co-receptors CXCR4 and/or CCR5



T-cell-line-tropic strain of HIV-1



Macrophage-tropic strain of HIV-1



Adapted from Fig. 3 of A. S. Fauci, *Nature* 384:529-533, 1996



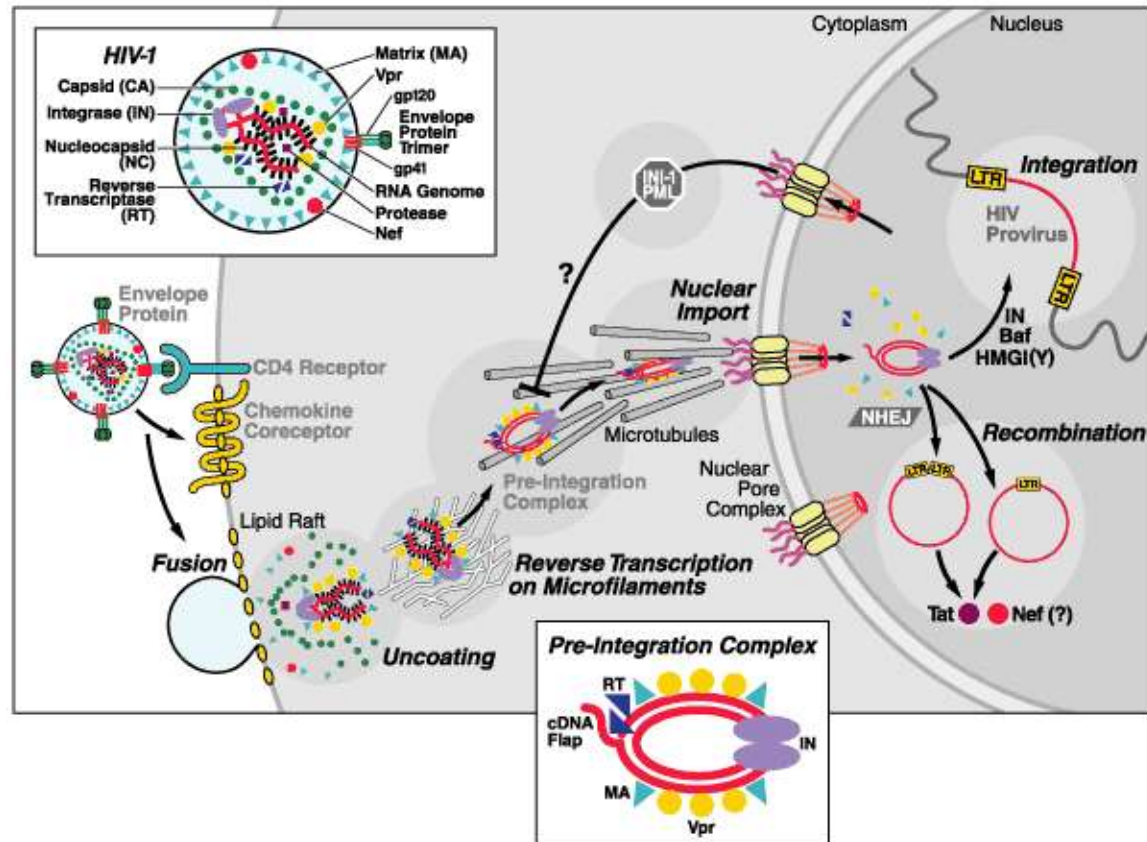
VIRAL REPLICATION

- Reverse transcriptase copies RNA into double stranded DNA
- DNA is integrated and replicated as provirus with host genome
- Provirus has its own promoter and signal that control transcription by host RNA polymerase
- Genomic RNA and spliced mRNAs are both produced
 - Later encode envelop glycoproteins and regulatory proteins



EVENTS AFTER ENTRY

- Uncoating, reverse transcription of the RNA genome,
- Nuclear import of the viral preintegration complex, and
- Integration of the double-stranded viral cDNA into the host chromosome, thus establishing the HIV provirus.

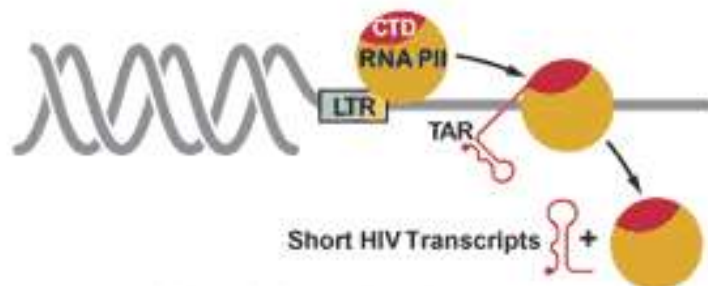


MECHANISMS OF LATENCY

Potential Mechanisms of Post-integration HIV Latency

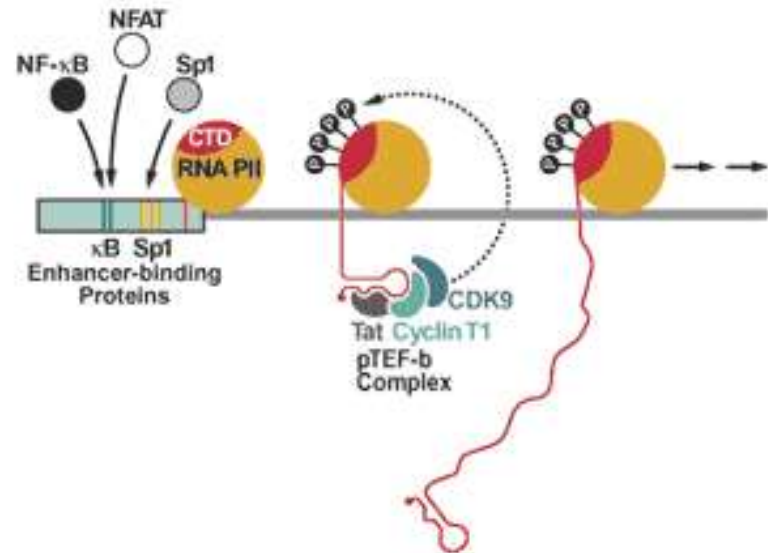


Integration into heterochromatin where transcription is repressed



Ineffective RNA PII elongation in the absence of Tat

Transcriptional Activation of HIV-1 Gene Expression

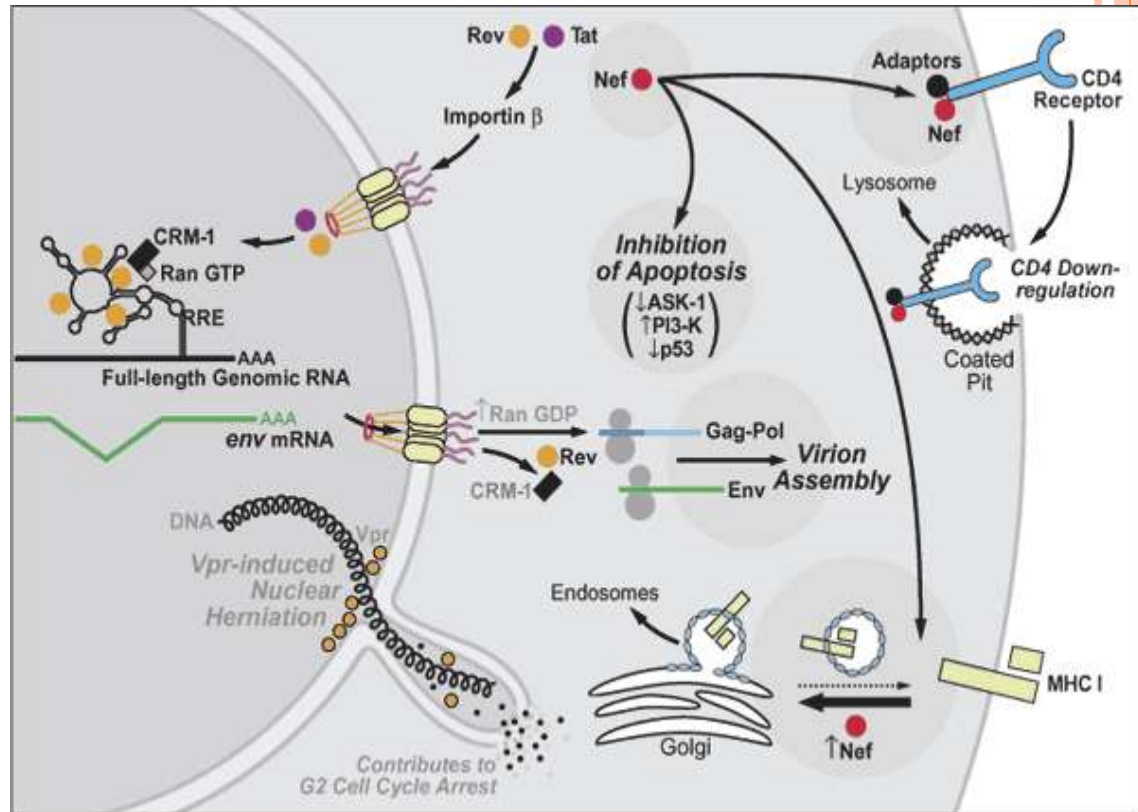


Tat and cyclin T1 binding to TAR activates CDK9, leading to phosphorylation of the C-terminal domain (CTD) of RNA PII and effective elongation



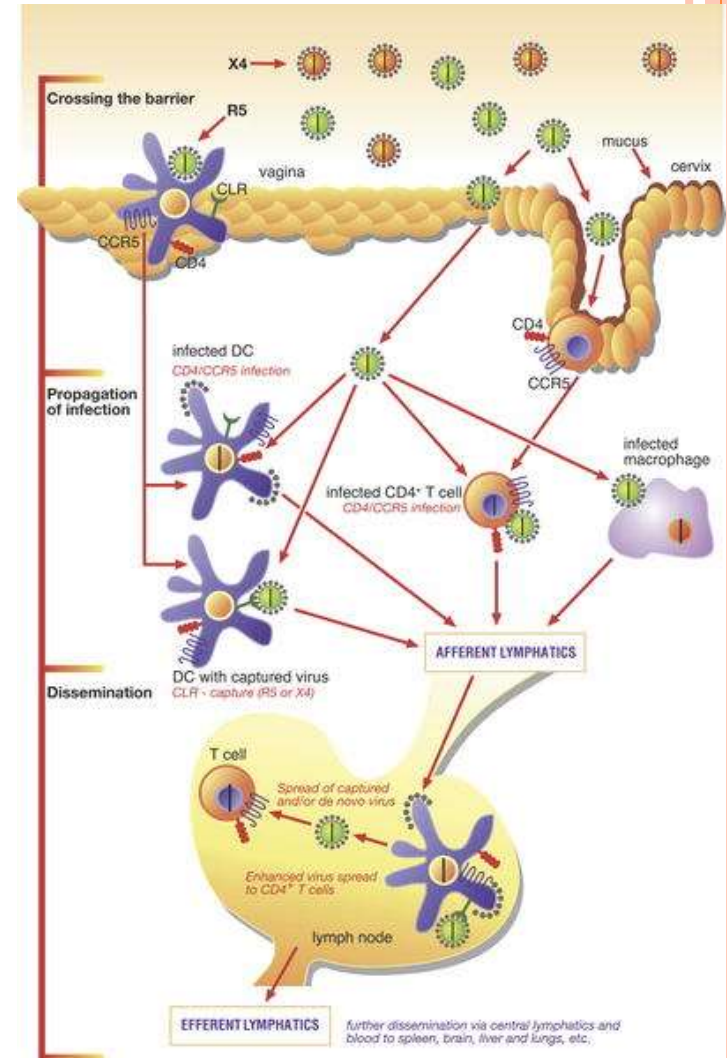
LATE EVENTS IN HIV-1 REPLICATION

- A summary of late events in the HIV-infected cell culminating in the assembly of new infectious virions.
- Highlighted are the roles of various viral proteins in optimizing the intracellular environment for viral replication including downregulation of CD4 and MHC I and inhibition of apoptosis by Nef, and the induction of G2 cell-cycle arrest by Vpr.
- A key action of the HIV Rev protein in promoting nuclear export of incompletely spliced viral transcripts that encode the structural and enzymatic proteins as well as the viral genome of new virions is also illustrated.



HIV-1 Pathogenesis

- Initial infection by Langerhans' and DCs
- Infection of local lymph nodes
- Infection of CD4+ lymphocytes & macrophages
- Spread to tissues;
- Viremia blood, virus in CSF
- Establish long-lived reservoirs in resting lymphocytes & tissue macrophages



HIV COURSE OF INFECTION

Often associated with acute viral syndrome and viremia

CD4⁺ T cells and Macrophages are main targets

Strong primary immune response

Slow but progressive decline in CD4⁺ T lymphocytes associated with hyperactive immune state

Ultimately development of

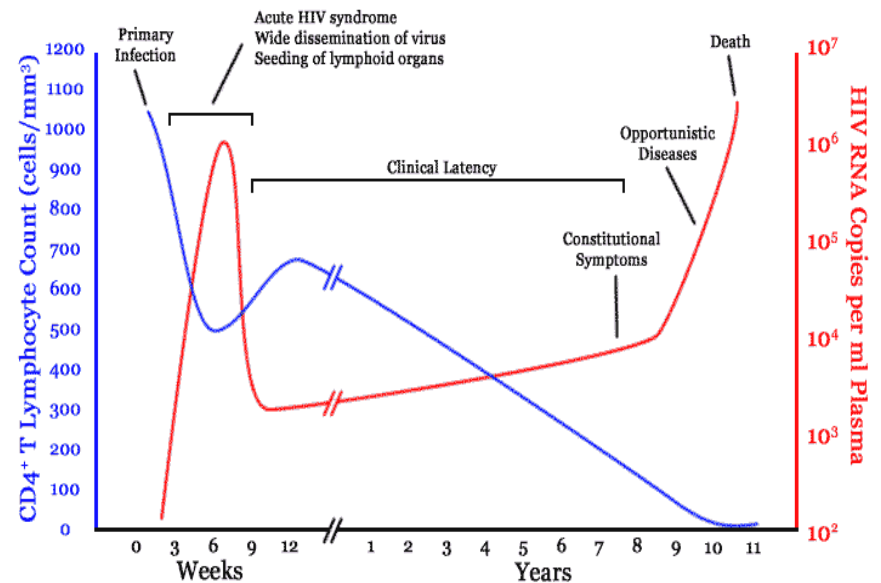
opportunistic infections

Neoplasms

Neurological complications

Cardiovascular events

Renal disease



KILLING OF CD4⁺ LYMPHOCYTES

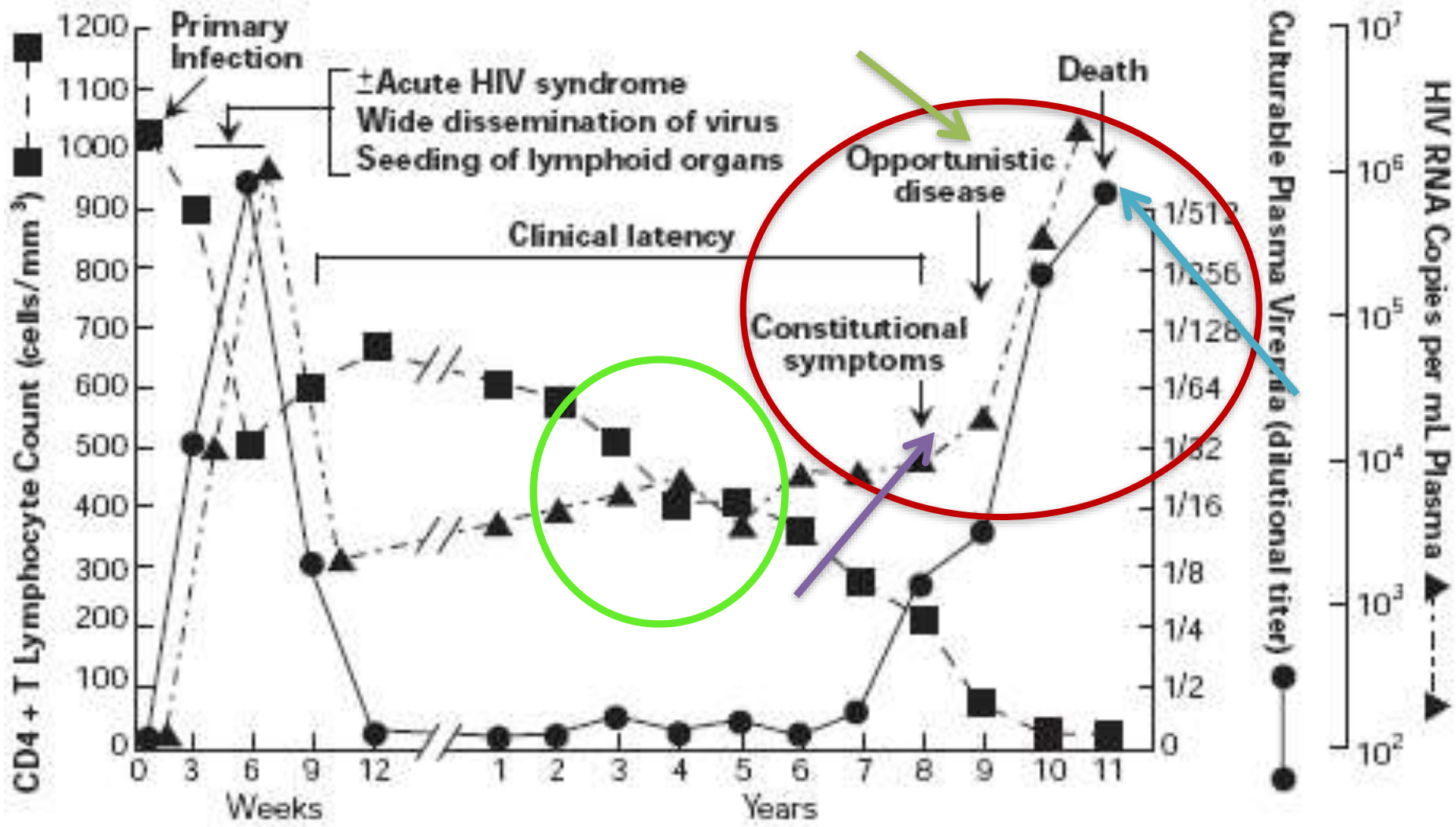
- Apoptotic mechanisms
 - Nef
- Non-apoptotic mechanisms
 - Direct
 - Tat
 - Envelop fusion
 - Indirect
 - CTLs,
 - Syncytia formation



HIV PATHOGENESIS AND PROGRESSION TO AIDS

- **CD4 cell depletion in GALT in the acute phase of HIV**
- Selective loss of Th17 cells
- Polyclonal B cell activation
- **Increased CD4 and CD8 turnover with decreased half lives.**
- Increased T-cells with activated phenotype
- Increased serum levels of proinflammatory cytokines.
- **Persistent activation generates of constant “viral targets”**

THE NATURAL HISTORY OF HIV INFECTION



CONDITIONS THAT DEFINE AIDS: INFECTIONS

■ Bacterial infections

- Disseminated MAC
- Pulmonary or EP Tuberculosis
- Recurrent bacterial pneumonia
- Salmonella sepsis

■ Fungal infections

- Candidiasis (esophageal, airway)
- EP coccidiomycosis
- EP cryptococcosis
- EP histoplasmosis
- Pneumocysts pneumonia

○ Parasitic infections

- Cryptosporidiasis c chronic diarrhea
- Isosporiasis c chronic diarrhea
- Toxoplamosis

○ Viral infections

- CMV (no liver, spleen or nodes)
- Herpes simplex (chronic ulcer, esophagus, airway)



HIV DISEASE SPECTRUM

- Acute infection
- Asymptomatic disease
- Symptomatic disease
- AIDS (CD4 <200 or Indicator disease)



PRIMARY (ACUTE) HIV INFECTION

- Acute febrile illness seen with acquisition of HIV infection
- **mononucleosis-like (fever, pharyngitis and LAM +/- rash) illness with or without aseptic meningitis associated with seroconversion to HIV**
- Typically 2-4 weeks after HIV exposure, can take longer
- Occurs in over 50% of patients although it is frequently unrecognized
- Diff Dx: Acute EBV, CMV, secondary syphilis, toxoplasmosis, influenza, parvovirus, Group A streptococcal pharyngitis.

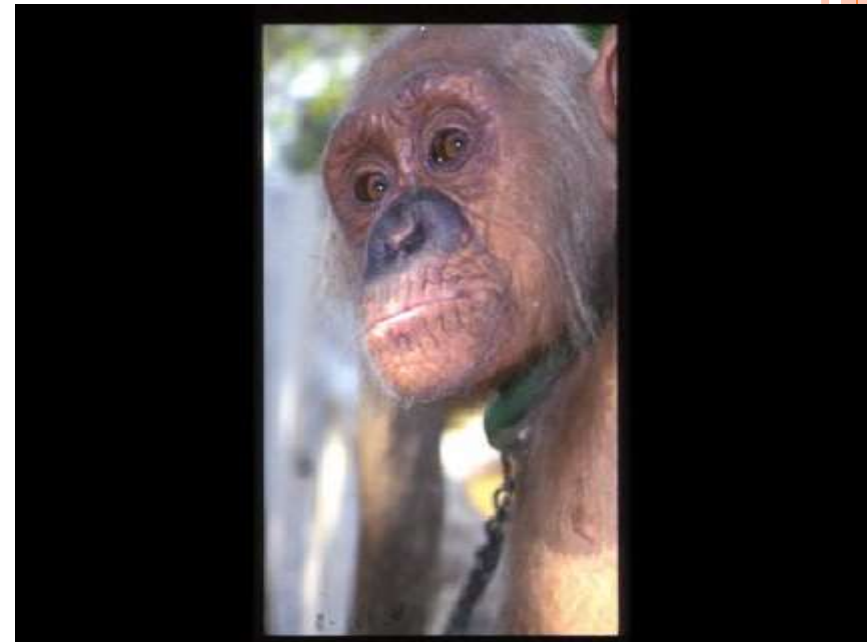
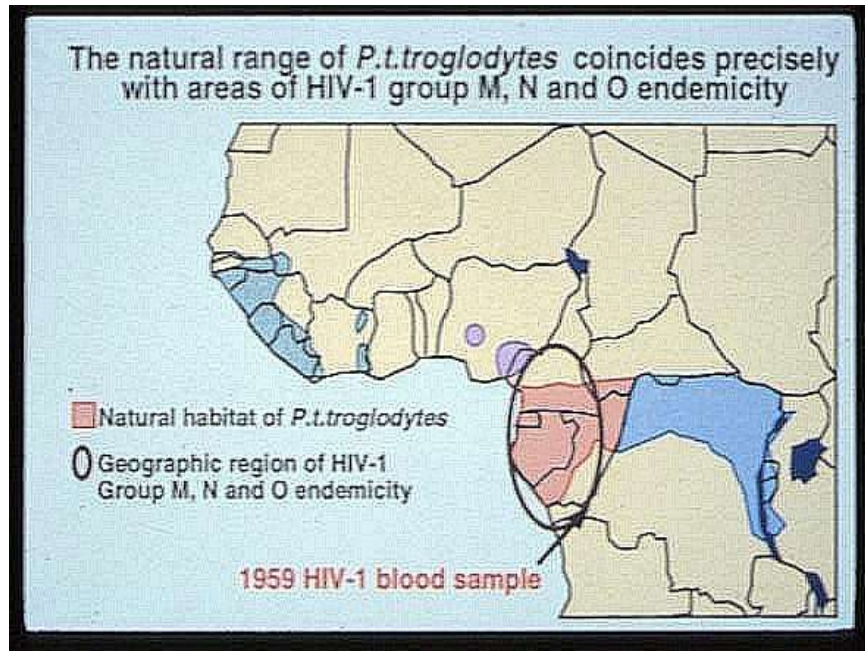
Diagnosis: HIV RNA in plasma. ELISA will be negative

- Dx: HIV RNA > 10,000 copies + indeterminate or negative HIV serology or recent seroconversion



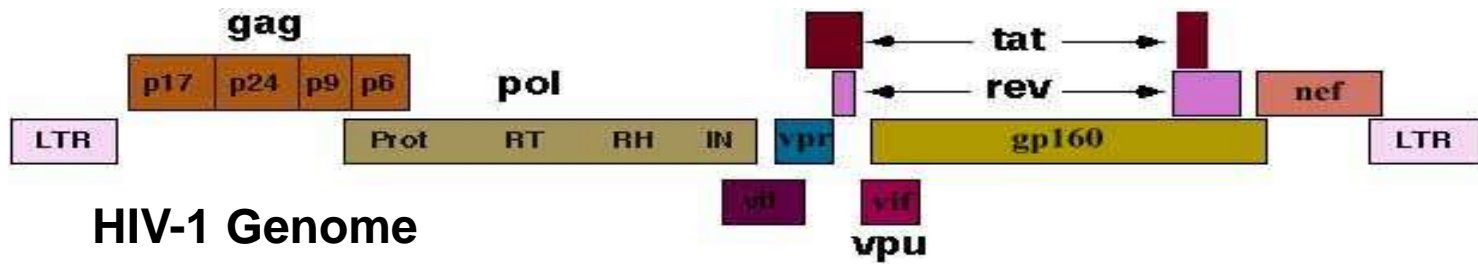
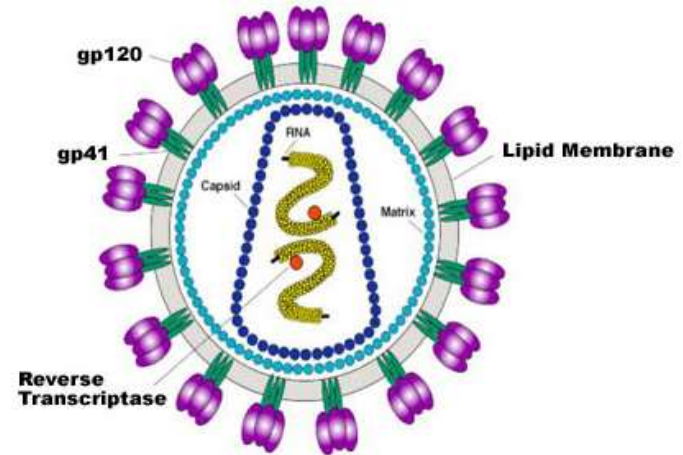
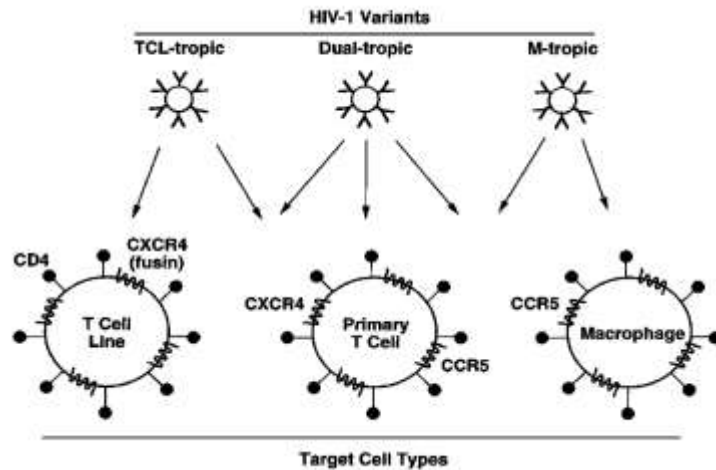
ORIGIN OF HIV

- HIV is a zoonosis (1 and 2)
- Origin of HIV-1 is SIVcpz from central black-faced chimpanzee (*pan t. troglodytes*)
- Humans probably first infected around 1930
- Oldest virus found in human specimen: 1959



HIV BIOLOGY

- Spherical, enveloped, diploid single-stranded positive sense RNA lentivirus
- HIV-1 attaches via gp120/gp41 (envelop) to the CD4 surface receptor and chemokine co-receptors CXCR4 and/or CCR5
- T-lymphocytes, myeloid cells (monocytes, dendritic cells), cells in the brain (perivascular macrophages, microglia, astrocytes, oligodendrocytes, BMVECs and neurons) express HIV-1 receptor/co-receptors (Martin-Garcia J., *et al.* Nat Rev Immunol, 2005)

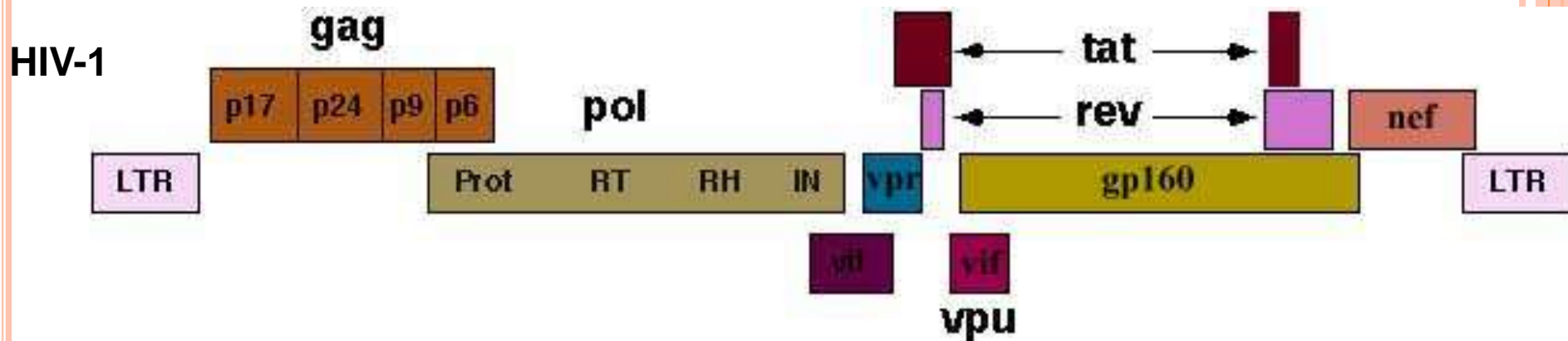
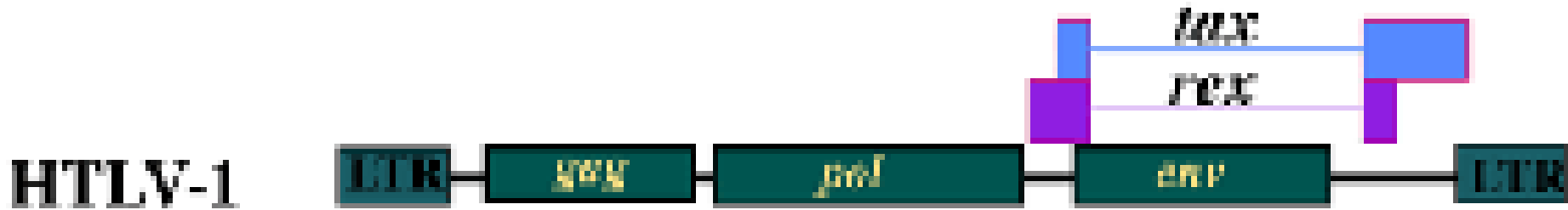


HIV-1 Genome

- Genome: Approximately 9-kilobases , 9 genes encoding 15 proteins



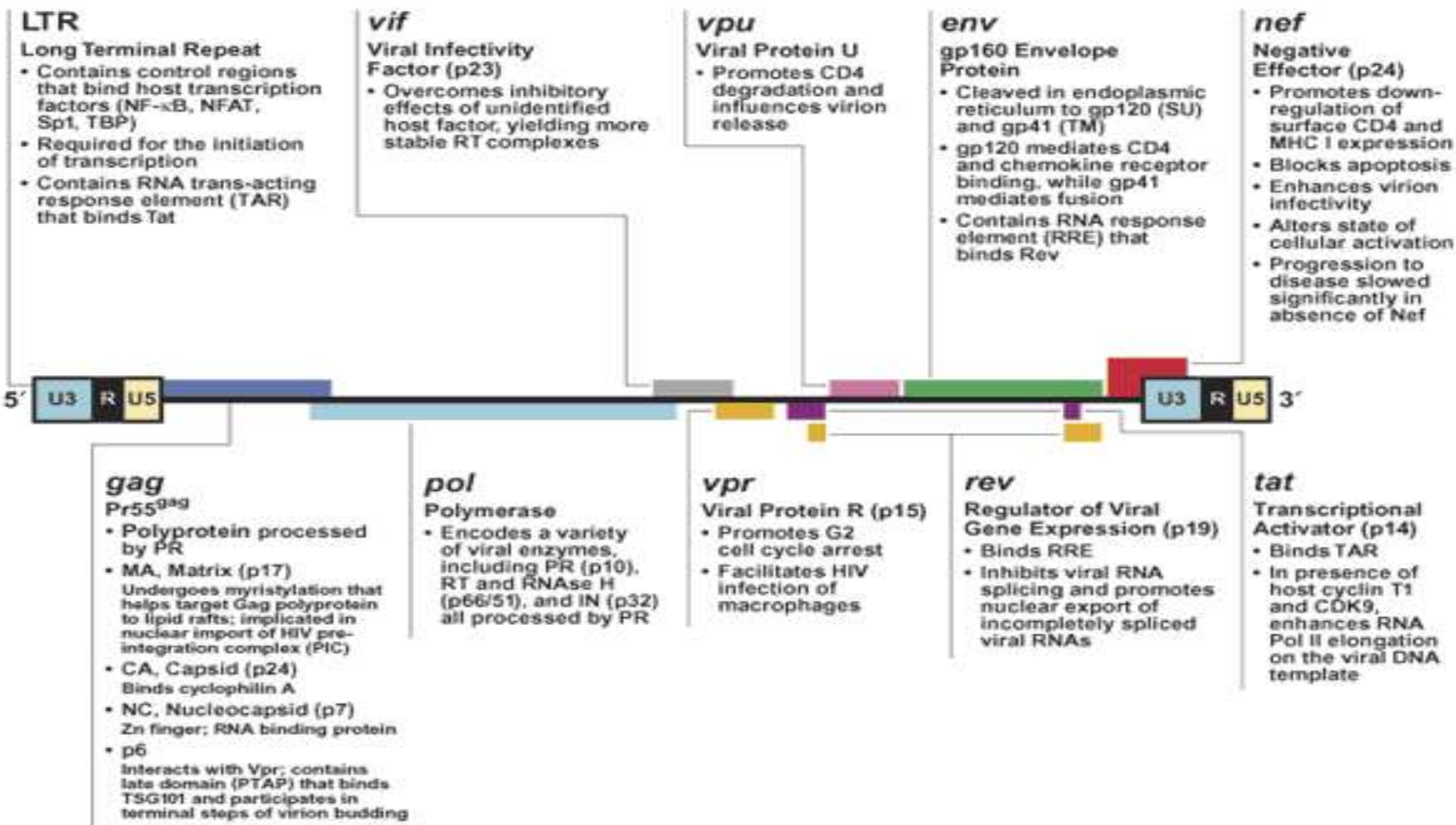
HIV-1 GENOME



overview of the organization of the (approximately) **9-kilobase** genome of the HIV provirus and a summary of the functions of its **9 genes** encoding **15 proteins**

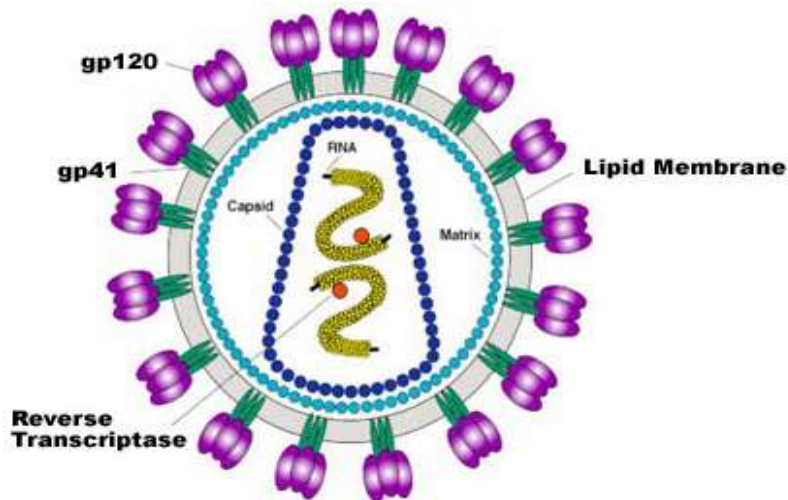
<http://hivinsite.ucsf.edu/InSite?page=kb-02-01-01>

HIV-1 GENOME

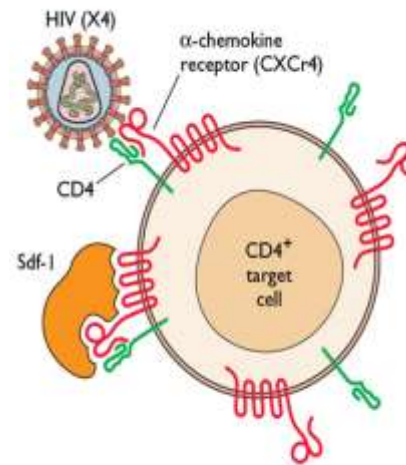


HIV-1 BINDING AND ENTRY

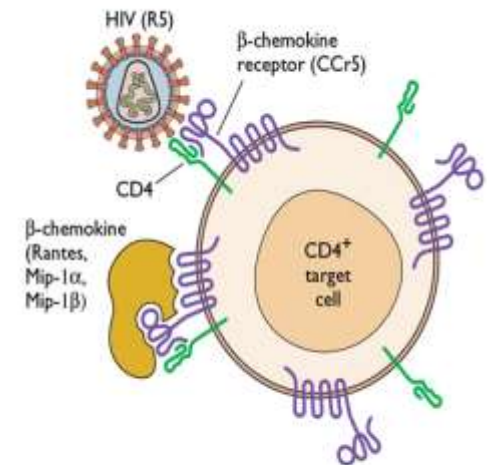
- HIV-1 attaches via gp120/gp41 to the CD4 surface receptor and chemokine co-receptors CXCR4 and/or CCR5



T-cell-line-tropic strain of HIV-1



Macrophage-tropic strain of HIV-1

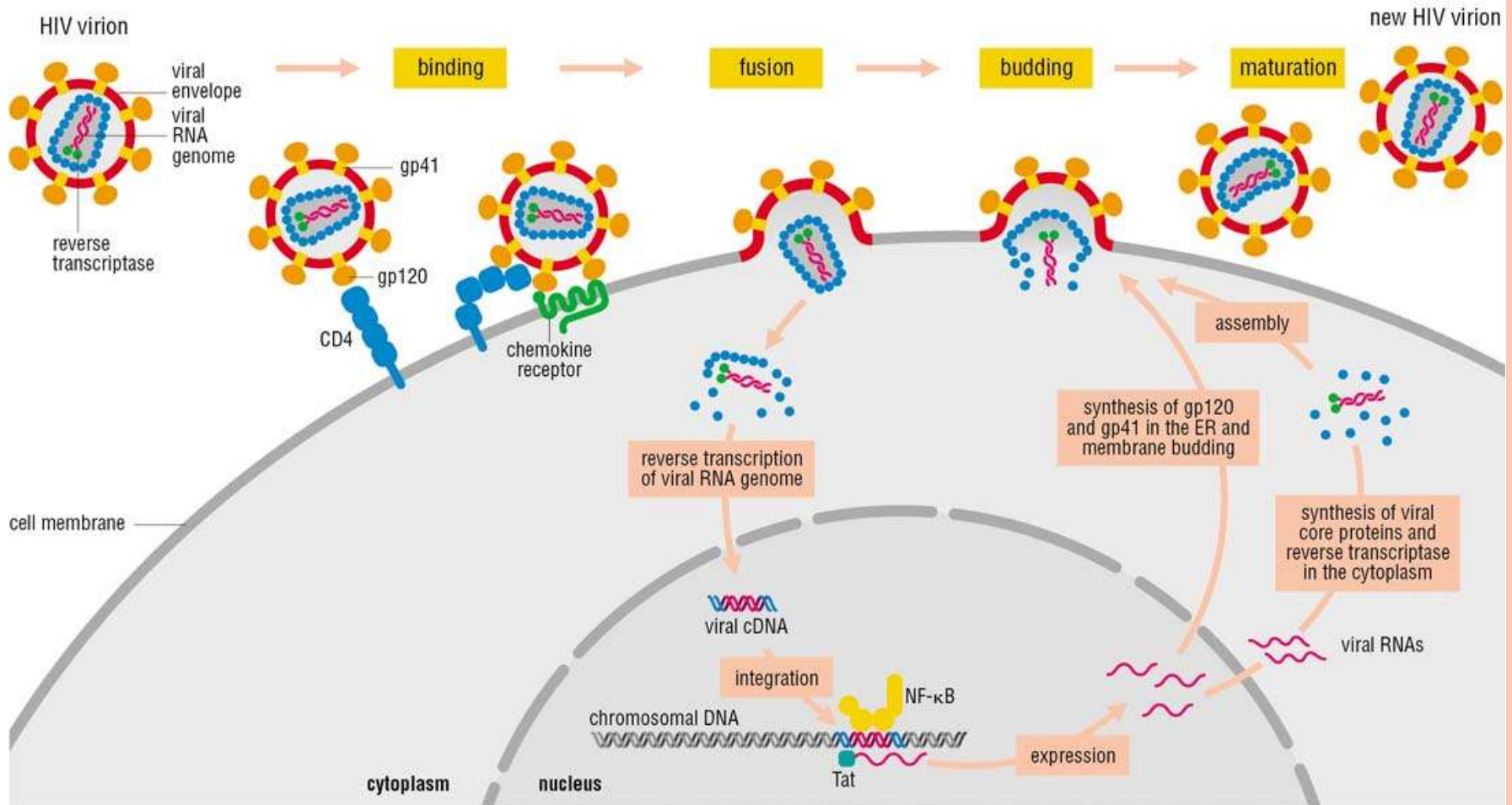


Adapted from Fig. 3 of A. S. Fauci, *Nature* 384:529-533, 1996, with permission



The life cycle of HIV

From **Immunity: The Immune Response in Infectious and Inflammatory Disease**
by DeFranco, Locksley and Robertson



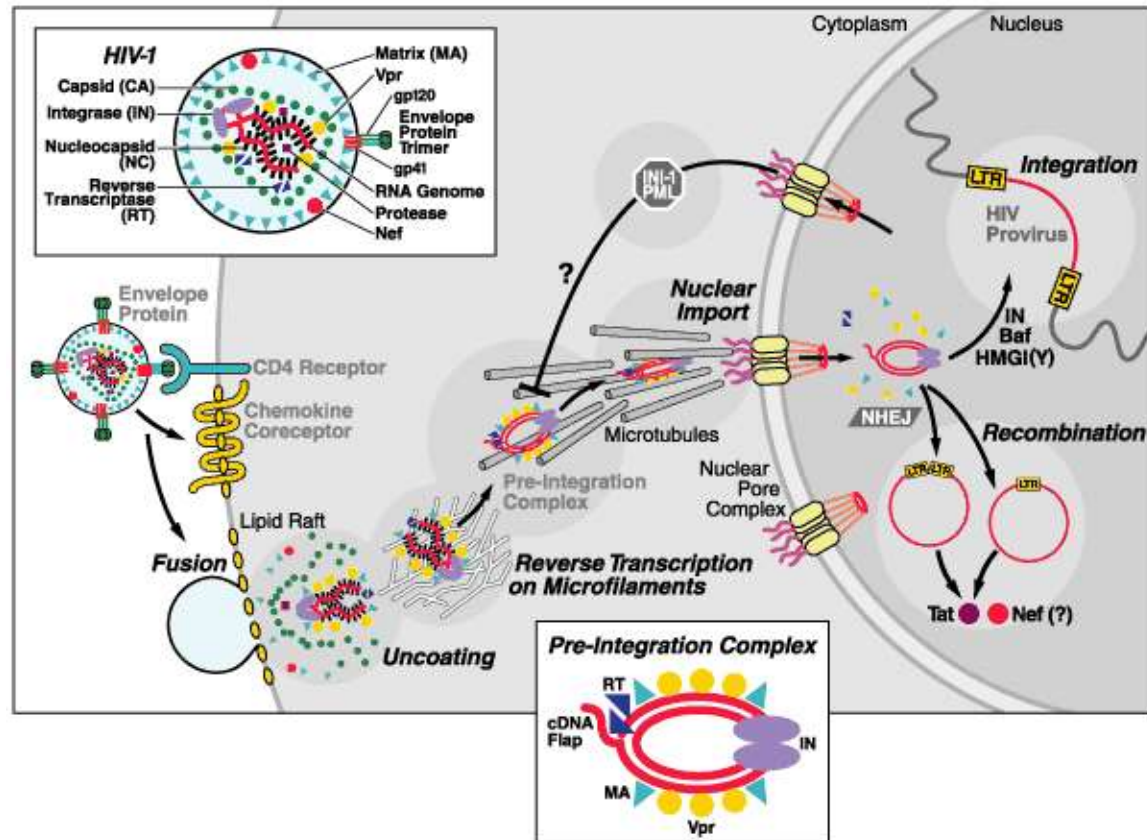
VIRAL REPLICATION

- Reverse transcriptase copies RNA into double stranded DNA
- DNA is integrated and replicated as provirus with host genome
- Provirus has its own promoter and signal that control transcription by host RNA polymerase
- Genomic RNA and spliced mRNAs are both produced
 - Later encode envelop glycoproteins and regulatory proteins



EVENTS AFTER ENTRY

- Uncoating, reverse transcription of the RNA genome,
- Nuclear import of the viral preintegration complex, and
- Integration of the double-stranded viral cDNA into the host chromosome, thus establishing the HIV provirus.

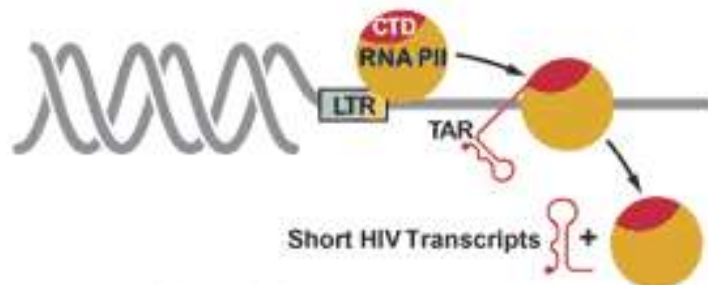


MECHANISMS OF LATENCY

Potential Mechanisms of Post-integration HIV Latency

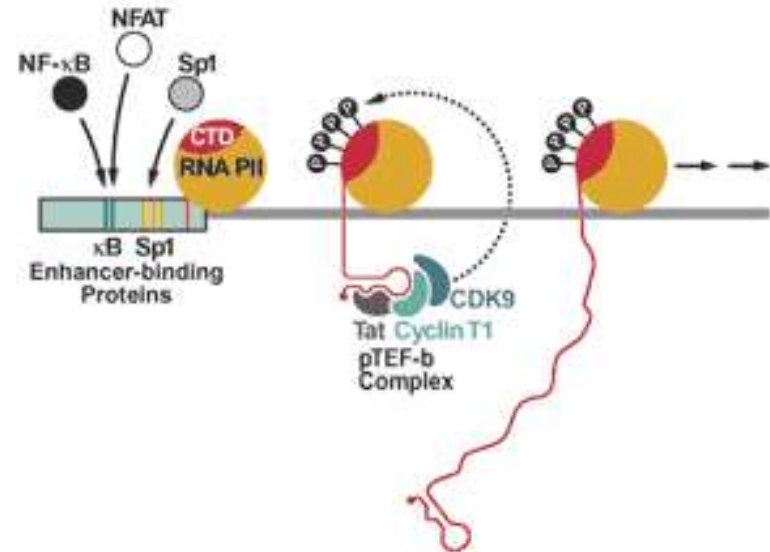


Integration into heterochromatin where transcription is repressed



Ineffective RNA PII elongation in the absence of Tat

Transcriptional Activation of HIV-1 Gene Expression

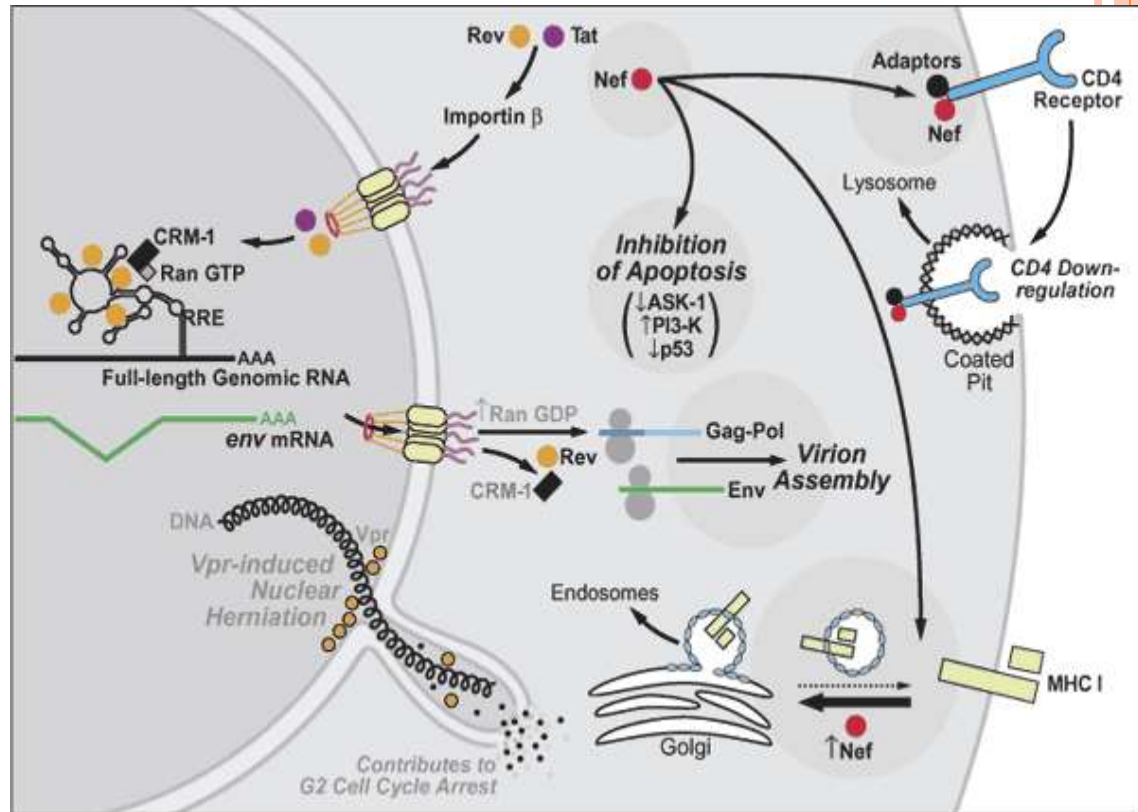


Tat and cyclin T1 binding to TAR activates CDK9, leading to phosphorylation of the C-terminal domain (CTD) of RNA PII and effective elongation



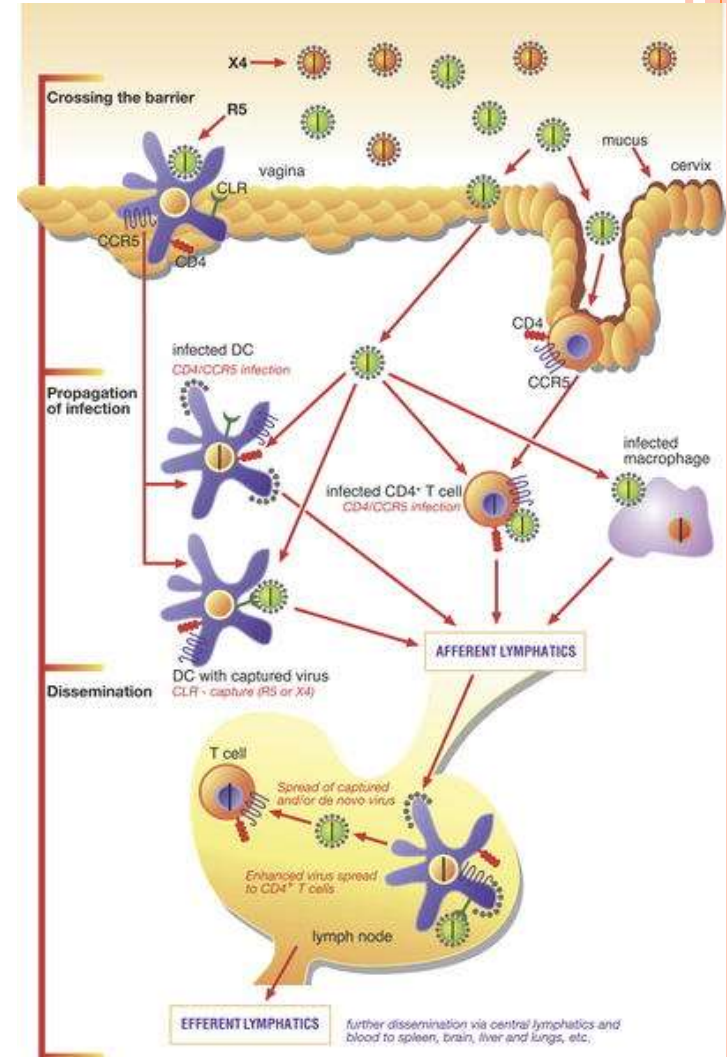
LATE EVENTS IN HIV-1 REPLICATION

- A summary of late events in the HIV-infected cell culminating in the assembly of new infectious virions.
- Highlighted are the roles of various viral proteins in optimizing the intracellular environment for viral replication including downregulation of CD4 and MHC I and inhibition of apoptosis by Nef, and the induction of G2 cell-cycle arrest by Vpr.
- A key action of the HIV Rev protein in promoting nuclear export of incompletely spliced viral transcripts that encode the structural and enzymatic proteins as well as the viral genome of new virions is also illustrated.



HIV-1 Pathogenesis

- Initial infection by Langerhans' and DCs
- Infection of local lymph nodes
- Infection of CD4+ lymphocytes & macrophages
- Spread to tissues;
- Viremia blood, virus in CSF
- Establish long-lived reservoirs in resting lymphocytes & tissue macrophages



HIV COURSE OF INFECTION

Often associated with acute viral syndrome and viremia

CD4⁺ T cells and Macrophages are main targets

Strong primary immune response

Slow but progressive decline in CD4⁺ T lymphocytes associated with hyperactive immune state

Ultimately development of

- opportunistic infections

- Neoplasms

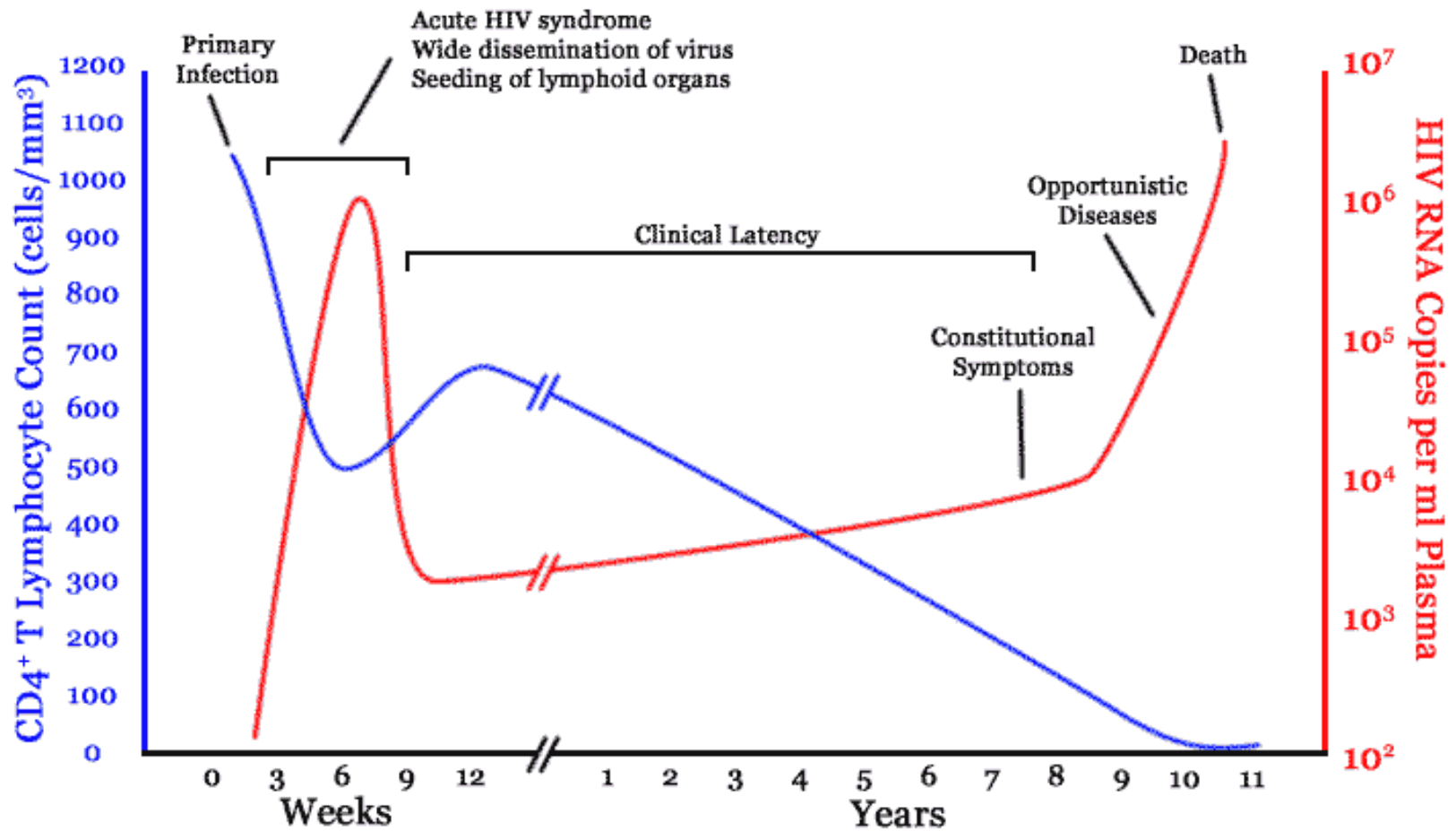
- Neurological complications

- Cardiovascular events

- Renal disease



HIV COURSE OF INFECTION

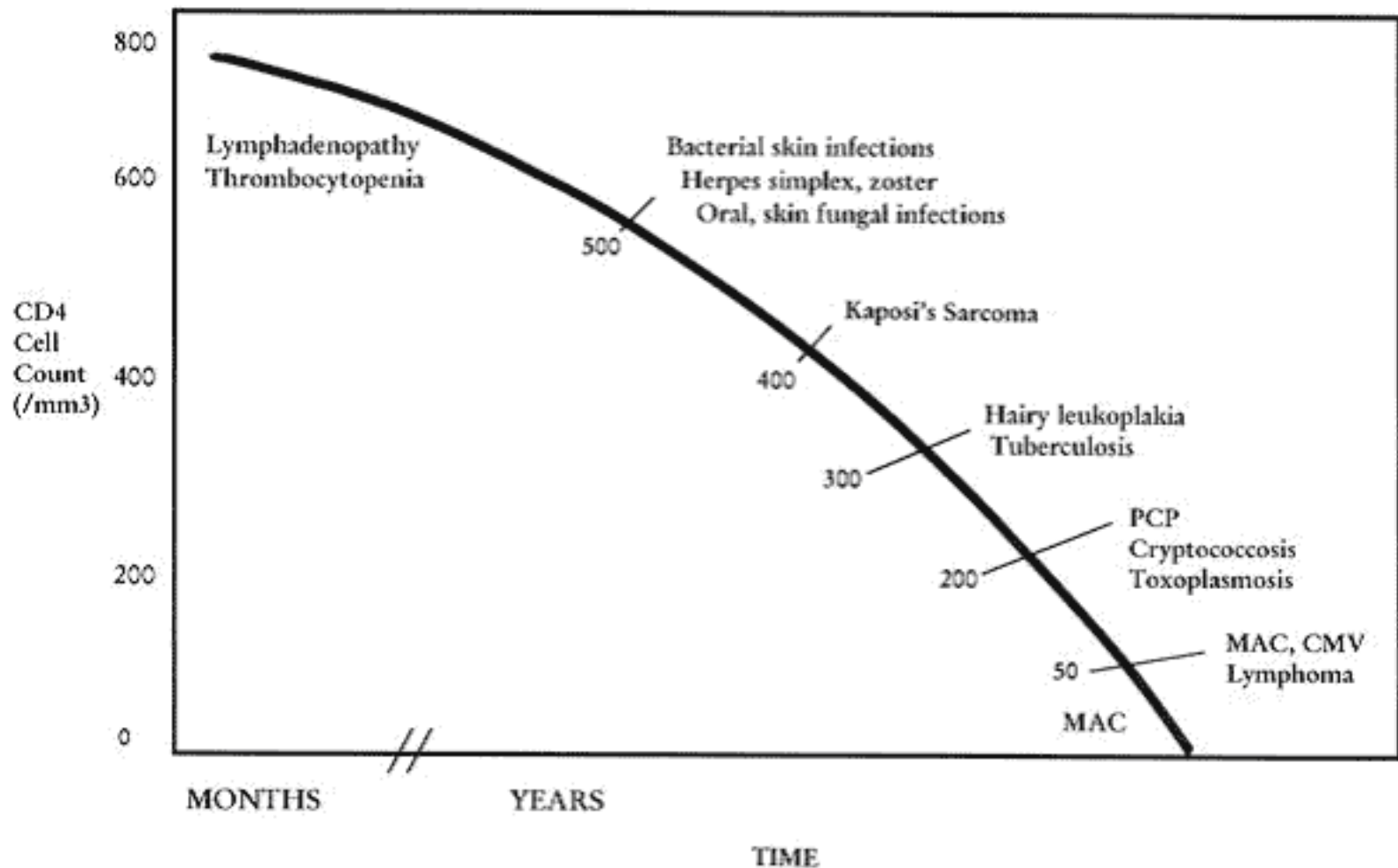


KILLING OF CD4⁺ LYMPHOCYTES

- Apoptotic mechanisms
 - Nef
- Non-apoptotic mechanisms
 - Direct
 - Tat
 - Envelop fusion
 - Indirect
 - CTLs,
 - Syncytia formation



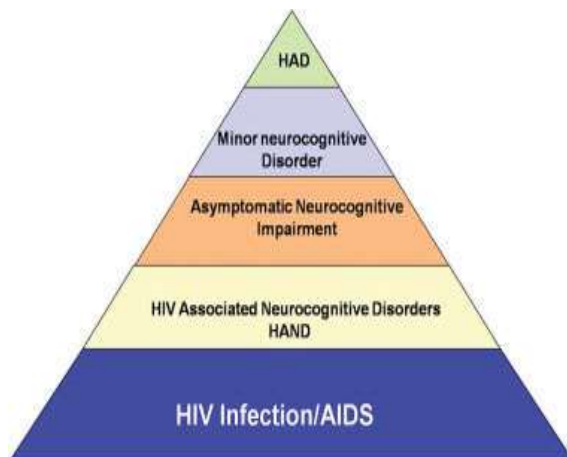
OPPORTUNISTIC INFECTIONS ACCORDING TO CD4 CELL COUNT



HIV-ASSOCIATED NEUROCOGNITIVE DISORDERS (HAND)

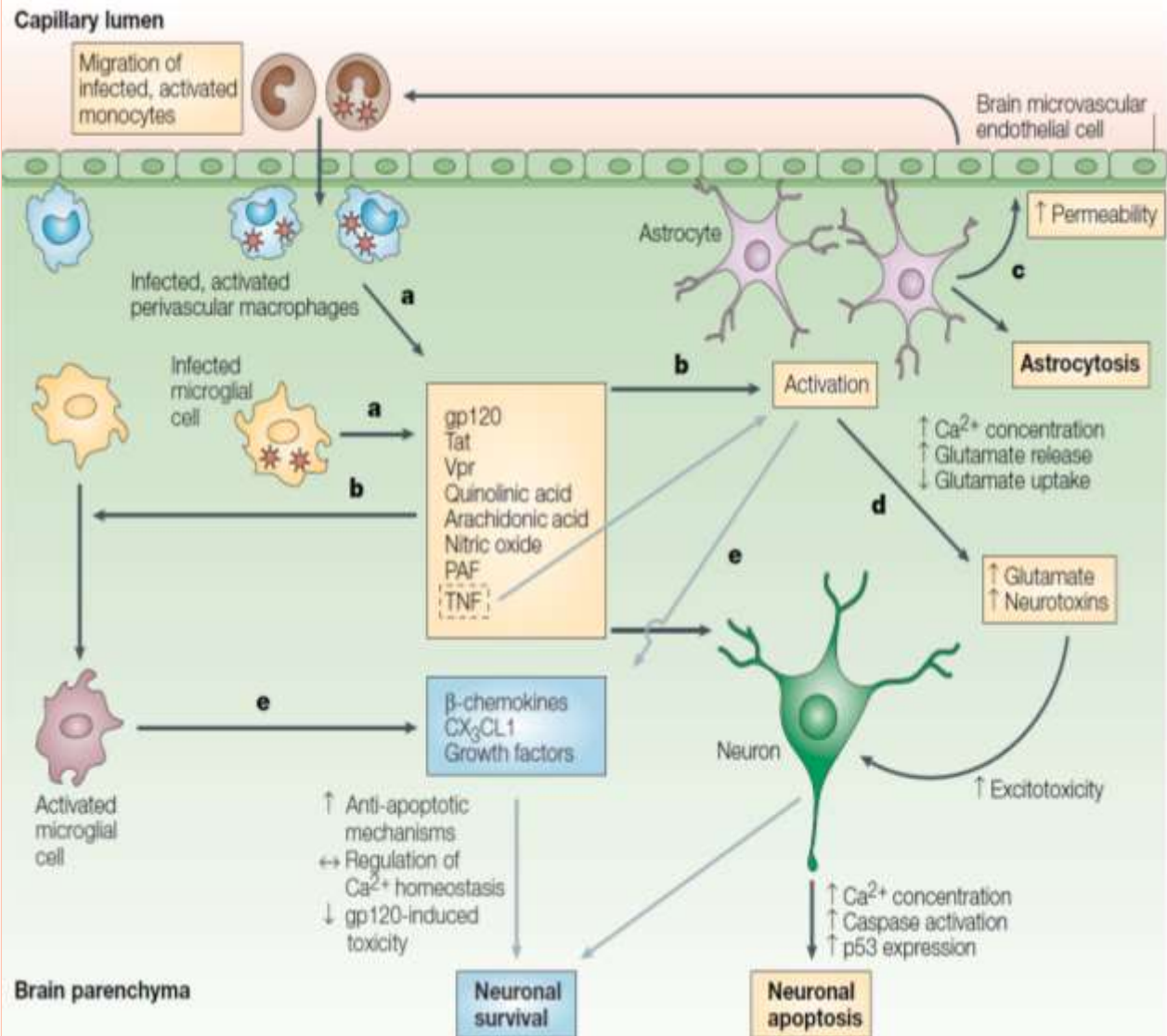
- Rise in the prevalence of human immunodeficiency virus (HIV)-associated neurocognitive disorders (HAND) despite use of highly active antiretroviral therapy (HAART).
 - Nosology: NC, ANI, MND and HAD (Antinori et al, 2007)
- Pathology is characterized by activation of microglia and astroglia and neuronal injury
 - Promoted by both systemic and central nervous system (CNS) inflammatory mechanisms
 - Viral products and chemokines/cytokines

Prevalence
HAND: 15-50%
ANI: 30%
MND: 20-30%
HAD: 2-8%



HIV ENTRY INTO THE CNS AND PATHOGENESIS

(‘TROJAN HORSE’ MECHANISM)

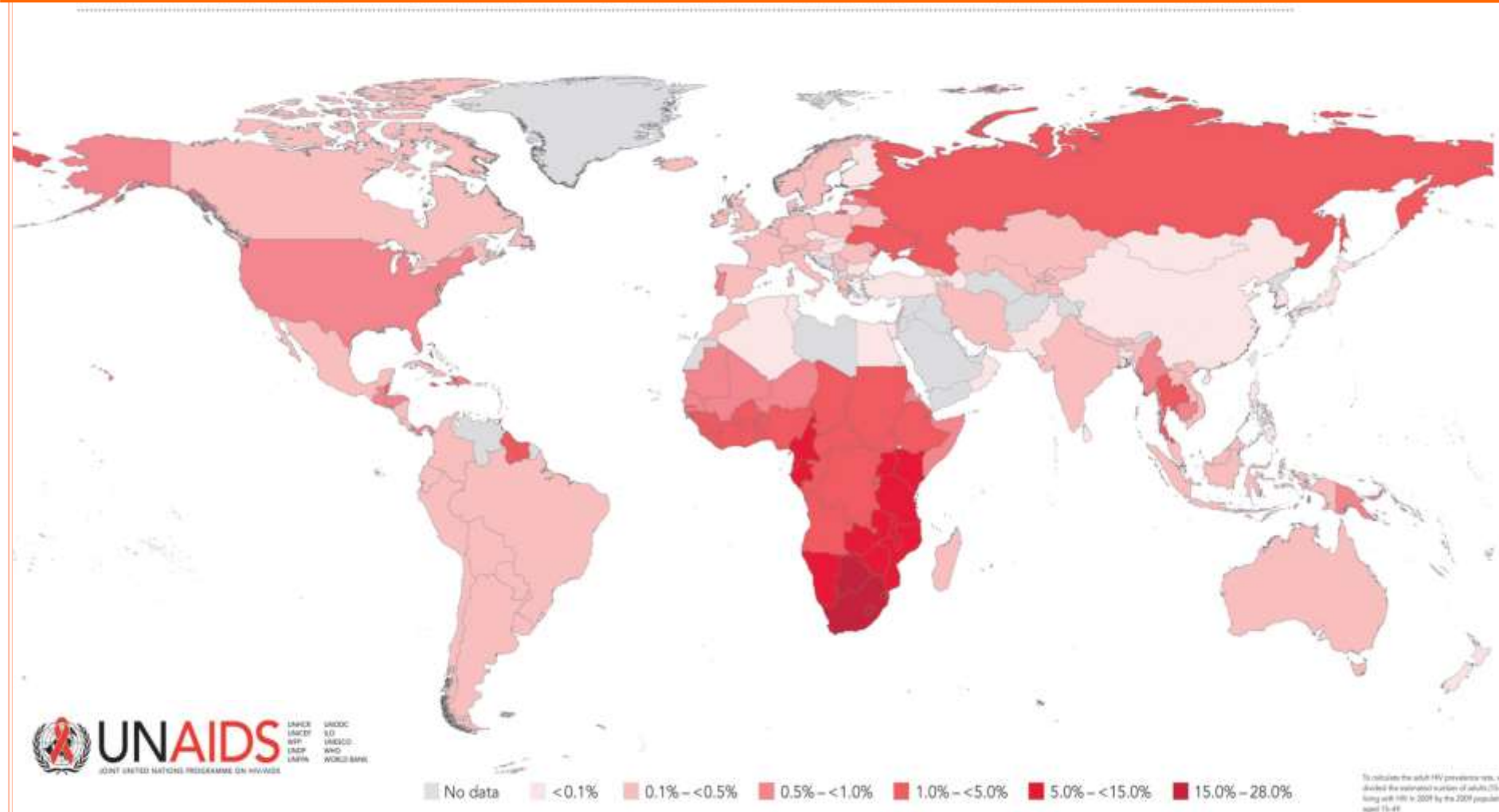


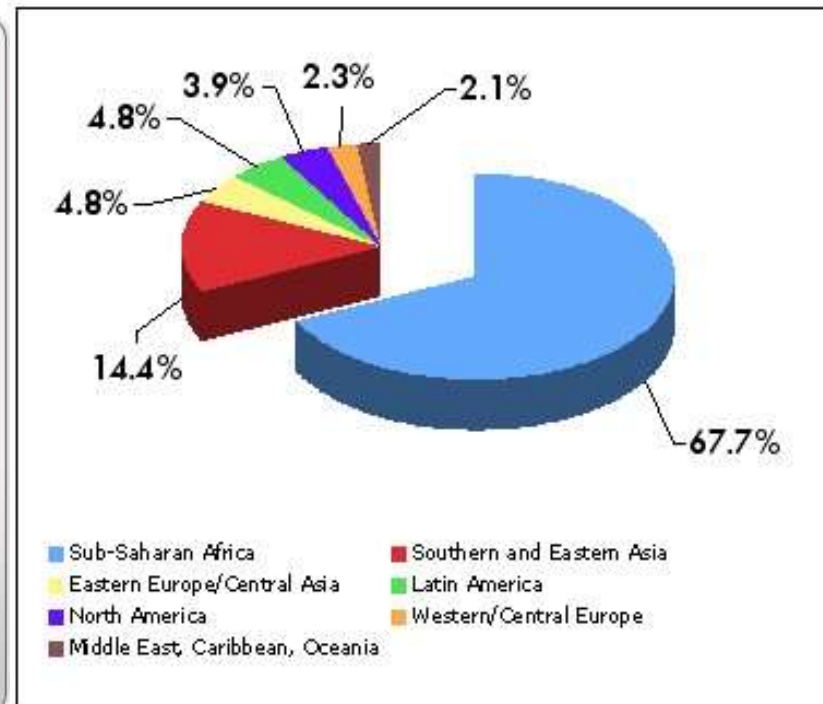
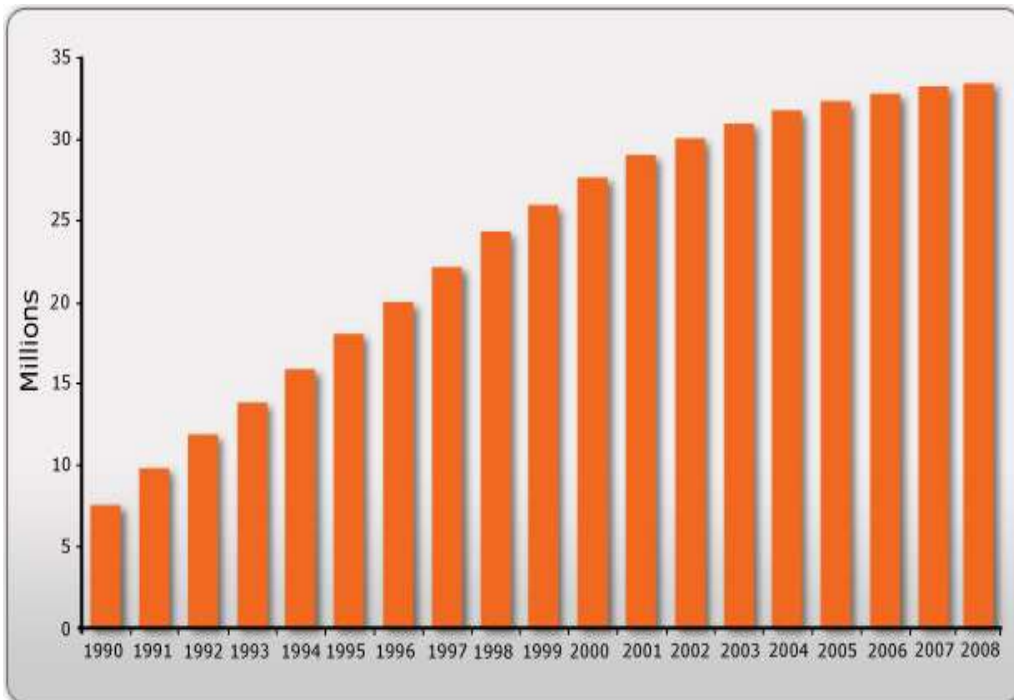
- Blood Brain Barrier/ Blood CSF barrier of the Choroid Plexus
- Entry via infected activated CD4+ T cells or monocytes
- Perivascular Macrophages and Microglia can be infected by HIV
- Neuronal injury:
 - Direct: tat, gp120, vpr
 - Indirect: TNF-α, NO, Glu, Ca²⁺

Martin-Gracia, *et al* 2002

2010: A global view of HIV infection

33.3 million people [31.4–35.3 million] living with HIV, 2009





Adults and children estimated to be living with HIV | 2011



Total: 34.2 million [31.8 million – 35.9 million]



Global summary of the AIDS epidemic | 2011

Number of people living with HIV

Total	34.2 million [31.8 million–35.9 million]
Adults	30.7 million [28.6 million–32.2 million]
Women	16.7 million [15.7 million–17.8 million]
Children (<15 years)	3.4 million [3.1 million–3.9 million]

People newly infected with HIV in 2011

Total	2.5 million [2.2 million–2.8 million]
Adults	2.2 million [2.0 million–2.4 million]
Children (<15 years)	330 000 [280 000–380 000]

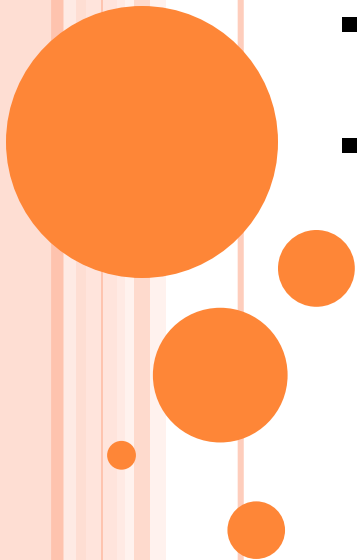
AIDS deaths in 2011

Total	1.7 million [1.6 million–1.9 million]
Adults	1.5 million [1.3 million–1.7 million]
Children (<15 years)	230 000 [200 000–270 000]

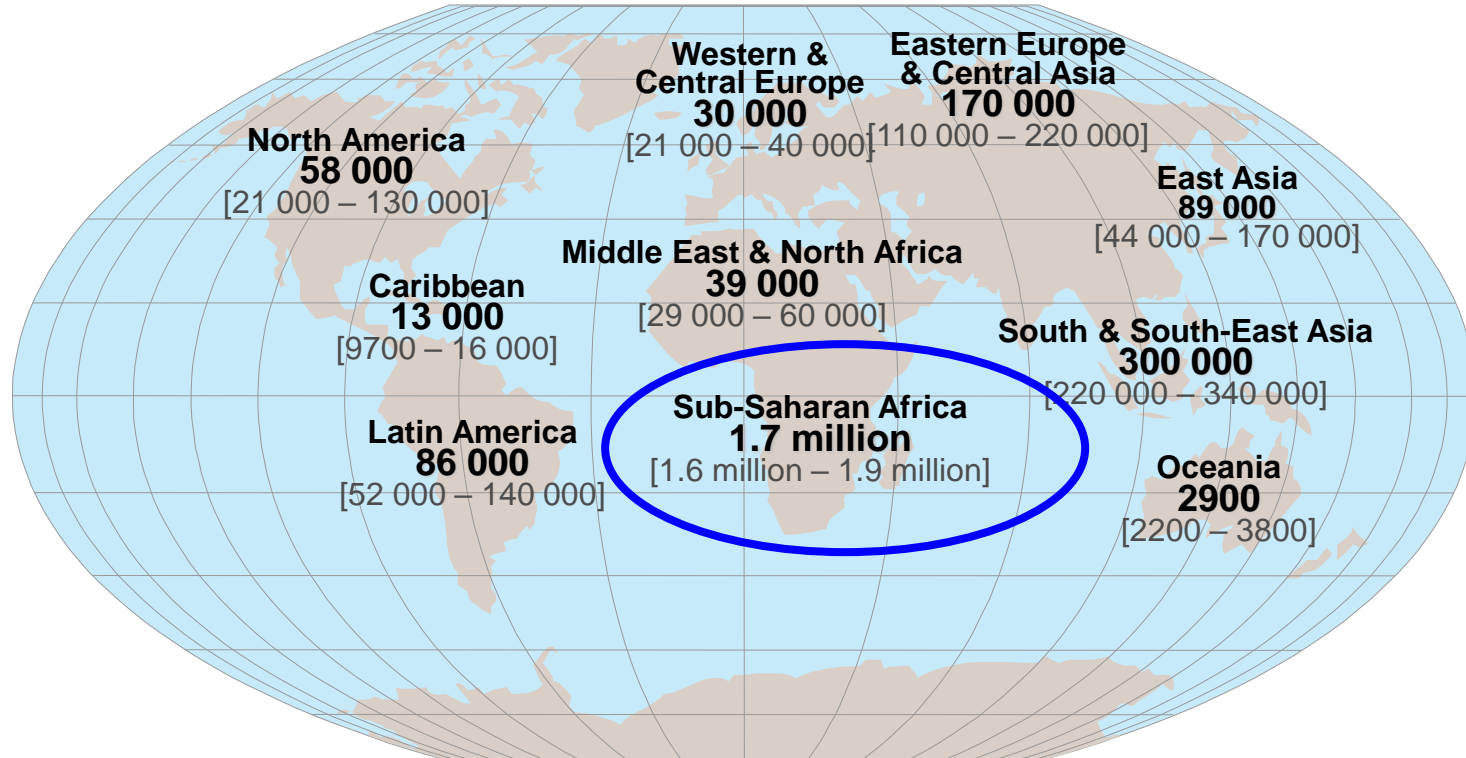


Over 7000 new HIV infections a day in 2011

- **About 97% are in low and middle income countries**
- **About 900 are in children under 15 years of age**
- **About 6000 are in adults aged 15 years and older, of whom:**
 - almost 47% are among women
 - about 41% are among young people (15-24)



Estimated number of adults and children newly infected with HIV | 2011



Total: 2.5 million [2.2 million – 2.8 million]



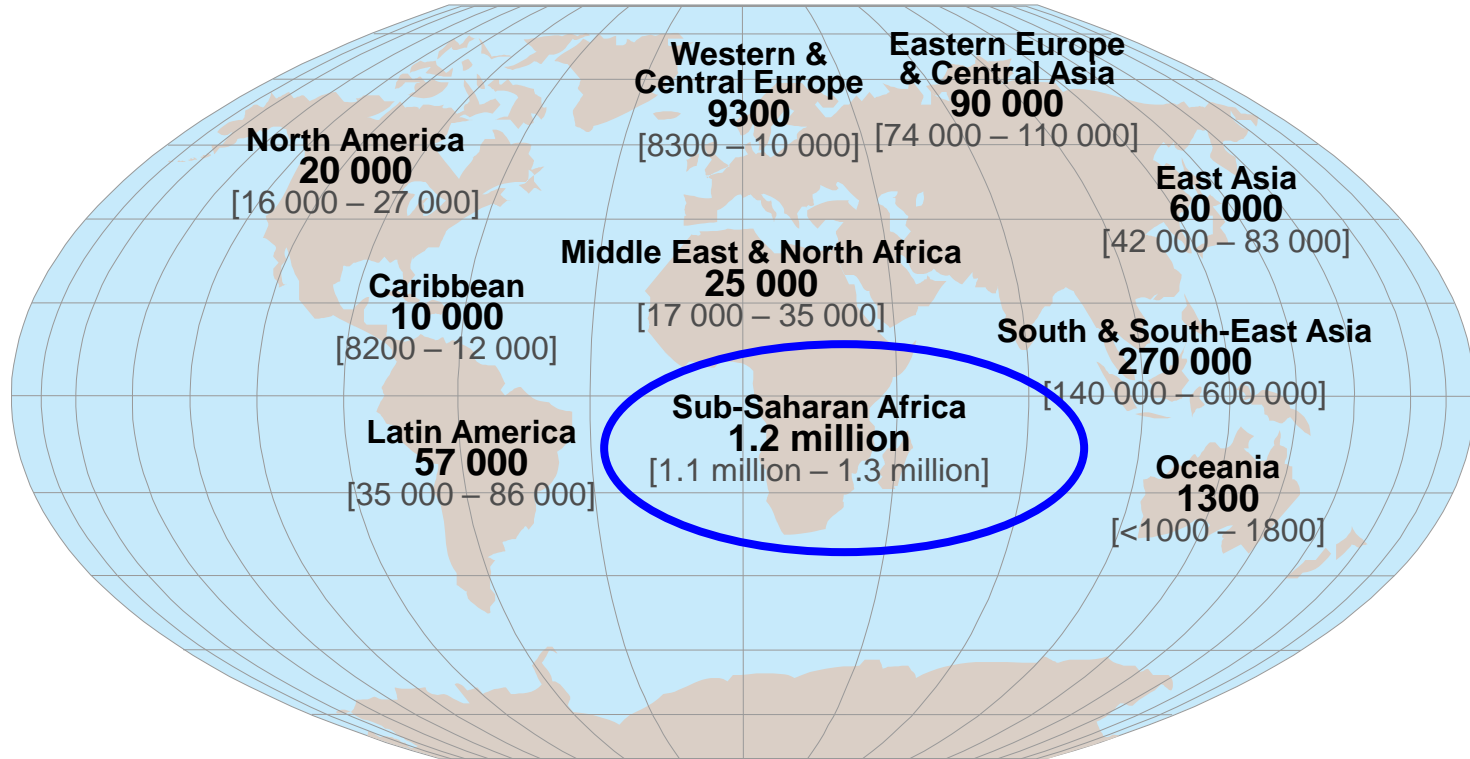
Adults and children estimated to be living with HIV | 2011



Total: 34.2 million [31.8 million – 35.9 million]



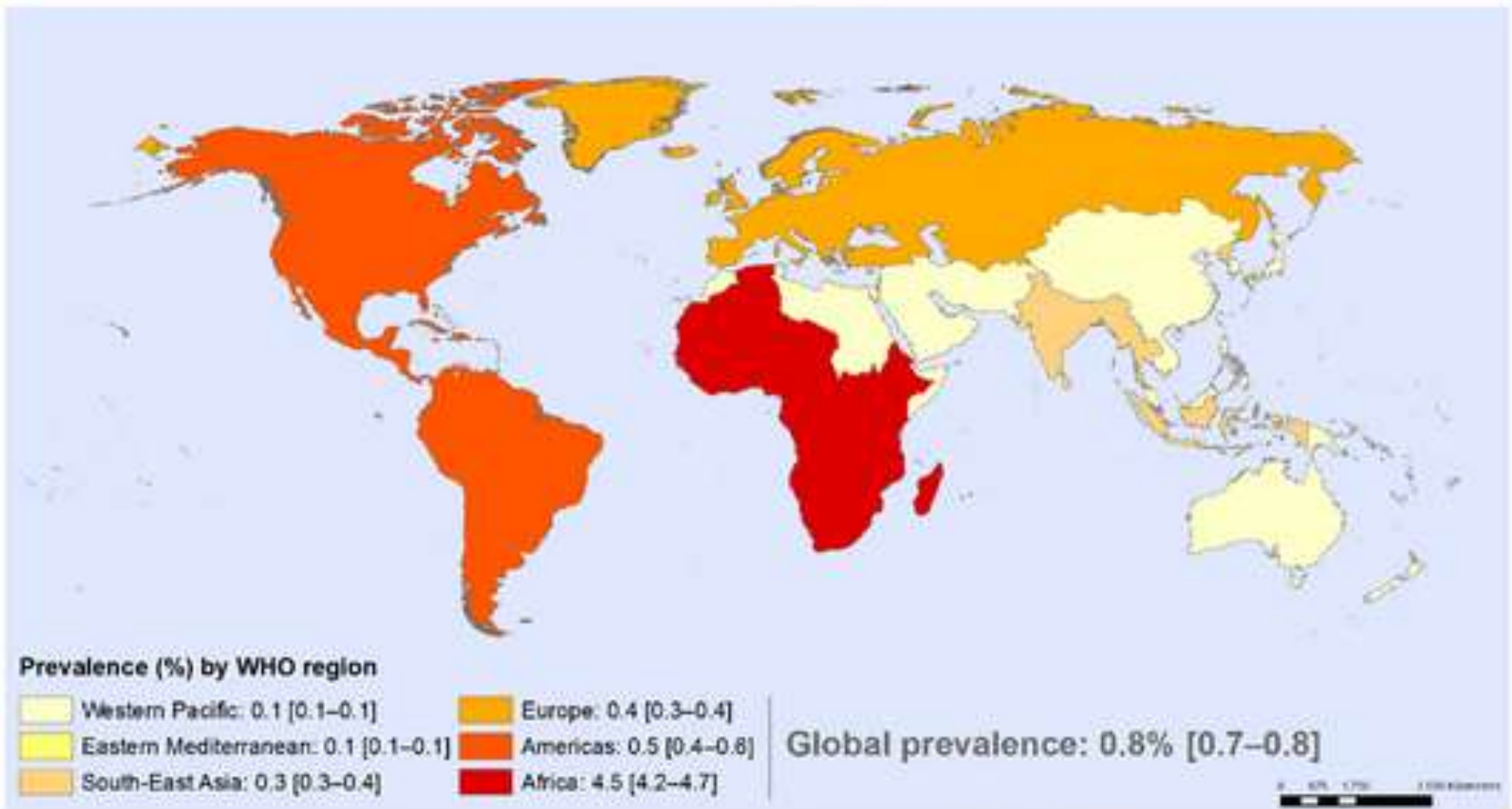
Estimated adult and child deaths from AIDS | 2011



Total: 1.7 million [1.6 million – 1.9 million]



Adult HIV prevalence (15–49 years), 2013 By WHO region



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Health Organization
Map Production: Health Statistics and
Information Systems (HSIS)
World Health Organization



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Global summary of the AIDS epidemic | 2013

Number of people living with HIV in 2013	Total	35.0 million	[33.1 million – 37.2 million]
	Adults	31.8 million	[30.1 million – 33.7 million]
	Women	16.0 million	[15.2 million – 16.9 million]
	Children (<15 years)	3.2 million	[2.9 million – 3.5 million]

People newly infected with HIV in 2013	Total	2.1 million	[1.9 million – 2.4 million]
	Adults	1.9 million	[1.7 million – 2.1 million]
	Children (<15 years)	240 000	[210 000 – 280 000]

AIDS deaths in 2013	Total	1.5 million	[1.4 million – 1.7 million]
	Adults	1.3 million	[1.2 million – 1.5 million]
	Children (<15 years)	190 000	[170 000 – 220 000]

GLOBAL AND REGIONAL DATA

Global summary of the AIDS epidemic | 2018

Number of people living with HIV	Total	37.9 million	[32.7 million–44.0 million]
	Adults	36.2 million	[31.3 million–42.0 million]
	Women (15+ years)	18.8 million	[16.4 million–21.7 million]
	Children (<15 years)	1.7 million	[1.3 million–2.2 million]

People newly infected with HIV in 2018	Total	1.7 million	[1.4 million–2.3 million]
	Adults	1.6 million	[1.2 million–2.1 million]
	Children (<15 years)	160 000	[110 000–260 000]

AIDS-related deaths in 2018	Total	770 000	[570 000–1.1 million]
	Adults	670 000	[500 000–920 000]
	Children (<15 years)	100 000	[64 000–160 000]

○ GLOBAL HIV STATISTICS

- 18.2 million [16.1 million–19.0 million] people were accessing antiretroviral therapy (June 2016)
- **36.7 million** [34.0 million–39.8 million] people globally were living with HIV (end 2015)
- 2.1 million [1.8 million–2.4 million] people became newly infected with HIV (end 2015)
- 1.1 million [940 000–1.3 million] people died from AIDS-related illnesses (end 2015)
- 78 million [69.5 million–87.6 million] people have become infected with HIV since the start of the epidemic (end 2015)
- 35 million [29.6 million–40.8 million] people have died from AIDS-related illnesses since the start of the epidemic (end 2015)



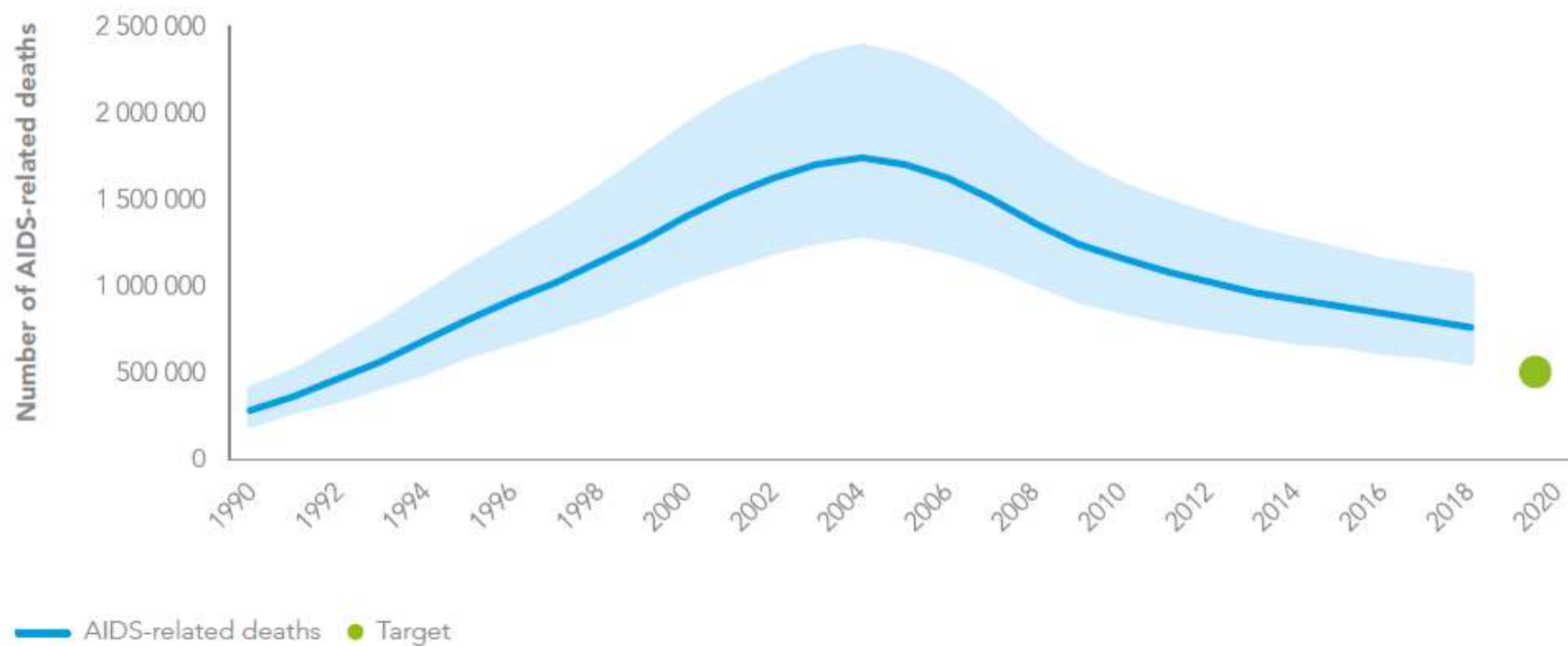
PEOPLE LIVING WITH HIV ACCESSING ANTIRETROVIRAL THERAPY



- As of June 2016, 18.2 million [16.1 million–19.0 million] people living with HIV were accessing antiretroviral therapy, up from 15.8 million in June 2015 and 7.5 million in 2010.
- In 2015, around 46% [43–50%] of all people living with HIV had access to treatment.
- In 2015, some 77% [69–86%] of pregnant women living with HIV had access to antiretroviral medicines to prevent transmission of HIV to their babies.
- **New HIV infections**
 - Worldwide, 2.1 million [1.8 million–2.4 million] people became newly infected with HIV in 2015.
- New HIV infections among children have declined by 50% since 2010.
 - Worldwide, 150 000 [110 000–190 000] children became newly infected with HIV in 2015, down from 290 000 [250 000–350 000] in 2010.
- Since 2010 there have been no declines in new HIV infections among adults.
 - Every year since 2010, around 1.9 million [1.9 million–2.2 million] adults have become newly infected with HIV.
- **AIDS-related deaths**
- AIDS-related deaths have fallen by 45% since the peak in 2005.
 - In 2015, 1.1 million [940 000–1.3 million] people died from AIDS-related causes worldwide, compared to 2 million [1.7 million–2.3 million] in 2005.
- **HIV/tuberculosis**
- Tuberculosis-related deaths among people living with HIV have fallen by 32% since 2004.
 - Tuberculosis remains the leading cause of death among people living with HIV, accounting for around one in three AIDS-related deaths.
 - In 2014, the percentage of identified HIV-positive tuberculosis patients who started or continued on antiretroviral therapy reached 77%.



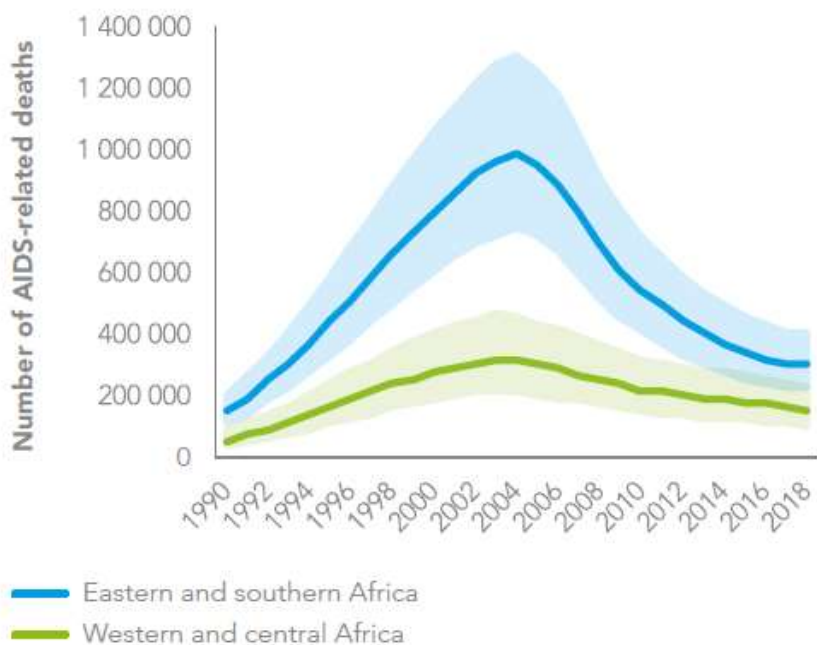
FIGURE 2.1 Number of AIDS-related deaths, global, 1990–2018 and 2020 target



Source: UNAIDS 2019 estimates.

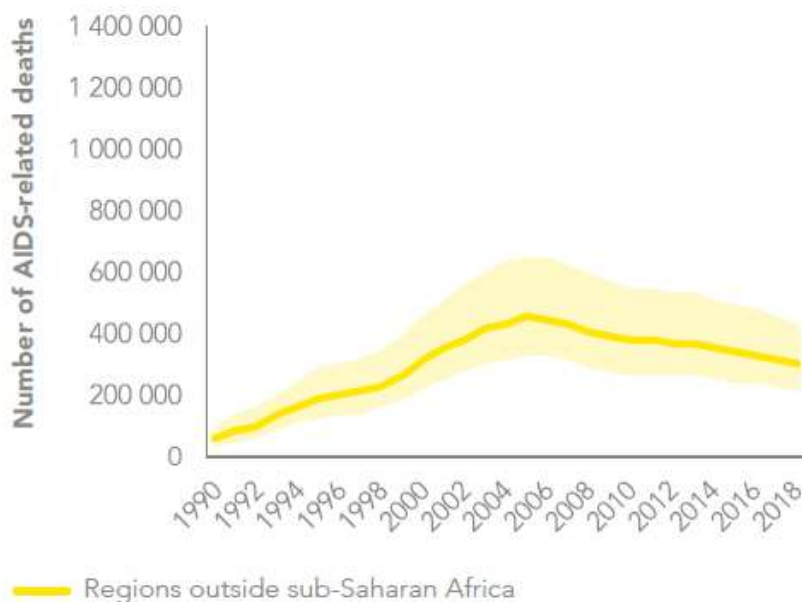


FIGURE 2.2 Number of AIDS-related deaths, eastern and southern Africa and western and central Africa, 1990–2018



Source: UNAIDS 2019 estimates.

FIGURE 2.3 Number of AIDS-related deaths, regions outside sub-Saharan Africa, 1990–2018



Source: UNAIDS 2019 estimates.



INVESTMENTS



- At the end of 2015, US\$ 19 billion was invested in the AIDS response in low- and middle-income countries (not including the countries that have recently transitioned into high-income categories).
- Domestic resources (USA) constituted 57% of the total resources for HIV in low- and middle-income countries in 2015.
- Recent updated UNAIDS estimates indicate that US\$ 26.2 billion will be required for the AIDS response in 2020, with US\$ 23.9 billion required in 2030.

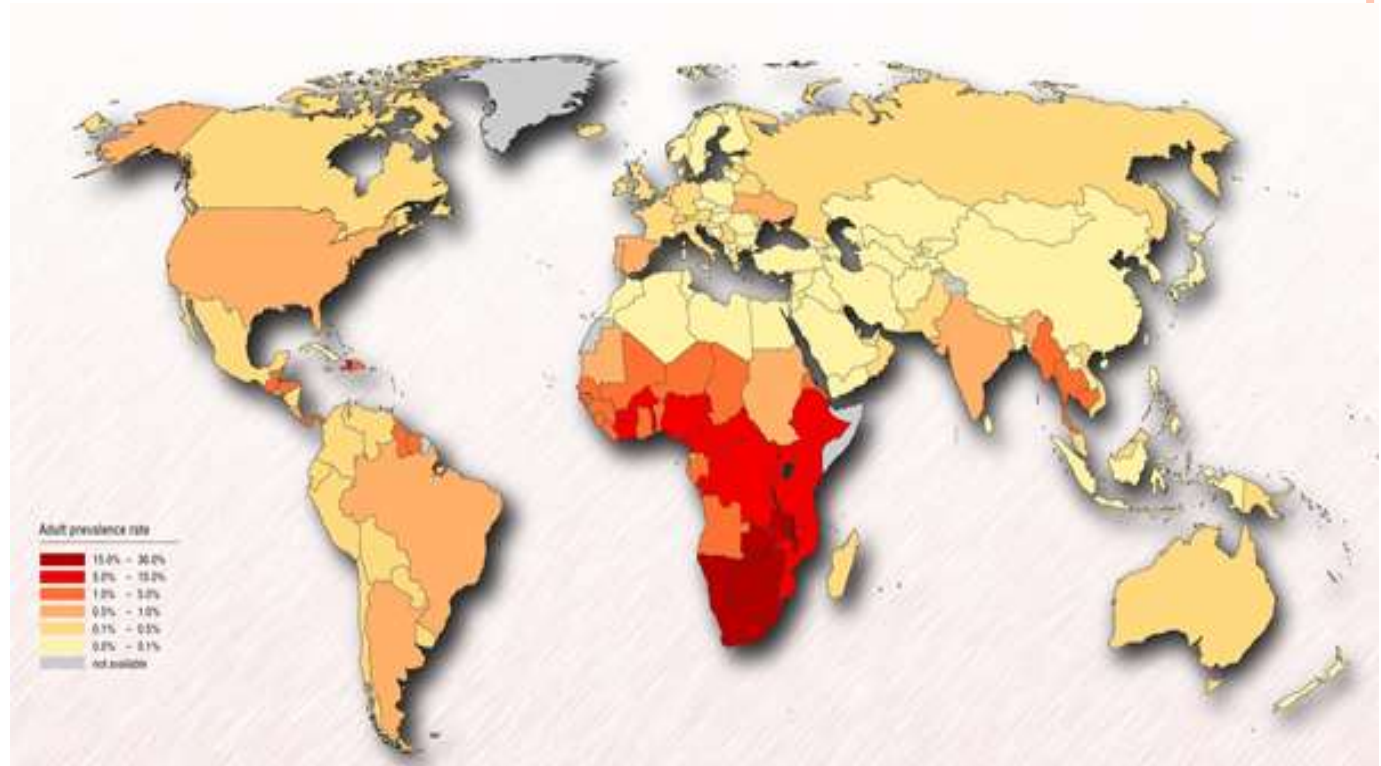


TOP 10 CAUSES OF DEATH IN THE WORLD

	Deaths in millions	% of deaths
Coronary heart disease	7.20	12.2
Stroke	5.71	9.7
Lower RTI	4.18	7.1
COPD	3.02	5.1
Diarrheal diseases	2.16	3.7
HIV/AIDS	2.04	3.5
TB	1.46	2.5
Lung cancer	1.32	2.3
Road accidents	1.27	2.2
Prematurity	1.18	2.0
		Source : WHO

HIV INFECTION-MEN TO WOMEN RATIOS

- US
 - 14 : 1
- Haiti
 - 3 : 1
- Africa
 - 1 : 1



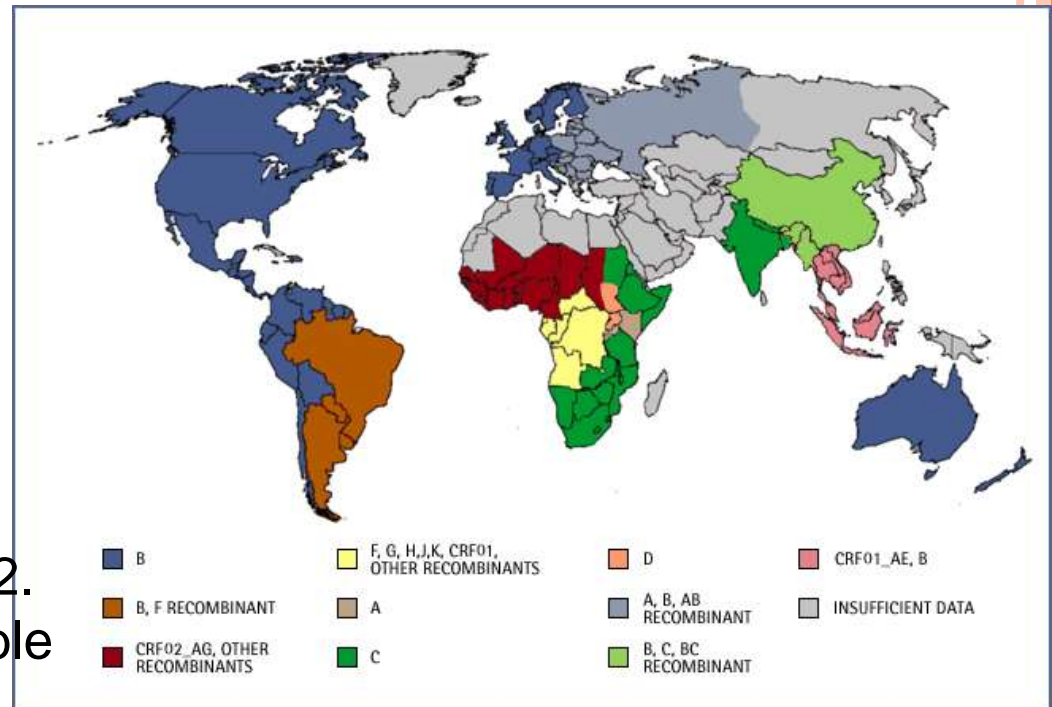
HIV GENOTYPES

○ HIV-1

- Group M
 - Subtypes or clades A-K
- Group N (West Africa)
- Group O (West Africa)
- Group P (newly identified)

○ HIV-2

- ELISA tests identify HIV 1 and 2.
- Earlier diagnostic tests had trouble identifying Groups N and O.



- Pathways to drug resistance can be different among subtypes
- HIV-2 runs a chronic and less pathogenic course and is intrinsically resistant to NNRTIs



HIV-1 VARIATION

- **HIV-1 is the most genetically variable of all human pathogens:**
 - Rapid replication rate ($\sim 10^{10}$ virions/day)
 - High mutation rate (~ 1 substitution/genome/round of replication)
 - Recombination events ($\sim 7-30$ crossovers/genome/round of replication)
- HIV-1 in the infected individual exists as a swarm of highly related but non-identical viral genomes termed “quasispecies”
- Genetic variability leads to increasingly more complex challenges for:
 - Therapeutic strategies (potentially more and unique resistance patterns)
 - Vaccine development (specific vaccine preparations for specific viral subtypes)



HIV TRANSMISSION FACTORS

- AIDS (the more advanced disease, greater chance of transmission)
- Active STD
- Presence of Genital lesions
- Frequency and type of unprotected sex
- Circumcision
- **Viral load**



VIRAL TRANSMISSION AND DISSEMINATION

- Viral transmission: exchange of body fluids, blood, semen, vaginal secretions, breast milk
- HIV-1 enters the host within cells (CD4+ lymphocytes/macrophages) or as cell-free viral particles
- Enters through skin, mucosal surfaces of genital tract



HOW IS HIV TRANSMITTED?

Infectious bodily fluids

- Blood (including menstrual blood)
- Semen
- Vaginal secretions
- Breast milk

- Sexual routes of transmission
 - vaginal sex
 - anal sex
 - oral sex (very low risk)
- Non-sexual routes of transmission
 - Sharing needles
 - Needle sticks
 - Hemophilia and other blood products
 - Artificial insemination
- Mother to child transmission



TRANSMISSION IN THE HEALTH CARE SETTING

- Pooled analysis of prospective studies of health care workers with occupational exposure suggests risk is approximately 0.3% (95% CI, 0.2% - 0.5%)¹
- Presence or absence of key risk factors influences risk
- Transmission from healthcare workers to patients extremely rare

1. Bell DM. Am J Med 1997;102(suppl 5B):9-15.



ESTIMATED PER-ACT RISK FOR ACQUISITION OF HIV BY EXPOSURE ROUTE

Exposure Route	Risk per 10,000 exposures
Needle-sharing injection drug use	67
Receptive anal intercourse	50
Percutaneous needle stick	30
Receptive penile-vaginal intercourse	10
Insertive anal intercourse	6.5
Insertive penile-vaginal intercourse	10
Receptive oral intercourse	1
Insertive oral intercourse	0.5



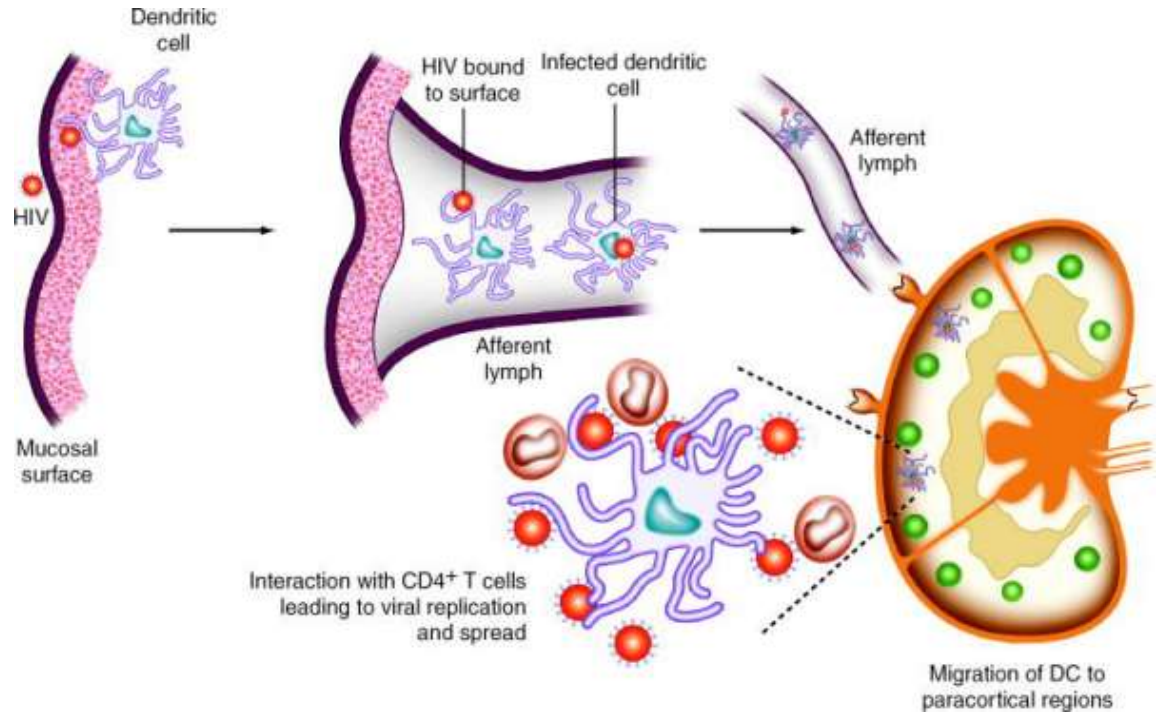
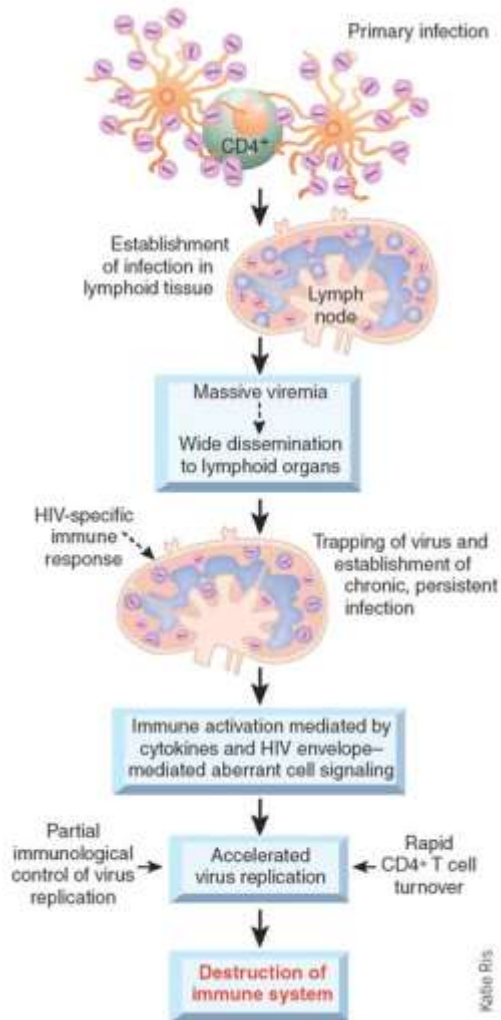
CONDOMS



- Condoms are highly protective (preventable fraction is about 80%-90%)
- Female condoms probably also effective, but data limited



Initial infection and dissemination of HIV infection.



Dendritic cells (DCs) at the site of exposure transport HIV to the paracortical regions of draining lymphoid tissues, leading to infection of CD4⁺ T cells and dissemination of HIV infection. (from Weissman D, Fauci AS. *Clin Microbiol Rev.* 1997;10:358.)

WHY AIDS DOES NOT FIT THE PARADIGM FOR CLASSIC VACCINE DEVELOPMENT

- Many vaccines mimic natural immunity
 - No recovered AIDS patients
- Most vaccines prevent disease, not infection
 - HIV infection remains latent for a long time
- Most vaccines protect for many years for organisms that change very little over time
 - HIV mutation rate is very high
- Most vaccines are either live attenuated or whole killed organisms
 - HIV does not retain antigenicity when killed, use of live attenuated virus raises a lot of safety concerns
- Most vaccines prevent against infections that are rarely encountered
 - HIV may be encountered daily by high risk groups
- Most vaccines protect through the mucosal surfaces of the GI and respiratory tracts
 - HIV infection is through the genital tract
- Many vaccines have been tested for safety and efficacy in animal models
 - There is no suitable animal model for HIV/AIDS

Obstacles to the development of HIV vaccine

- Antigenic diversity and hyper-variability of the virus
- Transmission of disease by mucosal route
- Integration of the viral genome into the host cell chromosome
- Transmission of the virus by the infected cell
- Latency of the viral infection
- Sequestration of the virus in the CNS
- Progressive dysfunction and destruction of the immune system of the host
- No spontaneous recovery from natural infection in spite of high level immune responses of the host



HIV VACCINE STRATEGIES

- Preventative Vaccine
- Therapeutic Vaccine
- Immune Responses
 - Antibody responses
 - Cell-mediated responses
 - Both antibody and cell-mediated responses



IDEAL HIV VACCINE

- Efficacy in mucosal parenteral route
- Excellent safety profile
- Single dose administration
- Long lived protection after vaccination
- Low cost
- Stable under field conditions (no cold chain)
- Ease of transportation and administration
- Ability to protect against diverse viral isolates



Vaccine Strategies Under Study

Vaccine constituents	Status	Advantages	Disadvantages
Vaccines eliciting anti-HIV antibodies			
Viral surface proteins, such as gp120	In phase I and II trials, which examine safety	Safe and simple to prepare	Vaccine-elicited antibodies have failed to recognize HIV from patients
Whole, killed HIV	Not under study in humans	Should present HIV surface proteins in a relatively natural conformation; simple to prepare	Slight risk that preparations might include some active virus; inactivated virus might shed its proteins and become ineffective
Pseudovirions (artificial viruses containing HIV surface proteins)	Close to phase I trials	Present HIV surface proteins in a relatively natural conformation	Difficult to produce and to ensure long-term stability



Vaccine Strategies Under Study

Vaccine constituents	Status	Advantages	Disadvantages
----------------------	--------	------------	---------------

Vaccines eliciting cellular responses			
Live vector viruses (non-HIV viruses engineered to carry genes encoding HIV proteins)	In phase II trials	Makers can control amount and kinds of viral proteins produced	Complicated to prepare; current vaccines elicit modest immune response
Naked DNA containing one or more HIV genes	In phase I trials	Simple and inexpensive to prepare	Some worry that integration of HIV genes into human cells could harm patients
HIV peptides (protein fragments)	In phase I trials	Simple to prepare	Do not elicit strong immune response

Vaccine Strategies Under Study

Vaccine constituents	Status	Advantages	Disadvantages
----------------------	--------	------------	---------------

Vaccines eliciting antibody and cellular responses			
Combinations of elements, such as pure gp120 protein plus canarypox vector	In phase II trials	Should stimulate both arms of the immune response at once	Complicated to prepare
Live, attenuated HIV	Not under study in humans; being assessed in nonhuman primates	Most closely mimics HIV; may interfere with ability of infectious HIV to replicate	Vaccine virus potentially cause AIDS



Types of Experimental HIV Vaccines:

- **Peptide vaccine:** made of tiny pieces of proteins from the HIV virus.
- **Recombinant subunit protein vaccine:** made of bigger pieces of proteins that are on the surface of the HIV virus. Examples of a recombinant subunit protein are gp120, gp140, or gp160 produced by genetic engineering.
- **Live vector vaccine:** non-HIV viruses engineered to carry genes encoding HIV proteins. The genes are inserted into another vector, which carries them into the body's cells. The genes in turn produce proteins that are normally found on the surface of the HIV virus. This type of vaccine most resembles the HIV virus but is not harmful. Many vaccines used today, like the smallpox vaccine, use this approach.
- **DNA vaccine:** uses copies of a small number of HIV genes which are inserted into pieces of DNA called plasmids. The HIV genes will produce proteins very similar to the ones from real HIV.
- **Vaccine combination:** uses any two vaccines, one after another, to create a stronger immune response. Often referred to as "prime-boost strategy."
- **Virus-like particle vaccine (pseudovirion vaccine):** a non-infectious HIV look-alike that has one or more, but not all, HIV proteins.



Novel designs for an HIV-1 vaccine

Design	Limitations
Plasmid DNA	Limited immunogenicity in humans
Live, recombinant vectors:	
Pox viruses	
Vaccinia	Dissemination in immunosuppressed vaccinees
MVA, NYVAC	Limited experience in humans
Canary pox	Limited immunogenicity in humans at achievable dosages
Gene-deleted adenovirus	Pre-existing immunity to adenovirus may limit immunogenicity
alphaviruses, adeno-associated virus	Limited experience in humans
Envelope subunit immunogens	No elicitation of neutralizing antibodies



HIV vaccine candidates in clinical trials

Product	Company	HIV subtype	Phase
Peptide			
C4-V3 peptide	Wyeth	B	1
V3 peptide	CIBG	B	1
p17	Cel-Sci	B	1
Recombinant subunit			
Bivalent rgp 120	VaxGen	B+E	3
Bivalent rgp 120	VaxGen	B	3
Oligomeric gp 140	Aventis	E	1
rgp 120	Chiron	E	1
p24	Chiron	B	1
DNA			
gag	Merck	B	1
env-rev	Wyeth	B	1
Live viral vector			
Vaccinia - env-gag-pol	Therion	B	1
Canarypox - env-gag-pr	Aventis	B	1
Live bacterial vector			
Salmonella -env	Univ Md.	B	1
Combinations (Vector prime + subunit boost)			
Canarypox - env-gag-pr, nef, pol	Aventis	E	2
• rgp 120	Chiron	E	
• rgp 120	Vaxgen	B+E	
Canarypox - env-gag-pr	Aventis	B	2
Salmonella - env	Univ. Med	B	1
(DNA prime+vector boost)			
DNA-gag, pol	Wyeth	B	1
• Canarypox-env, Gag-pr	Aventis	B	1
DNA-env-rev	Wyeth	B	1

HIV vaccine candidates in clinical trials Developing World

Product	Company	HIV subtype	Phase	Site
Peptide				
V3 peptide	CIBG	B	1	Cuba
Recombinant subunit				
Bivalent rpg 120	VaxGen	B+E	3	Thailand
Live viral vector				
Canary pox - env-gag-pr	Aventis	B	1	Uganda
Live bacterial vector				
Salmonella - env	Univ. Md.	B	1	
Combinations (Vector prime + subunit boost)				
Canary pox - env-gag-pr, • Nef, pol rpg 120	Aventis	E	2	Thailand
• rgp 120	Chiron	E		
• rgp 120	VaxGen	B+E		
Canary pox - env gag-pr	Aventis	B	2	Haiti, Trinidad, Brazil

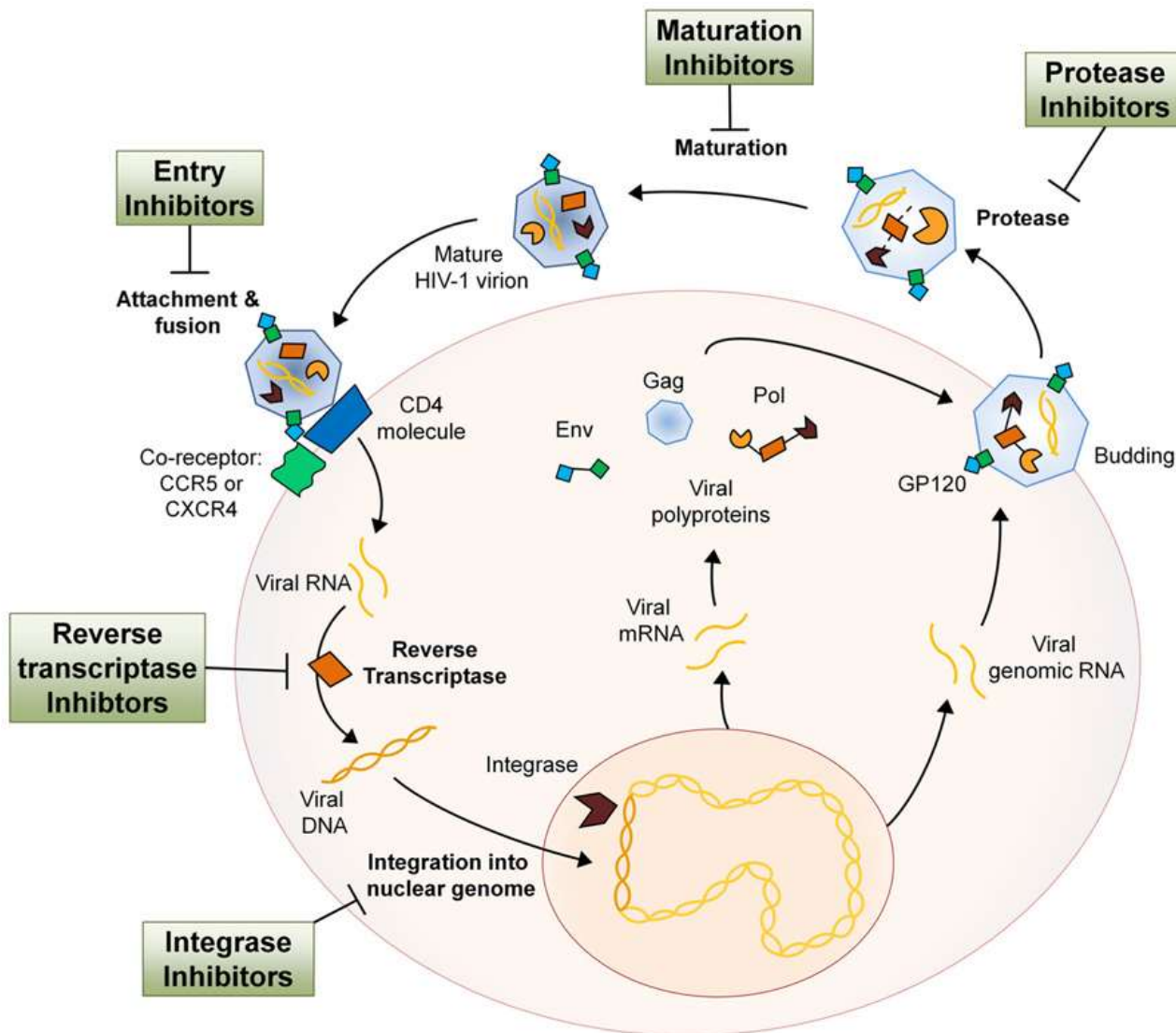


HIV vaccines currently in clinical trials (2002-03)

Vaccine Sponsor	Trial ID	Vaccine type	Description
Phase III			
VaxGen	Vax 004	AIDSVAX B/B	Gp 120 protein vaccine, clade B, being tested in Thailand
Phase II			
NIAID/Aventhis/VaxGen	HVTN 203	ALVAC vCP 1452	Canarypox vaccine (expressing <i>gag</i> , <i>env</i> and <i>pol</i>) with or without AIDSVAX B/B gp 120 boost)
Phase I/II			
NIAID/Aventhis/VaxGen	PACTG 326/ACTG 326	ALVAC vCP 1452	Canarypox vaccine with or without gp 120 boost in babies of HIV positive mothers
Phase I			
ANRS/AVANTIS	VAC 12	LP HIV 1	Lipopeptide (LIPO-4T) express in <i>gag</i> , <i>pol</i> and <i>nef</i> -also includes a CD4 epitope
ANRS/AVANTIS	VAC 10	ALVAC-vCP 1452 +/- lipopeptide	Canarypox and lipopeptides (LIPO-5T, LIPO-6T) separately and in combination
FIT Biotech	NA*	GTU-Nef DNA	HIV-1 <i>nef</i> gene expressed in DNA plasmid vaccine
IAVI/KAVI/DT	IAVI 004	MVA-HIVA	Recombinant vaccinia viral vector, clade A, single dose Kenya
IAVI/MRC	IAVI 005	MVA-HIVA	Recombinant, vaccinia viral boost to volunteers previously vaccinated with DNA prime in IAVI 001
IAVI/MRC/Cobra	IAVI 001	P Thr-HIVA	HIV-1 clade A, DNA vaccine, dose response United Kingdom
IAVI/MRC/IDT	IAVI 003	MVA, HIVA	Single dose of recombinant vaccinia viral vector. Clade A- United Kingdom
KAVI/IAVI Cobra	IAVI 002	P Thr-HIVA	HIV-1 clade A, DNA vaccine, dose response Kenya
Merck	NA	DNA	Dose response study of <i>gag</i> DNA, clade B
Merck	NA	MRKAd 5	None applying adenovirus vector, clade B, expressing <i>gag</i>
NIAID/Aventis	HVTN 093	ALVAC vCP 1452	Dose response study of canarypox vector, clade B
NIAID/Aventis/VaxGen	AVEG 038	ALVAC vCP 205	Canarypox vaccine boost (expressing <i>gag</i> , <i>pol</i> <i>env</i>) in volunteers who already received pox virus based vaccine
NIAID/Glaxo Smith Kline	AVEG 027	ALVAC vCP 205	Canarypox vaccine with or without VaxGen's gp 120, delivery method also evaluated
NIAID/Glaxo Smith Kline	HVTN 041	Nef-Tat-gp 120 +	Protein vaccine with ASO2A adjuvant (clade B)
WRAIR/Aventis	RV 138	ALVAC vCP 205	Canarypox vaccine delivered either in skin, muscle or dendritic cells in HIV-negative volunteers



STRATEGIES FOR DEVELOPMENT OF ANTIVIRALS

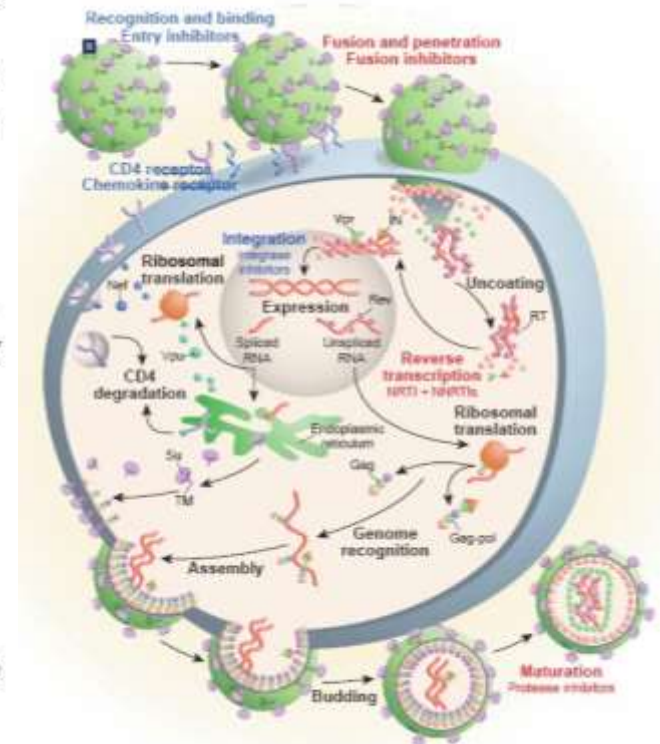


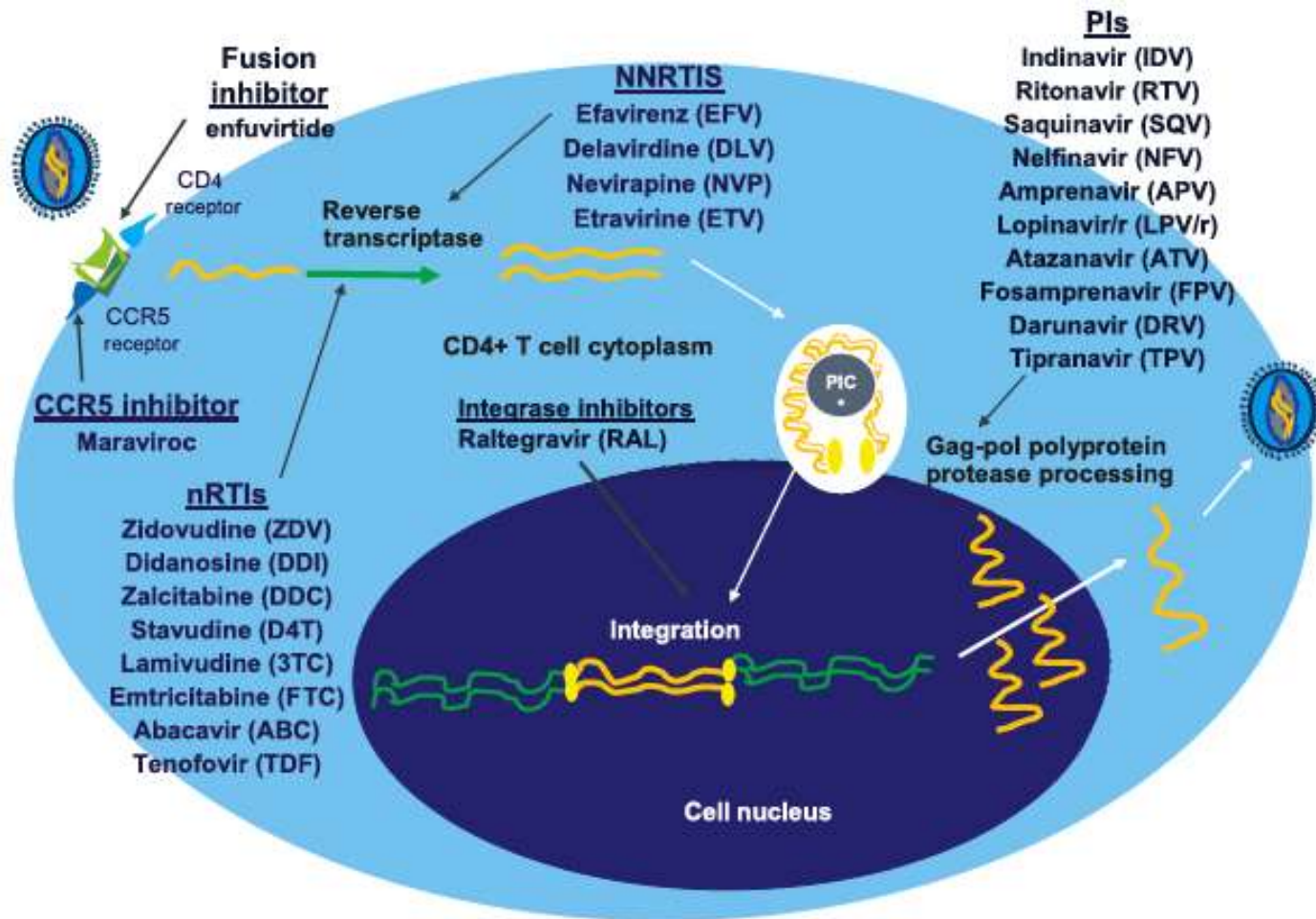
1. Attachment
2. Transcription
3. Regulation of transcription
4. Translation of viral mRNA
5. Posttranslational cleavage of viral proteins
6. Integration of viral DNA
7. Replication of proviral DNA
8. Assembly and maturation of virions
9. Virion release



Table 2 FDA-approved antiretroviral drugs available in the United States⁶⁵

Class	Generic name	Trade name	Date of FDA approval letter
Fusion inhibitors	Enfuvirtide (T-20)	Fuzeon	13 March 2003
NNRTIs	Delavirdine mesylate	Rescriptor	4 April 1997
	Efavirenz	Sustiva	17 September 1998
	Nevirapine	Viramune	21 June 1996
NRTIs	Abacavir sulfate	Ziagen	17 December 1998
	Abacavir sulfate + lamivudine + zidovudine	Trizivir	14 November 2000
	Didanosine (ddI)	Videx	9 October 1991
	Didanosine (ddI)	Videx EC	31 October 2000
	Lamivudine (3TC)	Epivir	17 November 1995
	Lamivudine + zidovudine	Combivir	26 September 1997
	Stavudine (d4T)	Zerit	24 June 1994
	Tenofovir disoproxil fumarate	Viread	26 October 2001
	Zalcitabine (ddC)	Hivid	19 June 1992
	Zidovudine (AZT, ZDV)	Retrovir	19 March 1987
Protease inhibitors	Amprenavir	Agenerase	15 April 1999
	Indinavir sulfate	Crixivan	13 March 1996
	Lopinavir + ritonavir	Kaletra	15 September 2000
	Nelfinavir	Viracept	14 March 1997
	Ritonavir	Norvir	1 March 1996
	Saquinavir	Fortovase	7 November 1997
	Saquinavir mesylate	Invirase	6 December 1995





ANTIRETROVIRAL CLASSES/DRUGS AND THEIR SITE OF ACTIVITY: NUCLEOSIDE AND NUCLEOTIDEREVERSE TRANSCRIPTASE INHIBITORS (NRTIs); NONNUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NNRTIs); PROTEASE INHIBITORS (PIs); FUSION INHIBITOR; CCR5 INHIBITOR; INTEGRASE INHIBITOR; *PRE-INTEGRATION COMPLEX (PIC) CLIN MICROBIOL INFECT 2009; 15 (SUPPL. 1): 69–73

AVAILABLE HIV ANTIRETROVIRAL AGENTS

○ Nucleoside reverse transcriptase inhibitors (NRTI)

- Abacavir/ABC
- Didanosine/ddI
- Lamivudine/3TC
- Emtricitabine/FTC
- Tenofovir/TDF
- Zidovudine/AZT
- *Stavudine/d4T
- *Zalcitabine

○ Non nucleoside reverse transcriptase inhibitors (NNRTI)

- Efavirenz/EFV
- Nevirapine/NVP
- Etravirine/ETR
- Rilpivirine/RPV
- *Delavirdine

○ Protease inhibitors

- Atazanavir/ATV
- Fosamprenavir/FPV
- Lopinavir/ritonavir LPV/r
- Nelfinavir/NFV
- Ritonavir (small r)
- *Indinavir/IDV
- Saquinavir/SQV
- Darunavir/DRV
- Tipranavir/TPV

○ Fusion inhibitors

- Enfuvirtide

○ CCR5 inhibitors

- Maraviroc/MVC

○ Integrase inhibitors

- Raltegravir/RAL
- **Elvitegravir/EVG**
- **Dolutegravir**



* Rarely used or discontinued due to adverse effects or pill burden



















Investigational drugs: New classes

Class		Drug	Phase	
Entry inhibitors	Attachment inhibitors	TNX-355 (anti-CD4 Ab)	II	
		BMS-488043	I	
	Coreceptor antagonists	CXCR4	AMD-070	I
			AL40-YV KRH-2931, -3945, -3140 T-22 TAK-779	Preclinical Preclinical Preclinical Preclinical
Coreceptor antagonists	CCR5	Maraviroc (UK-427,857)	III	
		PRO-140 AK602 TAK-220 TAK-652 SCH-D	II Preclinical Preclinical Preclinical On hold	
Integrase inhibitors		MK-0518 GS-9187 V-165 L-900564 Styrylquinolones	Phase III Phase II Preclinical Preclinical Preclinical	
Maturation inhibitors		PA-457 UK-201844	Phase II Preclinical	
Others		D-amino acid peptides RNase H inhibitors TAT-TAR antagonist Zinc finger inhibitors	Preclinical Preclinical Preclinical Preclinical	

COMBINATION ANTIRETROVIRAL THERAPY (cART)

- cART regiment generally consists of
 - Two NRTIs
 - One of the following
 - NNRTI
 - PI (boosted PI)
 - INSTI (Integrase inhibitor)
 - CCR5 antagonist (Fusion inhibitor)
- Selection should be individualized based on:
 - Virological and immunological efficacy
 - Toxicity (more especially reno-, hepato- and neurotoxicity)
 - Metabolic abnormalities (lipid, glucose etc metabolism)
 - Cardiovascular conditions
 - Pill burden
 - Dosing frequency
 - Drug interaction potential
 - Drug resistance
 - Comorbid condition



Drug	Class	Pill volume	Total pills per day	Dosing schedule	Combination product	Total pills per day	Dosing schedule	2006 sales [†]
Tenofovir	NRTI	300 mg		Once daily	  Truvada  Atripla		Once daily	1,125
Emtricitabine	NRTI	200 mg		Once daily			Once daily	174
Efavirenz	NNRTI	600 mg		Once daily		-	-	-
Abacavir	NRTI	300 mg		Once daily	  Bicton  TriVir  Zidovir		Once daily	396
Lamivudine	NRTI	300 mg		Once daily			Twice daily	478
Zidovudine	NRTI	300 mg		Twice daily			Twice daily	789

*From REF. 2. [†]Sales in US\$ millions are based on MIDAS sales data, IMS Health April 2007. NNRTI, non-nucleoside transcriptase inhibitor; NRTI, nucleoside transcriptase inhibitor.



COMPLICATIONS OF HIV THERAPY

- **Lipodystrophy**
- bleeding
- Bone marrow suppression
- **Cardiovascular disease**
- CNS disease
- Diabetes/insulin resistance
- Dyslipidemia
- GI effects
- **Hepatic toxicity**
- Hypersensitive reactions
- Lactic acidosis
- Myopathy
- **Nephrotoxicity**
- **Peripheral neuropathy**
- Rashes



PREFERRED HIV TREATMENT REGIMENS

- **NNRTI Based regimen**

- **EFV/TDV/FTC**

TDF should be used with caution in patients with renal insufficiency

- **PI Based regimen**

- **ATV/TDF/FTC**

- **DRV/TDF/FTC**

DTG/ABC/3TC should be used in patients who are HLA B*5701 negative

- **INSTI-based regimen**

- **DTG/ABC/3TC**

- **DTG/TDF/FTC**

Ref: DHHS Guidelines,
2013



ALTERNATIVE HIV TREATMENT REGIMENS

○ NNRTI Based regimen

- EFV/ABC/3TC
- RPV/TDF/FTC
- RPV/ABC/3TC

○ PI Based regimen

- ATV/ABC/3TC
- DRV/ABC/3TC
- FPV/ABC/3TC
- LPV/ABC/3TC
- LPV/TDV/FTC

○ INSTI-based regimen

- RAL/ABC/3TC

TDF should be used with caution in patients with renal insufficiency

DTG/ABC/3TC should be used in patients who are HLA B*5701 negative

Ref: DHHS Guidelines,
2013



COMMON HIV CO-MORBID CONDITIONS

- Haematological complications
 - Thrombocytopenia
 - Anaemia
 - Neutropenia
 - Eosinophilia
- Oncological complications
 - Kaposi's sarcoma
 - Non-hodgkin's lymphoma
 - Primary CNS lymphoma
 - Anal cancer
- Endocrine complications
 - Disorders of adrenal function
 - Hypogonadism
 - Thyroid disease
 - Pancreatitis
 - Hyperglycemia
 - Hypoglycemia
 - Ovarian complications
 - Bone disease
- Gastrointestinal complications
 - Nausea and vomiting
 - Anorexia
 - Diarrhea
 - Oral ulcers
- Renal complications
 - Nephropathy (HIVAN)
- Cardiac complications
 - Cardiomyopathy
 - Pericarditis/effusion
 - Endocarditis
- Pulmonary Complications
 - Pulmonary hypertension
 - Pneumonitis



COMMON HIV CO-MORBID CONDITIONS

- Musculoskeletal complications
 - Arthropathy
 - Myopathy
- Neurological complications
 - Distal sensory neuropathy
 - HIV associated dementia
- Psychiatric complications
 - Depression
 - Mania
 - Insomnia
- Dermatological complications
 - Skin infections
 - Viral
 - Bacteria;
 - Fungal



CONDITIONS THAT DEFINE AIDS: INFECTIONS

■ Bacterial infections

- Disseminated MAC
- Pulmonary or EP Tuberculosis
- Recurrent bacterial pneumonia
- Salmonella sepsis

■ Fungal infections

- Candidiasis (esophageal, airway)
- EP coccidiomycosis
- EP cryptococcosis
- EP histoplasmosis
- Pneumocysts pneumonia

○ Parasitic infections

- Cryptosporidiasis chronic diarrhea
- Isosporiasis chronic diarrhea
- Toxoplamosis

○ Viral infections

- CMV (no liver, spleen or lymph nodes)
- Herpes simplex (chronic ulcer, esophagus, airway)



OPPORTUNISTIC INFECTIONS IN HIV

- Progress has been made in improving the quality and duration of life for HIV infected persons
- HAART has restored immune function and chemoprophylaxis for OIs need not be lifelong.
- Stopping primary or secondary prophylaxis for certain pathogens was safe if HAART has led to an increase in CD4+ T lymphocyte counts above specified threshold levels.



Clinical Guidelines Portal



Home > Guidelines > Adult and Adolescent OI Prevention and Treatment Guidelines

Text Size

Collapse All | Expand All

- What's New
- Introduction
- PCP
- Toxo
- Cryptosporidia
- Microsporidia
- TB
- MAC
- Bacterial Respiratory
- Bacterial Enteric
- Bartonella
- Syphilis
- Candida
- ▶ Endemic Mycoses
- CMV
- ▶ Non-CMV Herpes
- HPV
- HBV
- HCV
- PML/JCV
- ▶ Geographic OIs
- ▶ Tables
- Figure: Immunization
- Appendix A: Preventing Exposure
- Appendix B: Abbreviations
- ▶ Appendix C: Panel Roster

Guidelines for the Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents

What's New in the Guidelines

(Last updated: April 16, 2015; last reviewed: April 16, 2015)

- ▶ Section Only PDF (114 KB)
- ▶ Full Guideline PDF (2.5 MB)
- ▶ Tables Only PDF (864KB)

Updates to the Guidelines for the Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents

The Guidelines for the Prevention and Treatment of Opportunistic Infections in HIV Infected Adults and Adolescents document was published in an electronic format that could be easily updated as relevant changes in prevention and treatment recommendations occur.

The editors and subject matter experts are committed to timely changes in this document because so many health care providers, patients, and policy experts rely on this source for vital clinical information. All changes are developed by the subject matter groups listed in the document (changes in group composition are also promptly posted). These changes are reviewed by the editors and by relevant outside reviewers before the document is altered. Major revisions within the last 6 months are as follows:

April 16, 2015:

1. **Hepatitis B Virus:** There is new information on techniques to evaluate the stage of liver fibrosis. New data on HBV immunization regimens is included in recommendations regarding the choice of the optimal immunization regimen. There are also new recommendations regarding the duration of anti-HBV therapy following initiation of ART.



TUBERCULOSIS

- Tuberculosis is the leading cause of death in the world
 - Kills 3million people annually
 - 18% of adults between 15-59yrs of age
 - 1/3 to ½ of world population is infected with *Mycobacterium tuberculosis*
- Immunopathogenesis: CD4+Th-1 cells secrete cytokines (IFN- γ) which recruit and activate macrophages
 - Formation of granulomatous tubercles
 - Production of lytic enzymes and inflammatory mediators
 - Collateral damage of surrounding tissue
 - Development of necrosis and calcification of lesions (Ghon complexes on X-ray)
 - Ability of bacilli to survive and grow in macrophages (immune evasion)

Pathogenic Mycobacteria

- ◆ *M. tuberculosis* complex:
 - *M. tuberculosis*, *M. bovis*, *M. microti*, *M. africanum*
 - pulmonary and extrapulmonary tuberculosis
- ◆ Non-tuberculous mycobacteria:
 - *M. avium-intracellulare*, *M. kansasii*, *M. scrofulaceum*, *M. fortuitum*, *M. ulcerans*, *M. marinum*
 - pulmonary and extra-pulmonary, some disseminated
 - also exist in free-living state
- ◆ *M. leprae*
 - skin, peripheral nerves, mucous membranes

M. tuberculosis Infection

◆ Transmission

- Most common: inhalation of droplet nuclei
 - ◆ < 10 organisms can produce disease
- Occasionally transmitted via GI tract or skin

◆ Probability of infection related to:

- Number of organisms in sputum of infected individual
- Frequency & efficiency of coughs
- Closeness of contact
- Adequacy of ventilation in contact area

M. tuberculosis Infection

- ◆ Risk factors for developing tuberculosis
 - Living in or originating from a developing country
 - Alcoholism
 - HIV Infection
 - Immunosuppression
 - Poverty and malnutrition
 - Close contact of patients with active infection
 - ◆ nursing homes, prisons, homeless shelters, hospitals

Hallmarks of disease caused by mycobacteria

- ◆ Disease develops slowly
- ◆ Disease follows a chronic course
- ◆ “Granulomatous” response
- ◆ High infectivity but low virulence for healthy individuals
- ◆ Immune response “double-edged sword”
 - Both immunity and pathology are the consequence of delayed type hypersensitivity/cell mediated immune responses to mycobacterial antigens

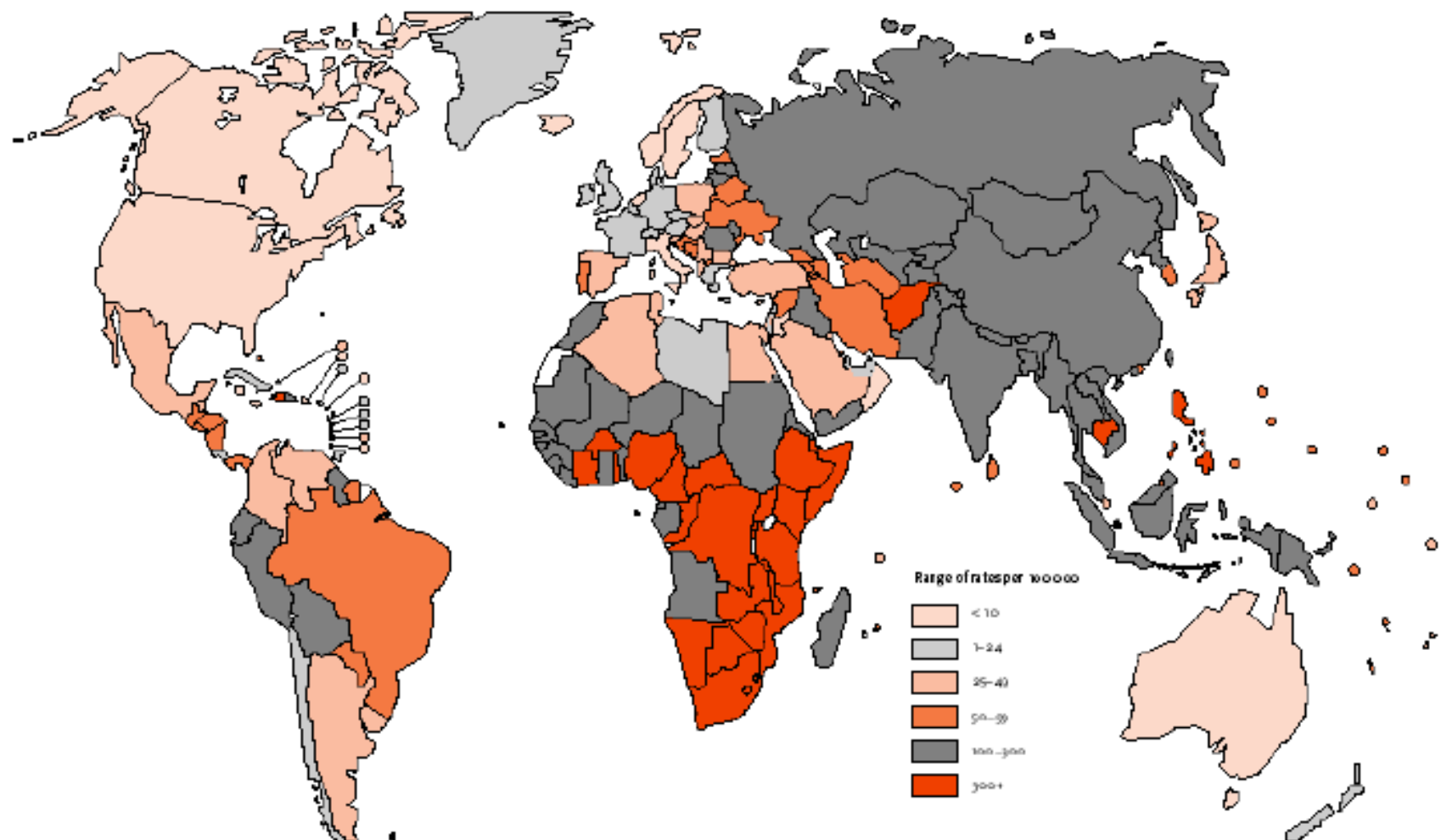
M. tuberculosis: Epidemiology

- ◆ “White Plague” epidemic
 - Industrial revolution, major period of urbanization (18th-19th centuries)
 - High mortality
 - ◆ 200-700 per 100,000 population per year
 - ◆ 20-30% of all deaths within urban centers
 - Predisposing sociological conditions
 - ◆ Poverty
 - ◆ Poor education, personal hygiene
 - ◆ Overcrowding
 - ◆ Social disruptions of public health programs due to war and economic depression

M. tuberculosis: Epidemiology

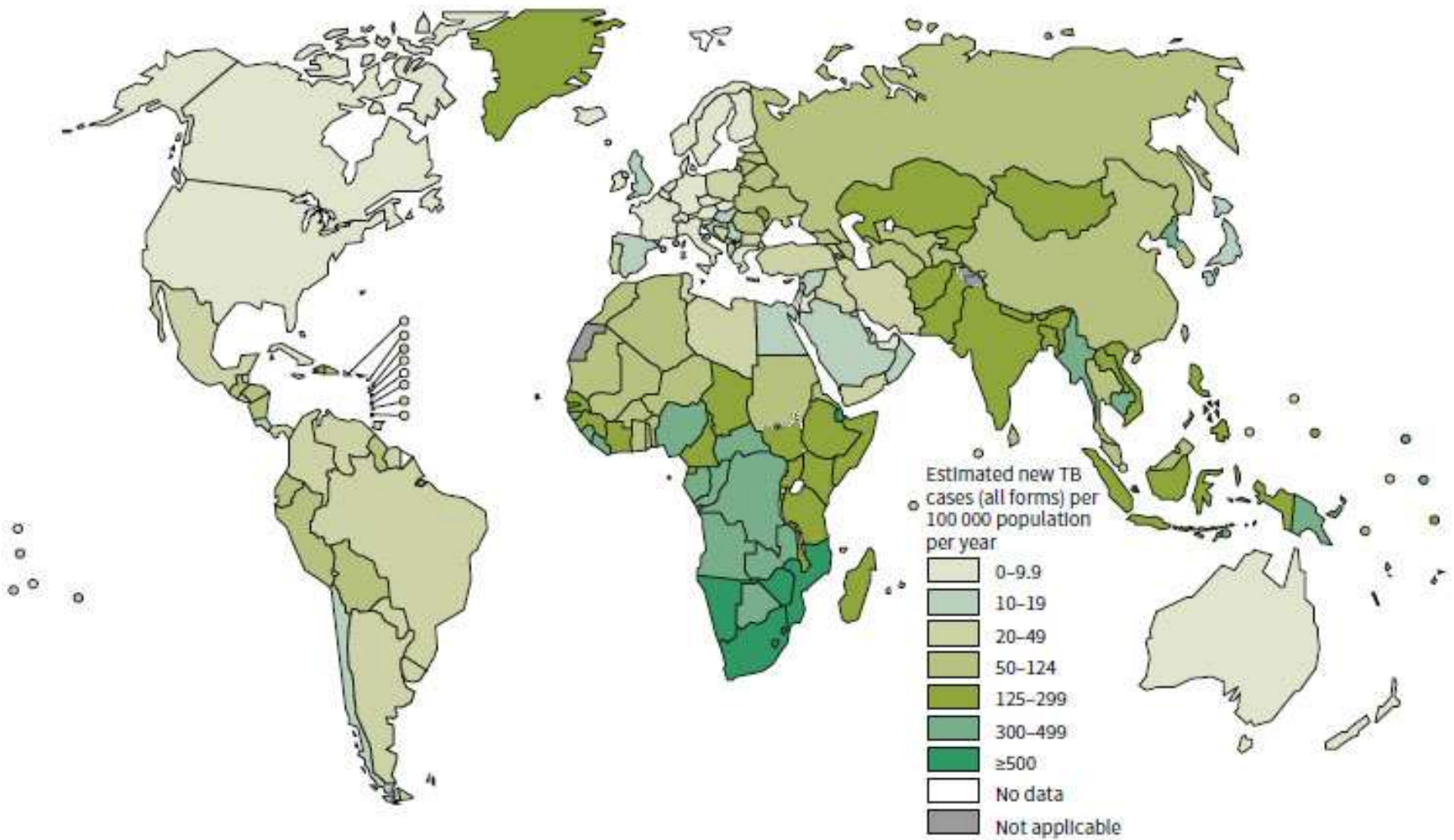
- ◆ Gradual decline during 20th century
 - Segregation of infected patients
 - TB testing of cows and milk pasteurization (*M. bovis*)
 - Primarily due to improved socioeconomic situation
 - ◆ Improved housing
 - ◆ Reduced crowding
 - ◆ Improved nutrition
 - ◆ Understanding of cause and transmission of disease
 - Decline **not** attributed to availability of chemotherapy and BCG vaccine

1. ESTIMATED TB INCIDENCE RATES, 2000



The data presented and the presentation of material on this map do not imply the approval of any opinion or behaviour on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dashed lines represent approximate boundaries for which there may not yet be full agreement.

Estimated TB incidence rates, 2013

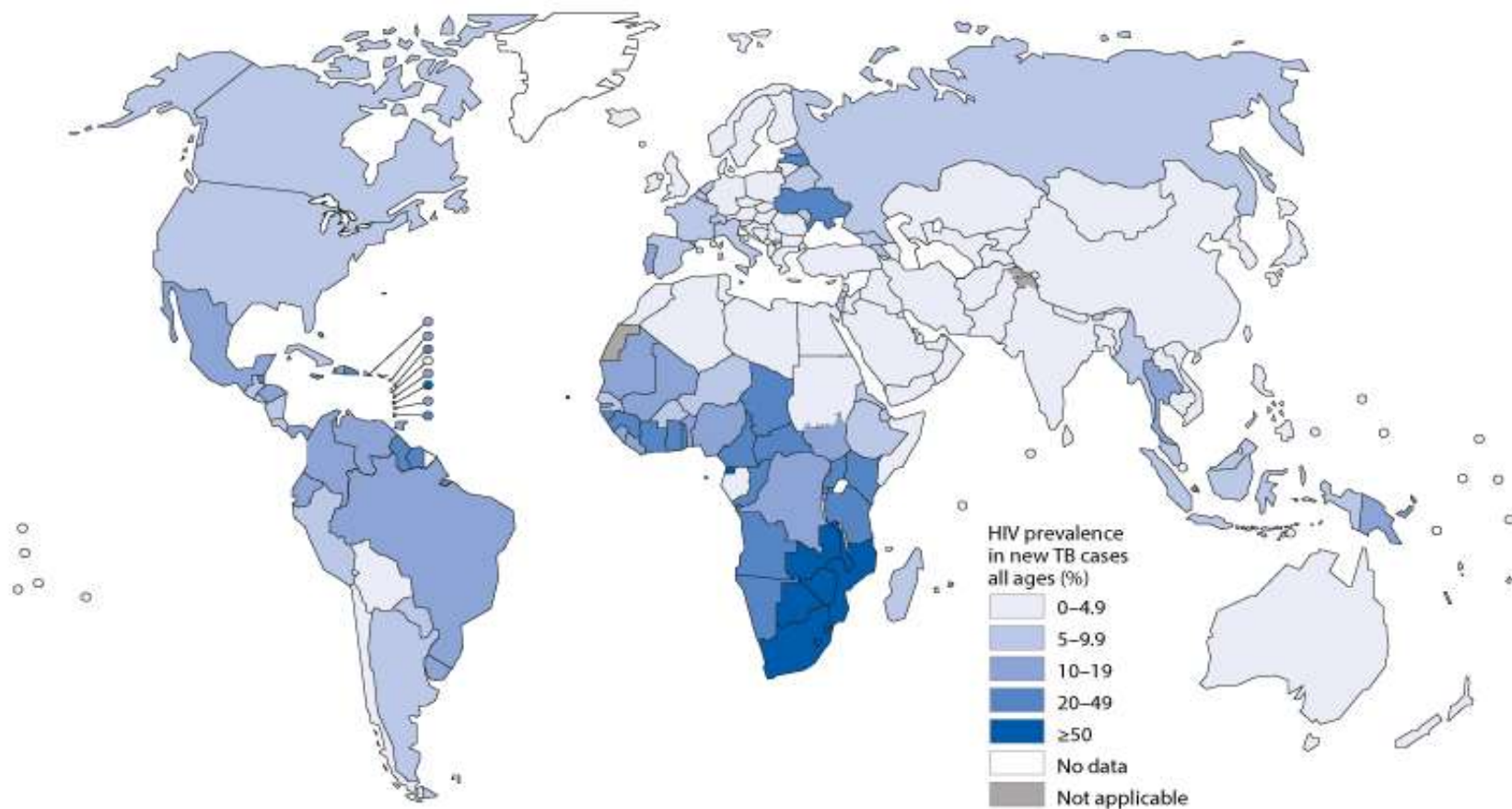


M. tuberculosis: Epidemiology

Global Impact

- ◆ *Mycobacterium tuberculosis*: leading cause of death due to infectious disease worldwide
 - approx. 1/3 - 1/2 of world's population infected with *M. tuberculosis*
 - ◆ 30 million with active disease
 - ◆ 8 million new cases per year
 - ◆ 3 million deaths per year
- ◆ Statistics may worsen in immediate future
 - Increased susceptibility to TB of AIDS patients
 - Development & spread of drug resistance
 - Decreased funding of control programs
 - Increase in incarcerated iv drug users & homeless population
 - Without new control measures, one billion new TB cases & 35 million deaths predicted between now and 2020

Estimated HIV prevalence in new and relapse TB cases, 2015



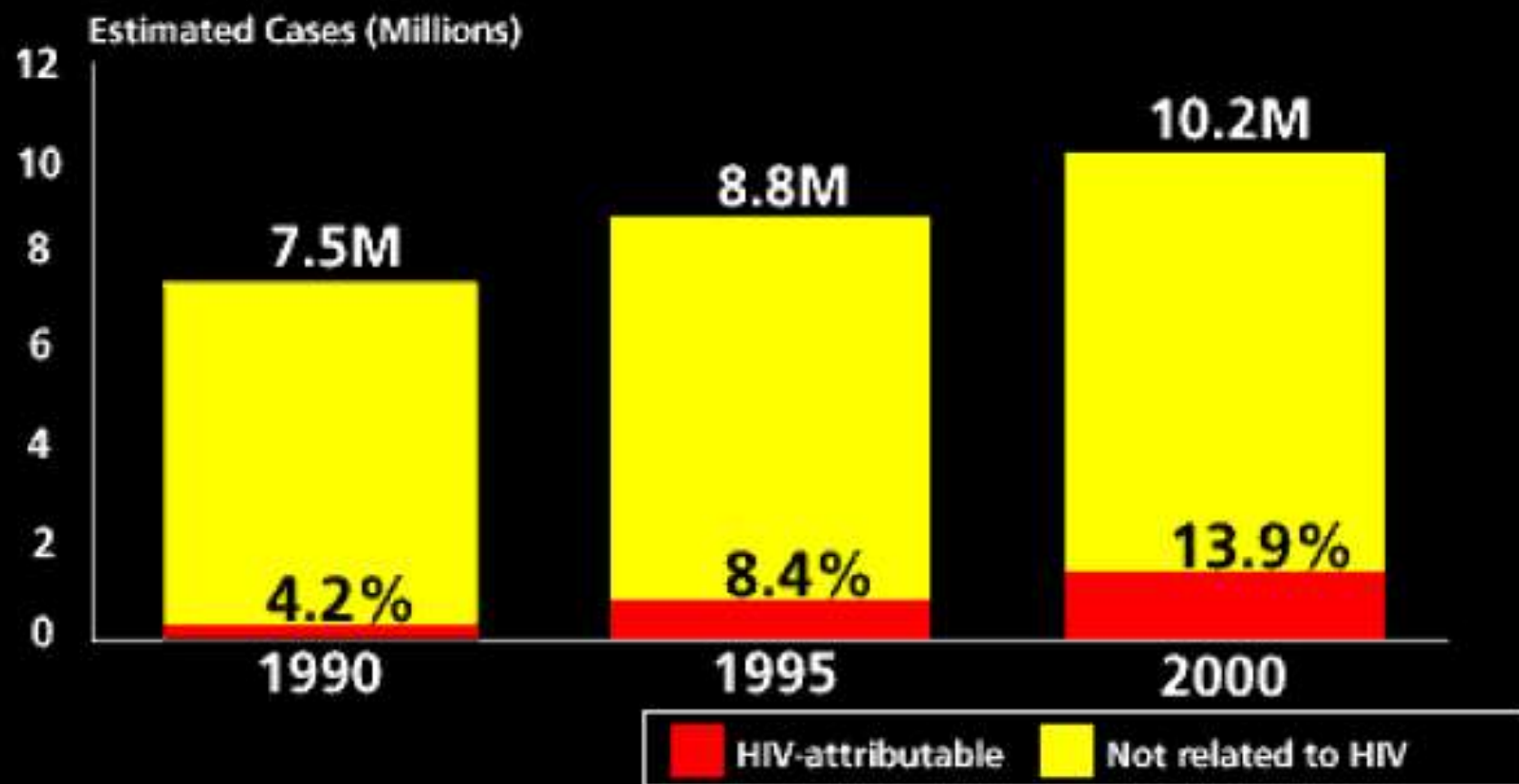
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Data Source: *Global Tuberculosis Report 2016*. WHO, 2016.

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TB Mortality and HIV Attributable TB

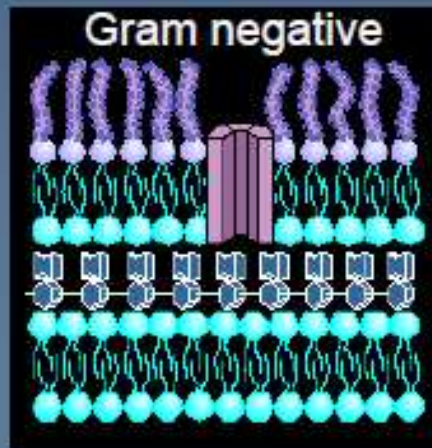
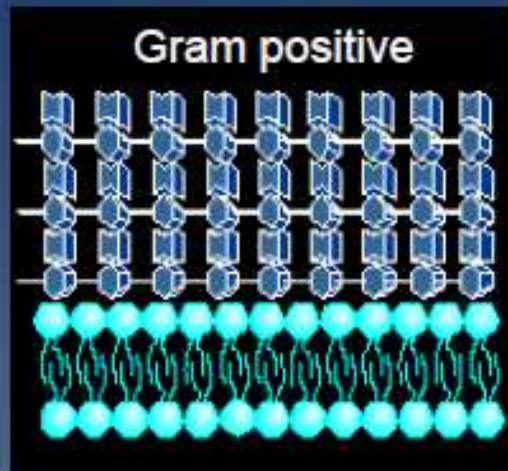


Properties of Mycobacteria

- ◆ Genera identified by acid-fast stain (AFB=Acid Fast Bacilli)
 - cell wall lipids make cell surface hydrophobic
 - resistant to staining with basic aniline dyes unless heat applied
 - once stained, resist decolorization with acid-ethanol
 - Ziehl-Neelsen stain allows visualization of mycobacteria



Mycobacterial cell wall



Lipid bilayer



Peptidoglycan



Lipid + LPS



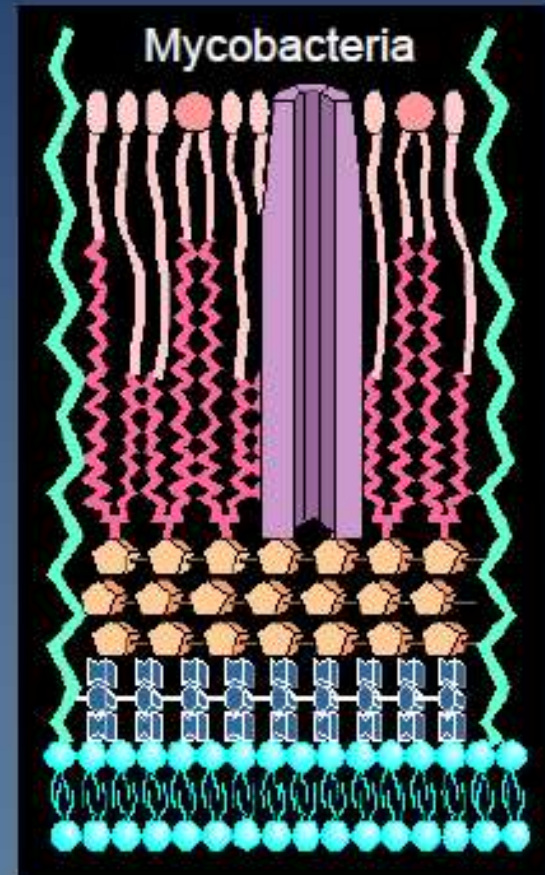
Mycolate



Acyl lipids



Arabinogalactan



LAM



Porins

M. tuberculosis: the organism

◆ Growth characteristics

- **Strict aerobes**, opt. growth 10% CO₂, pH 6.5-6.8
- Only *M. tuberculosis* produces niacin

◆ Cultural characteristics

- Slow-growing
 - ◆ **Mean generation time 12-24 hours (vs. *E. coli* 15-20 min)**
- Lowenstein-Jensen medium
 - ◆ selective medium: malachite green inhibits growth of non-mycobacterial contaminants
 - ◆ colonies appear in **3-6 weeks on solid media**
- **Liquid culture** in specialized media results in more rapid growth/detection (**5-12 d**)

M. tuberculosis: the organism

- ◆ Range of pathogenicity
 - Highly infectious, low virulence for healthy (immunocompetent) humans
 - Animal models:
 - ◆ Best animal model: guinea pigs
 - Highly virulent
 - Fatal in 6-12 wks
 - ◆ Mice
 - ◆ Monkeys in captivity (primate colonies)

M. tuberculosis: the organism

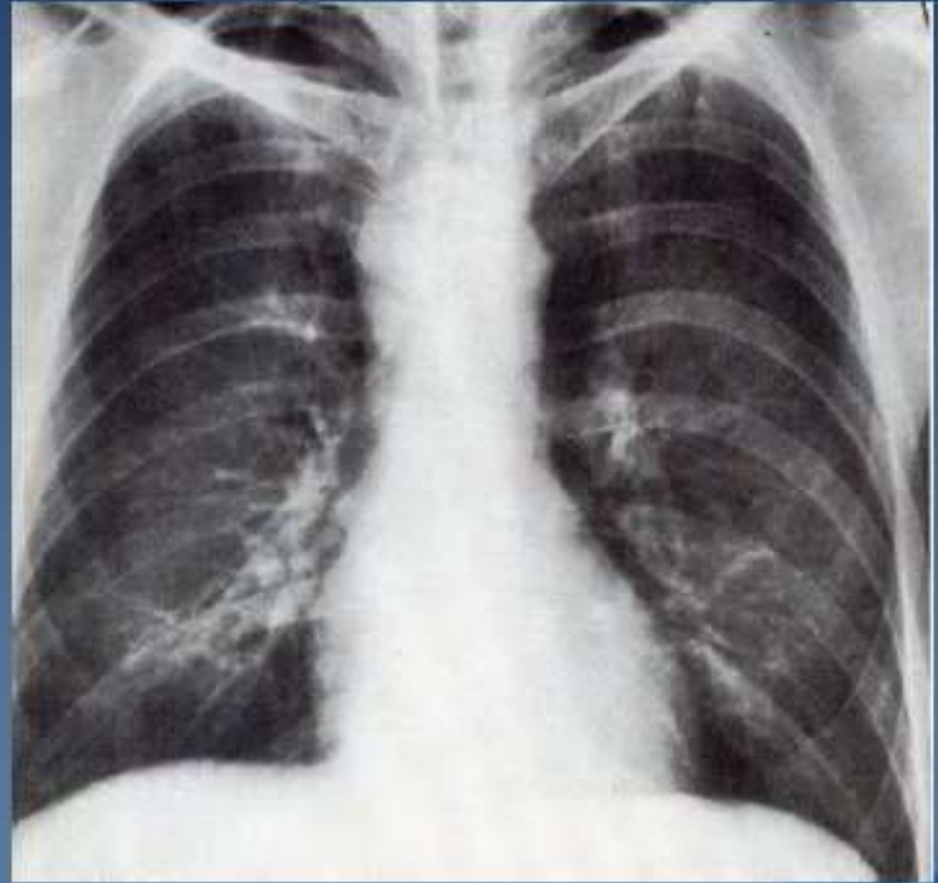
- ◆ Chemical/Environmental resistance
 - Resistant to drying
 - Resistant to most disinfectants (except formaldehyde and glutaraldehyde), acids, alkalis
 - Stability due to hydrophobic lipid surface
 - ◆ Also responsible for low permeability to many anti-microbial drugs (eg. β -lactams)
- ◆ Heat-sensitive
 - Killed in milk heated for 30 min @ 62 C (*M. bovis*)

Clinical Problem

- ◆ Three-year old Malia was seen by her GP with a 3-month history of malaise, reduced appetite and weight loss.
- ◆ Over the last month she had experienced episodes of fever and sweating at night and had developed a productive cough.
- ◆ Her mother was well but her father had a history of “chest trouble”.
- ◆ Relevant clinical manifestations
 - chronic productive cough
 - low-grade fever
 - night sweats
 - weight loss

Clinical Problem

- ◆ On examination, Malia was underweight for her age and coughed periodically.
- ◆ A respiratory examination revealed an area of dull percussion note, reduced air entry and wheezes over the right upper zone.
- ◆ A chest X-ray showed a middle & lower lung zone infiltrate with ipsilateral hilar adenopathy.



Clinical Problem

◆ Lab Results

- Haemoglobin
 - ◆ 11.2 g/dl (13.4-18.0)
- Zielhl-Neelsen stain of sputum smear
 - ◆ positive acid fast bacilli
- Sputum culture
 - ◆ positive for *M. tuberculosis*
- Intradermal PPD injection
 - ◆ Strong positive Mantoux test



Figure 3.2 Only the induration is being measured. This is **CORRECT**.

Diagnosis of Tuberculosis

◆ Chest radiography

– Primary disease

- ◆ single lung lesion in middle or lower right lobe
- ◆ enlargement of draining lymph nodes

– Reactivation disease

- ◆ single (cavitary) lesion in apical region
- ◆ multiple secondary tubercules
- ◆ unremarkable lymph nodes

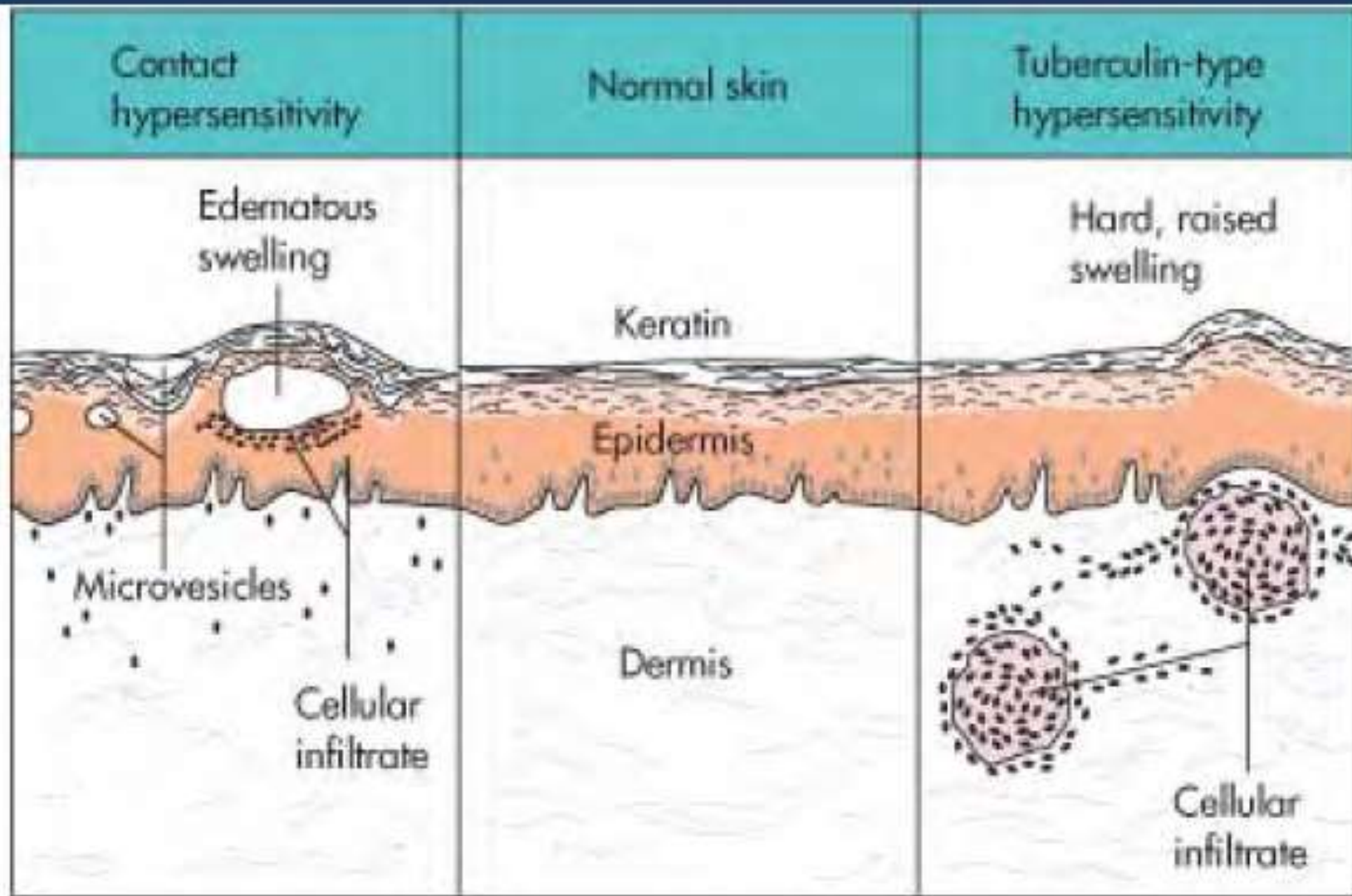
Diagnosis of Tuberculosis

◆ Tuberculin skin test

- Primarily used as a screening test for latent tuberculosis
- PPD - standardized preparation of *M. tuberculosis* culture filtrate proteins
- Mantoux test :
 - ◆ 1-5 TU administered intradermally
 - ◆ Skin reaction read after 48-72 h
 - ◆ Positive reaction: measured area of induration accompanied by erythema

◆ Tuberculin (PPD) skin test

- Useful to screen to exclude tuberculosis in countries with low level of disease, exposure (eg. Western countries such as US)
- *Not useful* in countries with high levels of tuberculosis and/or BCG vaccination



Guidelines for interpretation of Mantoux Test

Clinical Context	INDURATION			
	< 5 mm	5-10 mm	10-20 mm	>25 mm
Screening, non-endemic population	Negative	Suspicious of infection*	90% of patients have infection	Virtually all have infection
Screening, endemic population	Negative	Consistent with past infection*	Suspicious of infection	Suggestive of infection
Following BCG vaccination	Negative	Expected	Suspicious of infection	Suggestive of infection
Chest X-ray suggesting past infection	Negative	Consistent with past infection*	Suspicious of infection	Suggestive of infection
Suspicious clinical findings or contact with active tuberculosis	Negative	Suspicious of infection	90% of patients have infection	Virtually all have infection

*May also be due to cross-reactivity with other mycobacterial species.

Diagnosis of Tuberculosis

◆ Tuberculin (PPD) skin test

– Positive PPD test:

- ◆ exposure to *M. tuberculosis* or cross-reacting mycobacterium species

– Negative PPD test:

- ◆ no exposure to *M. tuberculosis*, or
- ◆ prehypersensitive stage of primary infection, or
- ◆ loss of reactivity with time (would be positive upon second skin test), or
- ◆ loss of reactivity due to severe disease, immunosuppression
 - severe disseminated tuberculosis (anergic)
 - other immunosuppressive diseases such as AIDS, measles

Summary

Diagnosis of Tuberculosis

- ◆ Clinical manifestations
- ◆ Tuberculin (PPD) skin test
- ◆ Abnormal chest radiograph
- ◆ Acid-fast bacilli in sputum or bronchoscopic specimens
- ◆ Culture of the organism from patient specimens
 - Identification of species
 - Drug sensitivity/resistance profile
- Lung biopsy and histological examination

Laboratory Diagnosis of Tuberculosis

- ◆ Confirmation and Species Identification
 - Isolation in pure culture
 - ◆ Solid and broth media
 - Selective medium containing antimicrobial agents (contaminated specimens)
 - ◆ Growth detected more rapidly (5-12 d vs. 3-4 w) using broth media & radio-labeled fatty acids
 - Cultural and biochemical tests for speciation
 - ◆ NAP differentiation test (p-nitro-a-acetyl-amino-hydroxypropiophenone)
 - *M. tuberculosis* – no growth
 - Non-TB mycobacteria – positive growth
 - Rapid alternatives for species determination:
 - ◆ Genetic probes
 - ◆ Fatty acid analysis

Laboratory Diagnosis of Tuberculosis

- ◆ Drug-susceptibility testing
 - Radiometric methods in presence/absence of antibiotic
 - More rapid than conventional sensitivity tests - 5-7 days after inoculated
 - Require primary culture of mycobacteria

Immunoprophylaxis: BCG vaccine

- ◆ Live vaccine
 - Derived from *M. bovis* strain
 - Attenuated by repeated subculture
 - Contraindicated in immuno-suppressed individuals
- ◆ Used in high risk situations, locations
- ◆ Highly varied efficacy
 - May not be very effective in preventing primary pulmonary tuberculosis
 - ◆ 0-85% in different vaccine trials
 - Clearly reduce no. of deaths among infected children
 - ◆ Reduces miliary tuberculosis
 - ◆ Reduces tuberculous meningitis

Variation in protection by BCG

- ◆ Hypotheses for variation in BCG efficacy against **primary tuberculosis**
 - Palmer: exposure to environmental mycobacteria may provide protection that is not improved upon by vaccination
 - Hart: strain differences between BCG preparations may be responsible for variation in efficacy
- ◆ Continued efforts to produce a better TB vaccine
 - Ideal TB vaccine
 - ◆ Control pulmonary (as well as disseminated) disease
 - ◆ Prevent establishment of latency
 - BCG may not contain key virulence factors/protective antigens of pathogenic *M. tuberculosis*

M. tuberculosis Virulence Factors

- ◆ Major classes of virulence factors
 - Surface ligands for macrophage receptors
 - ◆ Promote macrophage uptake of mycobacteria
 - Molecules that interfere with innate immune defenses of macrophages
 - ◆ Cell wall constituents & secreted molecules
 - ◆ Mechanisms:
 - Inhibition of phagosome:lysosome fusion
 - Exclusion of vesicular protease APT-ases → elevated pH
 - Resists killing by oxygen radicals
 - Molecules that interfere with adaptive immune response
 - ◆ Secreted molecules
 - ◆ Mechanisms:
 - Induce macrophage to produce inhibitory cytokines
 - Interfere with macrophage antigen processing and presentation
 - Induce *M. tb*-specific T cell apoptosis
 - Enzymes that enable *M. tuberculosis* to utilize alternative metabolic pathways during acute and chronic infection

M. tuberculosis Virulence Factors

◆ Cell wall constituents

- Wax D, Cord factor (TDM)
 - ◆ Production required for disease, persistence
 - ◆ Elicit granulomatous reactions in high conc.
- Sulfatides
 - ◆ Suppress production of superoxide anion & phagocytosis
 - ◆ Promote growth of mycobacteria within macrophages
- Lipoarabinomannan (LAM)
 - ◆ Inhibits CMI (blocks IFN- γ signaling in macrophages)
- Phenolic glycolipid I (PGL-I)
 - ◆ Protective capsule
 - ◆ ROI scavenger

Macrophage receptors used by *M. tuberculosis* for entry

Receptor	Mechanism
Complement Receptors	Activation of alternative complement pathway Scavenging C2a to produce C3 convertase
Mannose Receptor	Ligand: surface lipoarabinomannan Downregulated by IFN- γ
Pulmonary Surfactant Protein Receptors	Upregulated by IFN- γ
CD14	High affinity receptor for LPS, also binds lipoarabinomannan
Fibronectin Receptor	Ligand: fibronectin-binding molecules secreted by bacteria
Macrophage Scavenger Receptor	Unknown
Fc γ Receptors	Unknown

New Diagnostic Approach for Latent TB



- ◆ Utilization of RD1 interferon- γ based tests for in vitro laboratory diagnostic tests for latent TB
 - ESAT-6 and CFP-10 antigens cultured with whole human blood
 - Culture for 12 h \rightarrow measure IFN- γ release by ELISA (Quantiferon-TB) or ELISPOT (TSPOT)
 - Distinguishes between BCG-vaccinated individuals (neg) and individuals with latent TB (pos)
 - More rapid than TST, requiring only 1 doctor's visit
 - May supplement or eventually replace TST

Role of Glyoxylate Shunt Enzyme Isocitrate Lyase (ICL) in Establishment of Latent Infection

- ◆ ICL allows shift in bacterial metabolism in response to nutrient availability in macrophage
 - Mycobacteria use fatty acids rather than carbohydrates as carbon substrates during infection
 - ICL diverts acetyl-CoA from beta-oxidation of fatty acids into glyoxylate shunt pathway
 - Allows bacteria to synthesize carbohydrates from fatty acids (TCA cycle + glyoxylate shunt)
 - Upregulated during infection of macrophages
- ◆ ICL required for persistence of *M. tuberculosis* in lung

Immunity to *M. tuberculosis*

- ◆ Multiple T cell subsets required for acquired immunity to tuberculosis
 - CD4+ T cells – dominant role
 - ◆ Importance illustrated by increased TB susceptibility of HIV patients
 - CD8+ T cells
 - $\gamma\delta$ T cells (role unknown)
 - CD1 restricted $\alpha\beta$ TCR+ T cell (role unknown)
 - Different T cell subsets appear to recognize different subsets of *M. tuberculosis* antigens

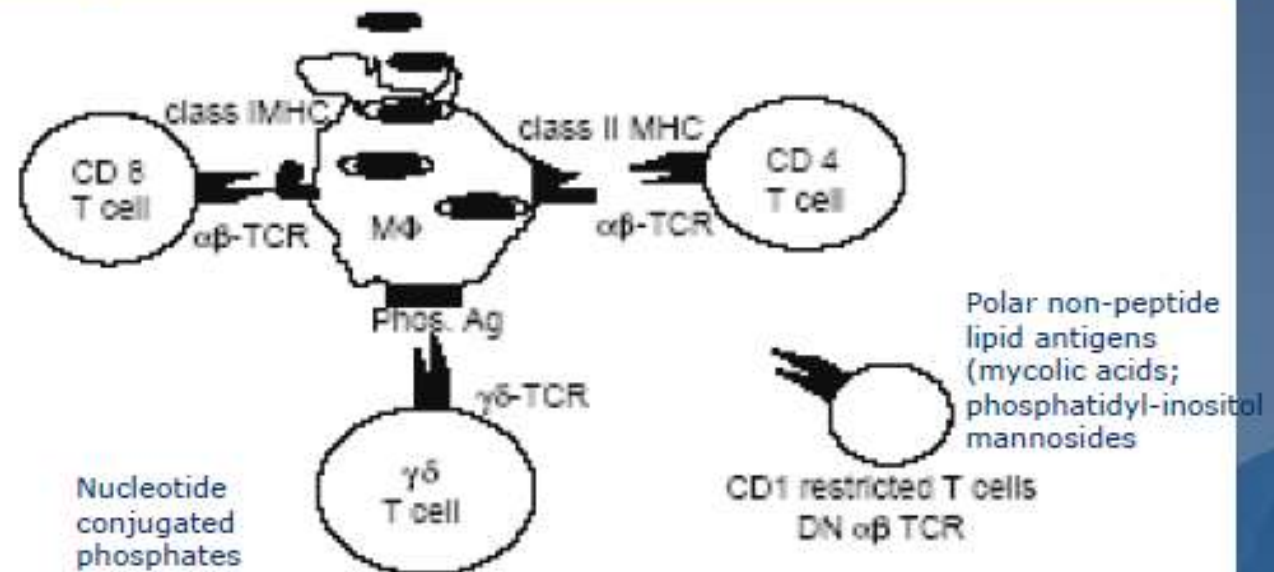


Figure 2 Many T cell subsets respond to *M. tuberculosis* infected macrophages.

Immunity to *M. tuberculosis*

- ◆ *M. tb*-specific CD4+ T cells
 - Secrete macrophage activating cytokines
 - ◆ IFN- γ - critical role in macrophage activation & regulation of APC function
 - ◆ TNF α/β
 - ◆ IL-12
 - CTL activity for *M. tb*-infected macrophages
 - Provide help for gd and CD8+ T cells through IL-2 secretion
- ◆ *M. tb*-specific CD8+ T cells
 - Secrete IFN- γ (less than CD4+ T cells)
 - CTL activity for *M. tb*-infected macrophages

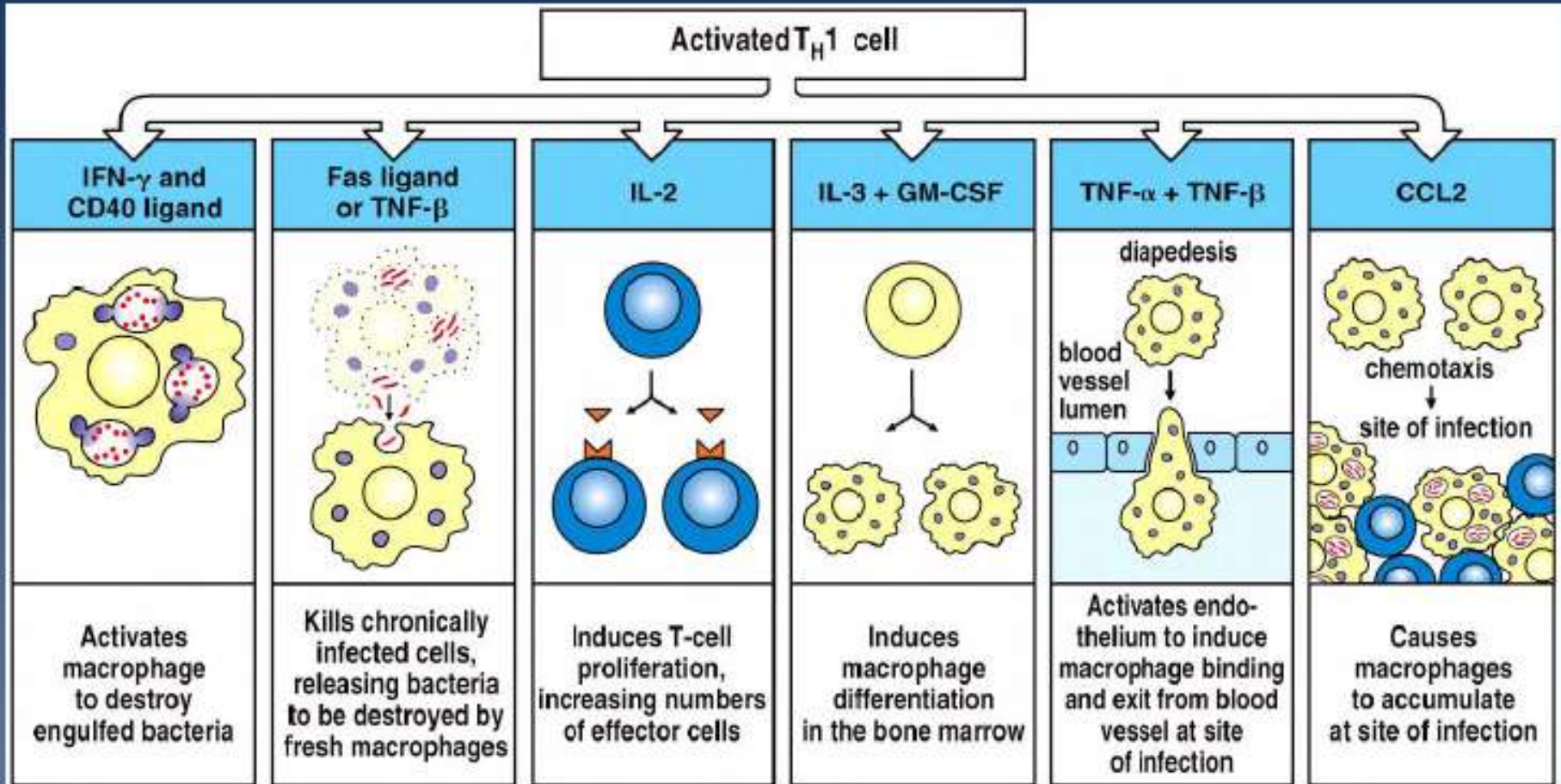
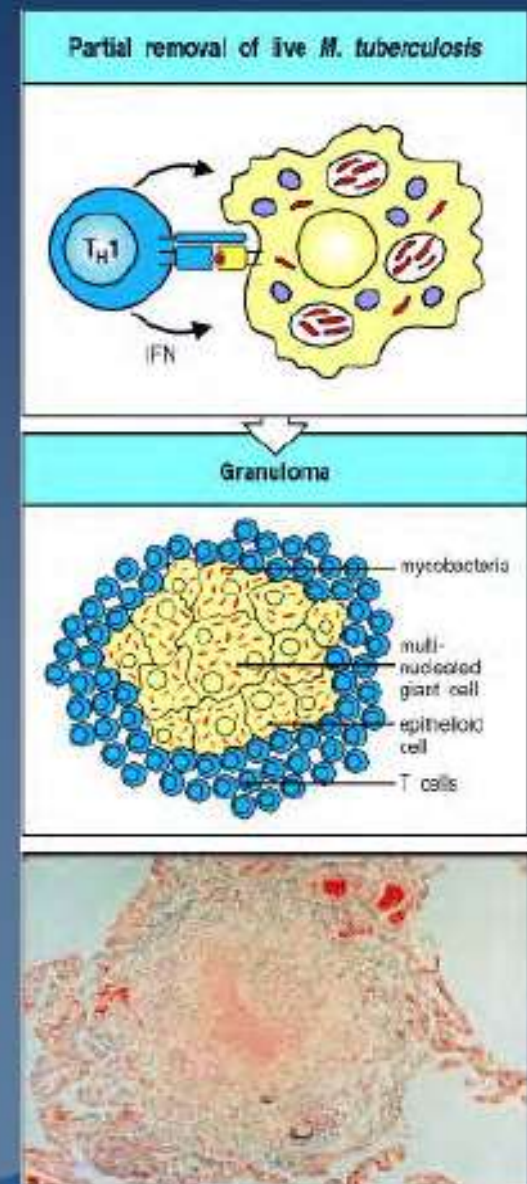


Figure 8-41 Immunobiology, 6/e. (© Garland Science 2005)

M. tuberculosis: Pathogenesis

- ◆ Development of cell-mediated immune response
 - Formation of classic tubercles at site of bacterial multiplication
 - Initiated by recognition of TB antigen-Ia complexes on macrophage surface by specific T lymphocytes
 - Microscopic granuloma
 - ◆ Multinucleated giant cells formed by fusion of macrophages (Langhans cells)
 - ◆ Epithelioid cells (activated macrophages)
 - ◆ Surrounded by lymphocytes and fibroblasts
 - ◆ Center of granuloma can necrotize if hypersensitivity intense (caseous)



M. tuberculosis: Pathogenesis of pulmonary tuberculosis

- ◆ Stage I: Inhaled bacillus may
 - multiply to form primary lesion, or
 - be eliminated by alveolar macrophages before any lesion produced
- ◆ Stage II: Small, caseous lesions may
 - progress to form macroscopic **tubercles**, or
 - heal/stabilize before detectable by radiograph.

M. tuberculosis: Pathogenesis of pulmonary tuberculosis

- ◆ Stage III: Large, caseous lesions may
 - grow and shed bacilli into blood and lymph, or
 - heal and stabilize
- ◆ Stage IV: Caseous lesions may liquefy and disseminate bacilli into bronchial tree
 - spread of disease via sputum
 - promote chronic, non-healing course

M. tuberculosis: Pathogenesis of miliary tuberculosis

- ◆ Most serious form of tuberculosis, fatal if untreated
- ◆ Many small tubercles visible throughout lungs (millet seed)
- ◆ Hematogenous spread of organism to many organs



Ineffective immune response, poor granuloma formation; occurs in immuno-compromised individuals

M. tuberculosis: Pathogenesis of reactivation tuberculosis

- ◆ Occurs in about 10% of those recovering from primary infection
- ◆ Western countries: most common in males over the age of 50
- ◆ Risk factors:
 - malnutrition, alcoholism, diabetes, old age
 - dramatic life change (loss of spouse)
 - AIDS

M. tuberculosis: Pathogenesis of reactivation tuberculosis

- ◆ Clinical characteristics:
 - Chronic fever & weight loss, night sweats, productive cough, hemoptysis
- ◆ Areas of high oxygen tension: apex of lung
 - Numerous lesions, large areas of caseous necrosis
 - Frequent erosion of small blood vessels
 - Sputum infectious
- ◆ Immunosuppressed patients (AIDS)
 - Systemic spread to other organs (miliary tuberculosis)

Genetic Control of Tuberculosis Susceptibility

- ◆ General observations
 - ◆ Most humans have high **innate** resistance
 - ◆ Racial differences in disease susceptibility
 - ◆ Related to duration of epidemics
 - ◆ Identical twin studies - similar TB susceptibilities
- ◆ Case-control studies:
 - IL-8
 - ◆ IL-8 -251A allele associated with TB disease (OR>3)
 - ◆ IL-8 level higher in patients who die from TB than survivors
 - Interferon gamma
 - ◆ IFN γ (+874A->T) polymorphism associated with susceptibility to tuberculosis
 - ◆ Associated with increased NFkappaB binding to +874T allele in controls
- ◆ Susceptibility genes also identified in mouse model
 - MHC complex, Bcg gene - natural-resistance associated macrophage protein (Nramp), Tbc gene

Treatment and Prophylaxis of *M. tuberculosis* infection

- ◆ First line anti-mycobacterial drugs
 - Combination: isoniazid, ethambutol, rifampin, pyrazinamide, streptomycin
 - Short term ambulatory therapy: 6-9 months
 - ◆ Rapid disappearance of bacilli from sputum
 - ◆ Eventual clearance of bacilli from tissues, prevent endogenous reactivation
- ◆ Second line anti-mycobacterial drugs
 - Para-Aminosalicylic acid
 - Ethionamide
 - Cycloserine
 - Fluoroquinolones
 - Kanamycin

Treatment of *M. tuberculosis* infection

- ◆ Double/triple therapy used for treatment of established tuberculosis
 - reduces problem of drug-resistance
 - patient non-infectious within 1-2 wks
 - initial therapy systemic, followed by several months of oral antibiotics
 - certain drugs effective within 9 months (isoniazid+rifampin)

Directly Observed Treatment, Short-course (DOTS)



- ◆ Patient compliance most important factor influencing treatment outcome
- ◆ DOTS established in high prevalence areas, poor compliance (102 countries)
 - Goals:
 - ◆ Break cycle of transmission
 - ◆ Prevent development of drug-resistant strains of TB
 - Provide staff and resources to diagnose, initiate & monitor treatment, record & report treatment progress, manage supplies

Antibiotic Prophylaxis

- ◆ Prophylactic chemotherapy with isoniazid alone
 - Suspected primary or latent infection with no clinical evidence of disease
 - indications for prophylaxis:
 - ◆ Positive X-ray
 - ◆ Close contact of infectious case, PPD+
 - ◆ Child who is close contact of infectious case, PPD-
 - ◆ Known, recent PPD conversion (eg. health care worker)
 - ◆ Immunosuppressed patient with some evidence of primary infection (PPD+, Xray+, other) - eg. AIDS pts
 - Single drug used: isoniazid due to small load of bacilli
 - Risk of hepatitis

Resistance to Anti-mycobacterial Drugs

Emergence of drug resistance due to poor compliance (CDC, 1991)

- 15% *M. tuberculosis* isolates in US resistant to one or more agents
- 3% of new cases and 7% of recurrent cases resistant to multiple agents
- Multiple drug resistance (MDR)
 - ◆ MDR strains confined primarily to large metropolitan areas (NYC)
 - ◆ associated with outbreaks assoc. with rapid progression, high mortality
 - ◆ result of stepwise accumulation of resistance to individual drugs
- Susceptibility testing mandatory for all initial isolates

M. tuberculosis Resistance to Isoniazid (INH)

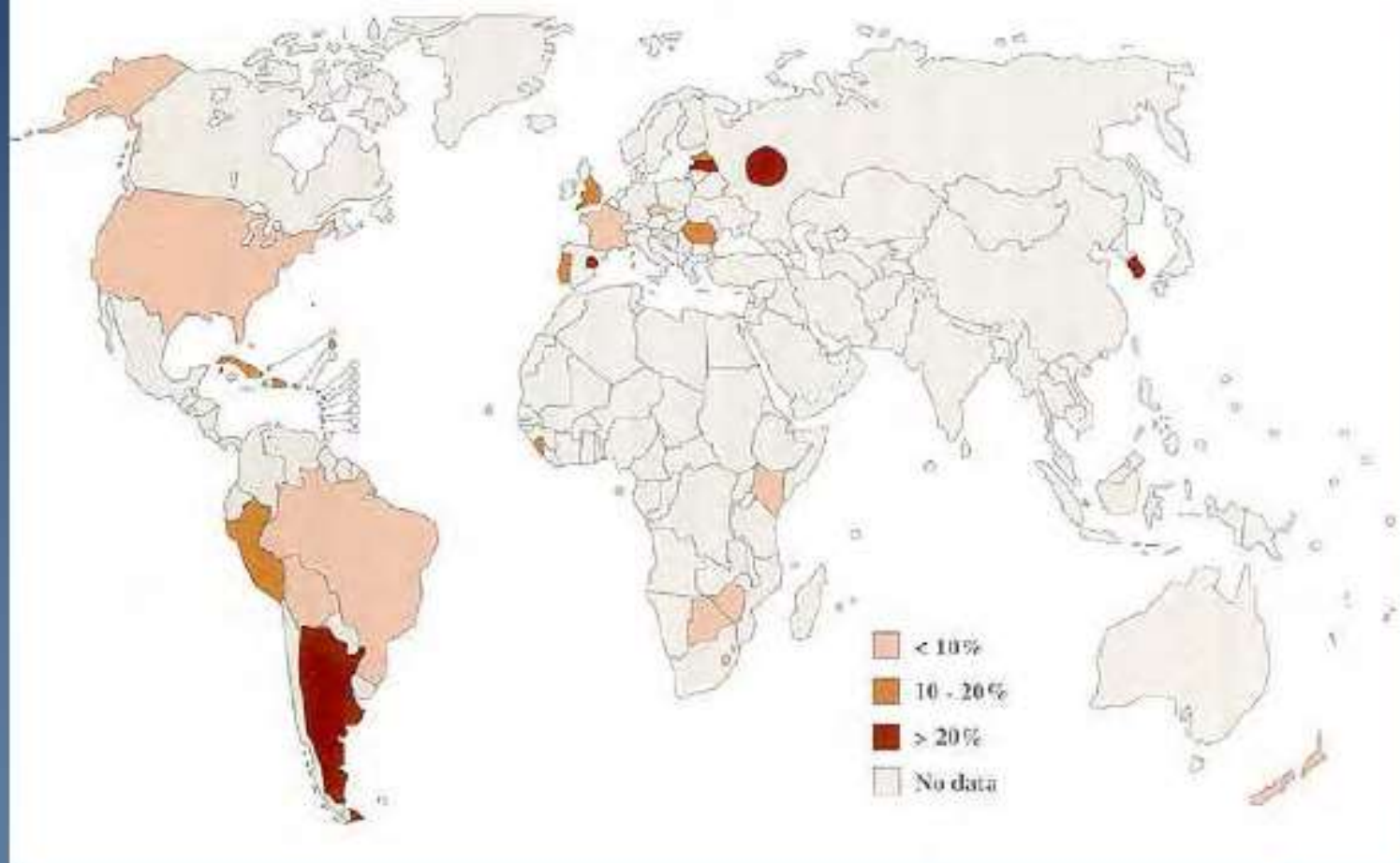
- ◆ Isoniazid targets mycolic acid synthesis pathway
- ◆ *katG* gene mutations associated with resistance in some organisms
 - INH is pro-drug, requires activation by mycobacterial catalase-peroxidase enzyme *katG*
- ◆ *kasA* gene mutations major contributor to drug resistance
 - encodes *b*-ketoacyl acyl carrier protein (ACP) synthase
 - functions in as tri-molecular complex of AcpM carrier protein, KasA, and INH
 - Kas A target of mutations in 5-20% INH resistant *M.tb*
- ◆ *InhA* gene mutations associated with resistance, but resistance profile not consistent with major role
 - encodes NADH-dependent acyl carrier protein reductase

Multiple Drug Resistance (MDR-TB)

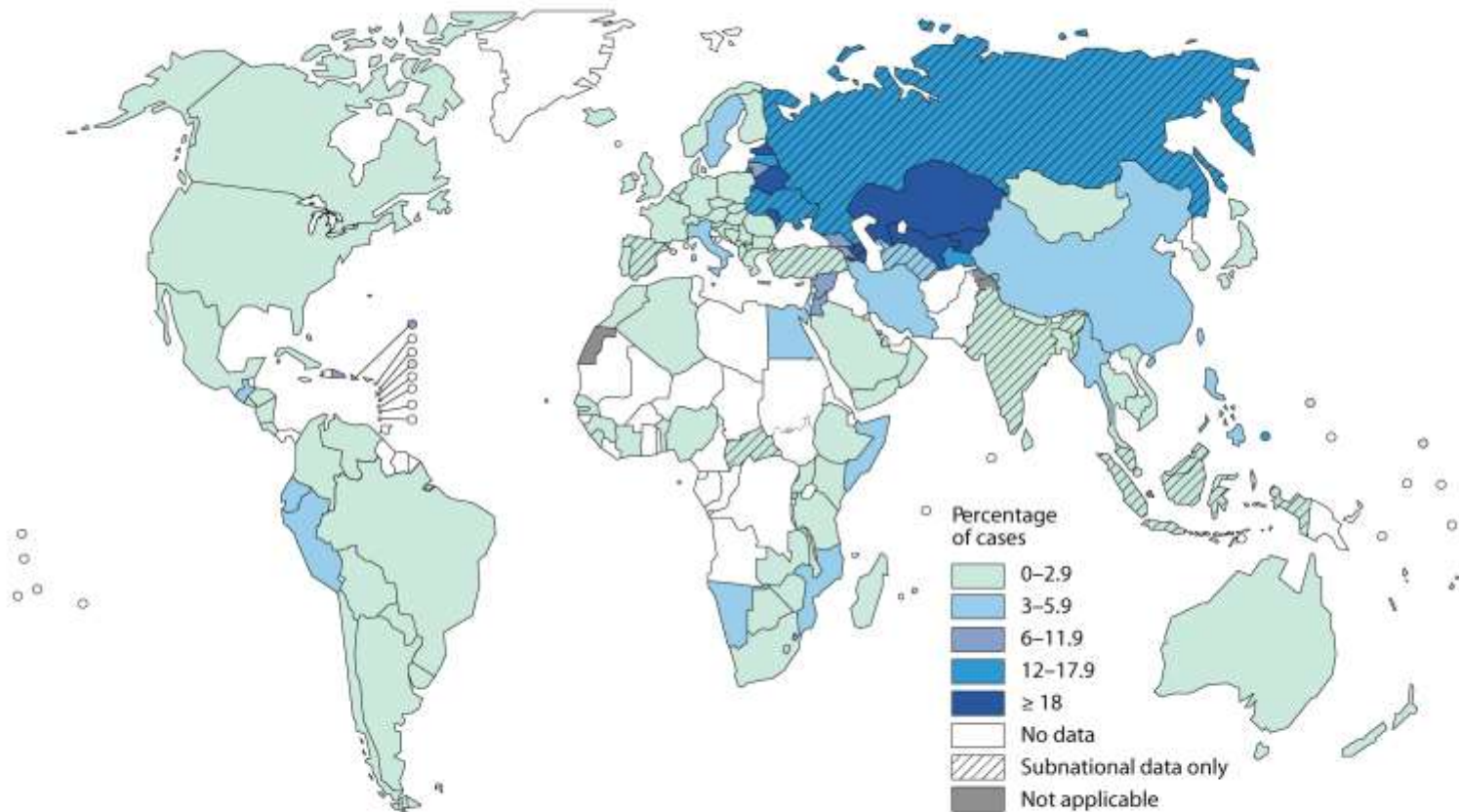
- ◆ Isoniazid resistance mutations
 - Catalase-peroxidase (*katG*) gene
 - ◆ Highest frequency @ codon for amino acid 315
 - Enoyl-ACP reductase (*inhA*) gene
 - ◆ Regulatory sequence
 - β -ketoacyl acyl carrier protein synthase (*kasA*) gene
 - Alkyl hydroperoxidases (*oxyR-ahpC/ahpC*) genes
 - ◆ Intergenic regulatory sequence
- ◆ Rifampin resistance mutations
 - RNA polymerase B (*rpoB*) gene
 - ◆ Focused within 69 bp region

Global Prevalence of MDR-TB

Prevalence of acquired MDR-TB in countries and regions surveyed, 1994-1997



Percentage of new tuberculosis cases with MDR-TB*



* MDR-TB: multidrug-resistant tuberculosis (resistance to, at least, isoniazid and rifampicin)

Note: Figures are based on the most recent year for which data have been reported, which varies among countries.

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Source: *Global Tuberculosis Report 2012*. WHO, 2012.



World Health Organization

Countries with XDR-TB




Study Questions

1. What are risk factors for tuberculosis and which of these are related to probability of infection and/or progression to active disease?
2. What is the immunological basis of the PPD skin test and how is it used as a screening test for latent tuberculosis? What are its limitations?
3. What is the relevance of the RD 1 region of the *M. tuberculosis* genome with respect to pathogenesis, immunity, and laboratory diagnosis?
4. How is the enzyme isocitrate lyase involved in *M. tuberculosis* virulence?
5. What are the innate and adaptive immune mechanisms involved in resistance to TB infection & disease? What are some mechanisms used by the bacterium to evade these protective responses?

Resources

- ◆ Murray, Rosenthal & Pfaller. Medical Microbiology, 5th edition, 2005.
 - Chapter 14
 - Chapter 29
- ◆ Boom, W.H. et al. Human immunity to *M. tuberculosis*: T cell subsets and antigen processing. Tuberculosis 83:98-106, 2003.
- ◆ Young, D.B. Building a better tuberculosis vaccine. Nat. Med. 9:503-504, 2003.
- ◆ McKinney, J.D. et al. Persistence of Mycobacterium tuberculosis in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. Nature 406:735-8, 2000.
- ◆ Munoz-Elias, E.J. and McKinney, J.D. Mycobacterium tuberculosis isocitrate lyases 1 and 2 are jointly required for in vivo growth and virulence. Nat. Med. 11:638-44, 2005.

BACTERIAL INFECTIONS

- **Streptococcus pneumoniae** remains the most common etiological agent of community-acquired bacterial pneumonia
 - With wider use of HAART and antimicrobial prophylaxis, the incidence of invasive pneumococcal disease among HIV-infected persons has declined in the post-HAART era.
 - Pneumococcal vaccination can reduce the risk of invasive pneumococcal disease in HIV-infected patients remains controversial. (23-valent polysaccharide pneumococcal vaccine vs. 9-valent conjugate vaccine)
 - **Non-typhoid Salmonella** incidence of HIV-associated community-acquired bacteraemia has declined in the post-HAART era.
 - The optimal duration of suppressive ciprofloxacin therapy to prevent recurrences of non-typhoid Salmonella bacteraemia remains unclear
- 

FUNGAL INFECTIONS

- ***Pneumocystis jiroveci* pneumonia** [formerly known as *P. carinii* pneumonia (PCP)] has declined and survival following severe PCP has improved in patients receiving HAART.
- HAART resultant increases of CD4 lymphocyte counts to >200 cells/mm³ for more than 3 months, the risk for pneumocystosis is sufficiently low to warrant discontinuation of prophylaxis.
- However, pneumocystosis continues to be a leading cause of community-acquired pneumonia, resulting in mortality among AIDS patients in the post-HAART era because of patient non-adherence to antimicrobial prophylaxis and failure to use antiretroviral therapy.
- **Candidiasis**-oral thrush
- **Cryptococcosis** has significantly decreasing in the post-HAART era.
- ***Histoplasma capsulatum*, *Coccidioides immitis* and *Penicillium marneffe***, usually develop in patients at an advanced stage of HIV infection, and relapse rates are high in those patients not receiving maintenance antifungal prophylaxis.
- Use of antiretroviral therapy and antifungal prophylaxis has been found to be associated with a reduced risk

VIRAL INFECTION

- Reactivation of latent **cytomegalovirus** (CMV) infection in patients with CD4 lymphocyte counts of <50 cells/mm³ is associated with decreased survival
- the incidence and mortality rate of CMV disease have declined with HAART and anti-CMV therapy.
- clinical studies suggest that it is safe to discontinue secondary prophylaxis against CMV in patients with an increase in CD4 counts in response to HAART
- Progressive multifocal leucoencephalopathy (PML), a demyelinating disease of the central nervous system caused by the human polyomavirus **JC virus**
- leading almost invariably to death a median of 4–6 months after diagnosis
- The use of HAART has been shown to prolong survival, improve neurological function and reduce the size of active PML lesions on radiographic images.



PARASITIC INFECTIONS

- **Toxoplasma** encephalitis (TE) is a life-threatening opportunistic infection of the central nervous system in patients at advanced stage of HIV infection.
- It is estimated that 20%–47% of the patients seropositive for *Toxoplasma gondii* in the pre-HAART era will ultimately develop TE.
- The risk for TE has declined in HIV-infected patients who receive trimethoprim/sulfamethoxazole prophylaxis and HAART with immune restoration.
- many studies have demonstrated that the occurrence of TE was rare after discontinuation of primary and secondary prophylaxis against pneumocystosis and TE in patients with restoration of immunity.



PARASITIC INFECTIONS

- ***Cryptosporidium parvum*** and ***microsporidia*** are the two common opportunistic parasites that cause chronic diarrhea and wasting in HIV-infected patients with CD4 counts <100 cells/mm³
- Antimicrobial agents have limited efficacy in preventing or eradicating infections with *cryptosporidia* or *microsporidia* among HIV-infected patients.
- Diarrhoea due to *microsporidia* and *cryptosporidia* resolved spontaneously with immune restoration among HIV-infected patients who responded to HAART



HIV-ASSOCIATED NEUROCOGNITIVE DISORDERS (HAND)

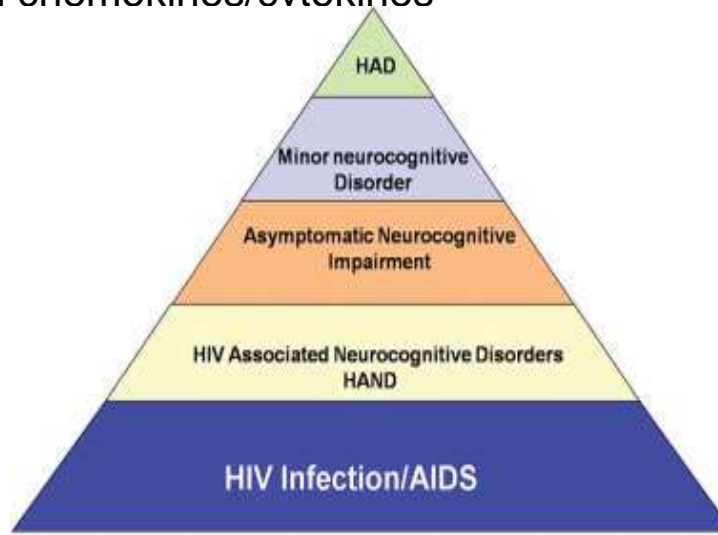
- Rise in the prevalence of human immunodeficiency virus (HIV)-associated neurocognitive disorders (HAND) despite use of highly active antiretroviral therapy (HAART).

- Nosology: NC, ANI, MND and HAD (Antinori et al, 2007)

Prevalence
HAND: 15-50%
ANI: 30%
MND: 20-30%
HAD: 2-8%

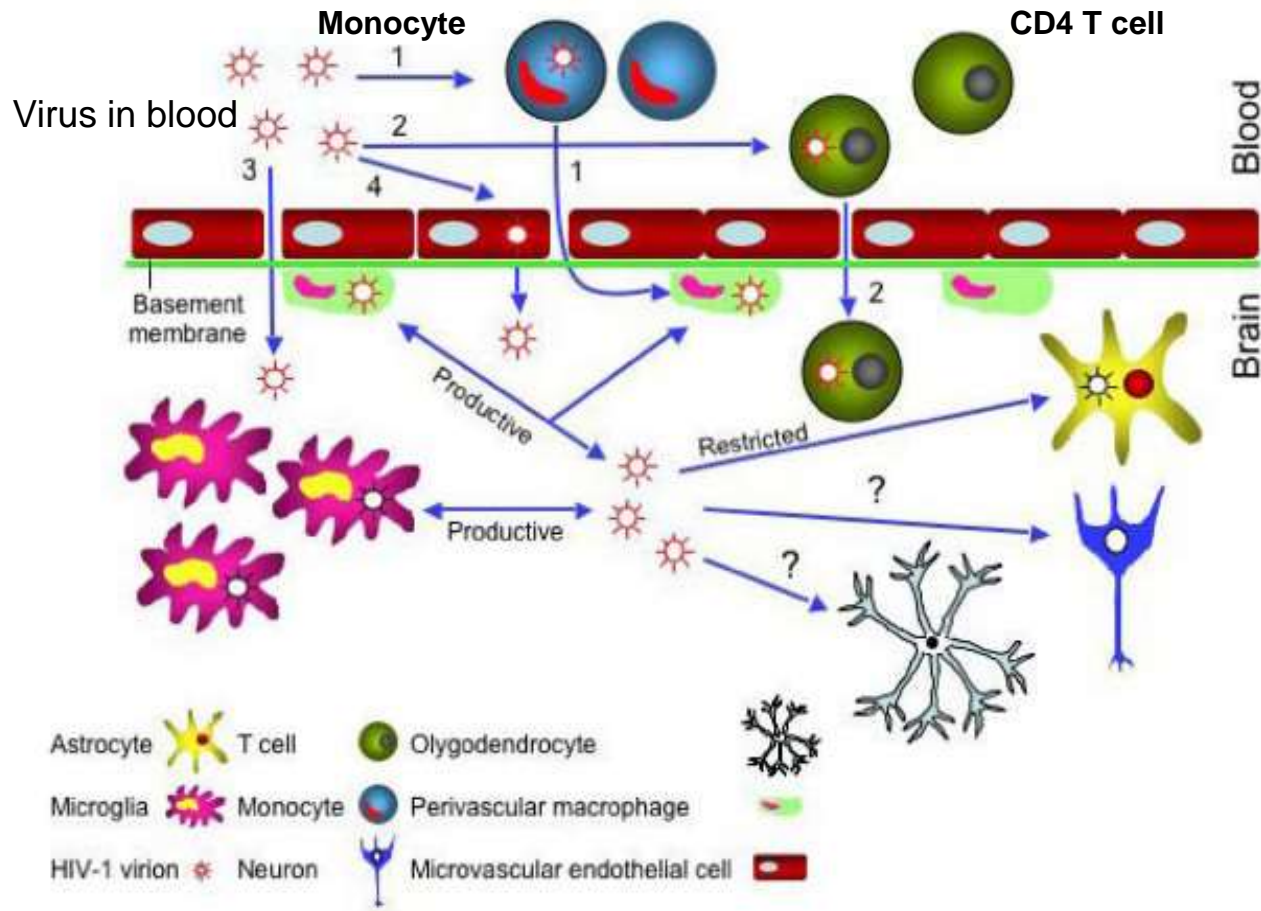
- Pathology is characterized by activation of microglia and astroglia and neuronal injury

- Promoted by both systemic and central nervous system (CNS) inflammatory mechanisms
- Viral products and chemokines/cytokines



HIV ENTRY INTO THE CNS AND PATHOGENESIS

(‘TROJAN HORSE’ MECHANISM)



- Blood Brain Barrier/ Blood CSF barrier of the Choroid Plexus
- Entry via infected activated CD4+ T cells or monocytes
- Perivascular Macrophages and Microglia can be infected by HIV
- Neuronal injury:
 - Direct: tat, gp120, vpr
 - Indirect: TNF- α , NO, Glu, Ca²⁺

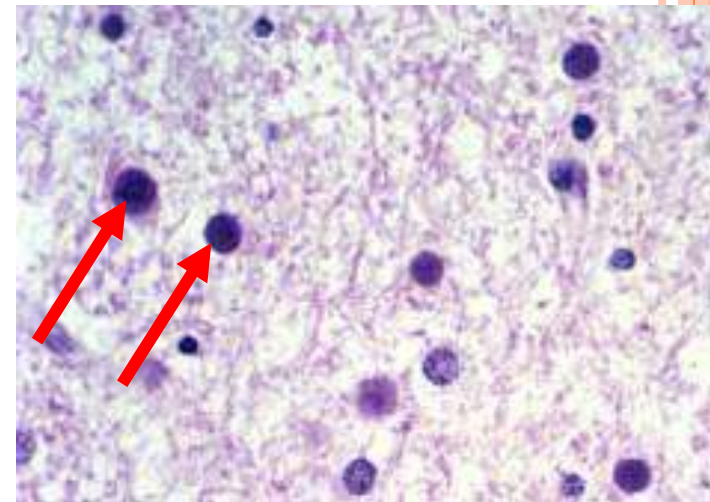
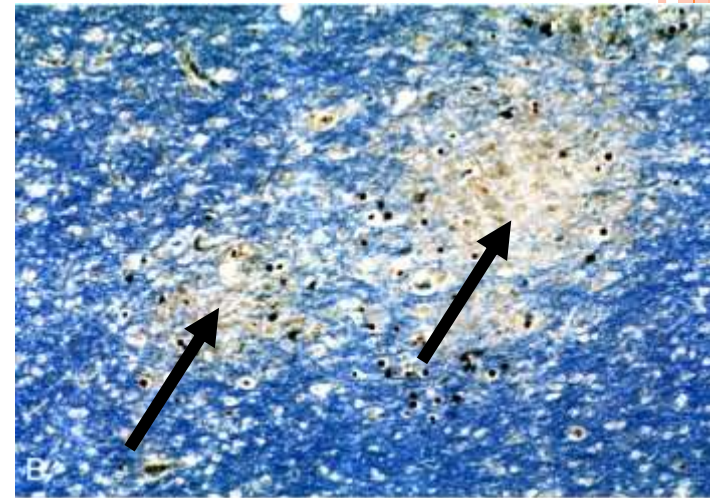
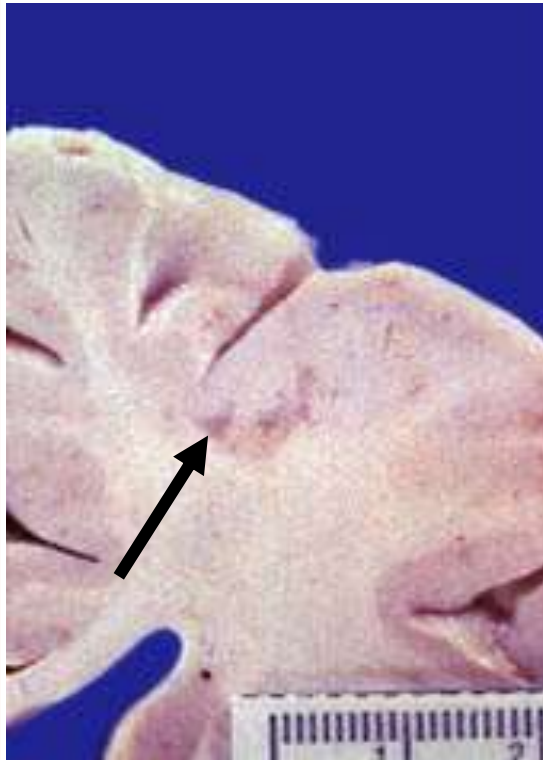
PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY

- PML is a demyelinating condition seen in AIDS patients or immunocompromised individuals
 - Reactivation of poliovirus (JC)
 - Targets oligodendrocytes



Progressive Multifocal Leukoencephalopathy

Black arrows - demyelination
Red arrows - viral inclusions

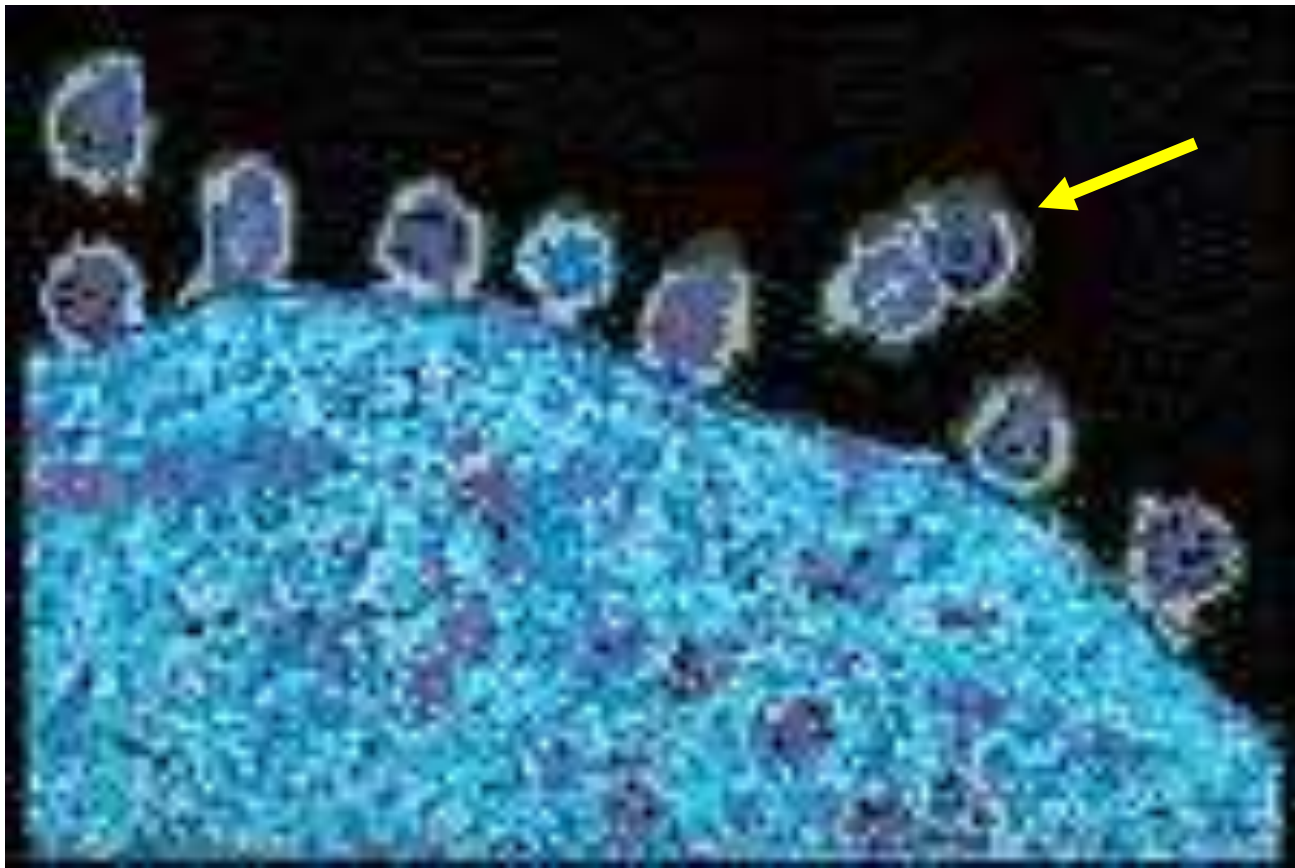


HIV ENCEPHALOPATHY AND HIV DEMENTIA COMPLEX

- Chronic immune activation and inflammation in the brain
- Neuronal injury from viral proteins and immune mediators
- Major cause of neurological complications in AIDS patients
- Less common in the HAART era but as patients live longer the prevalence continues to rise



It's All About the Macrophage Neuropathology of HIV



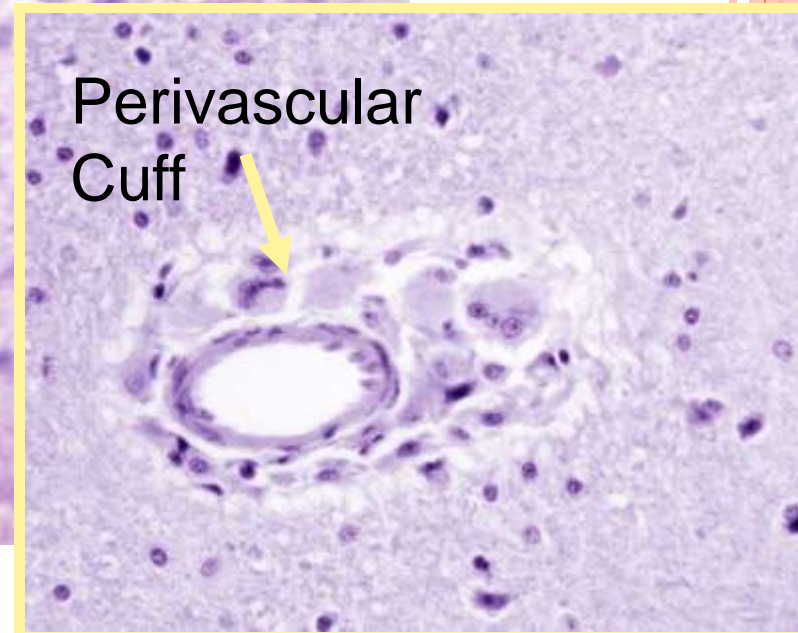
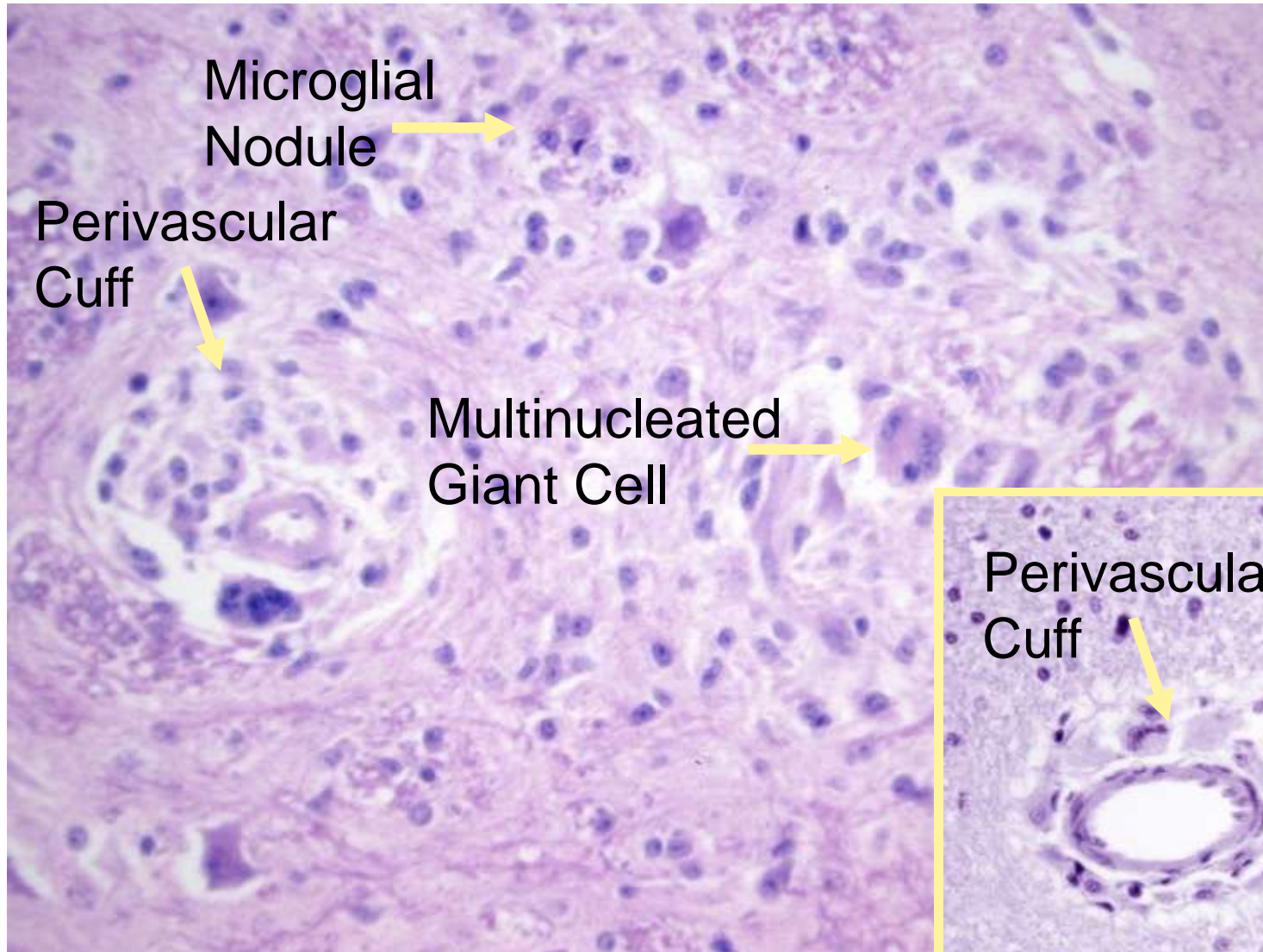
Classical HIV Encephalitis

Major Features:

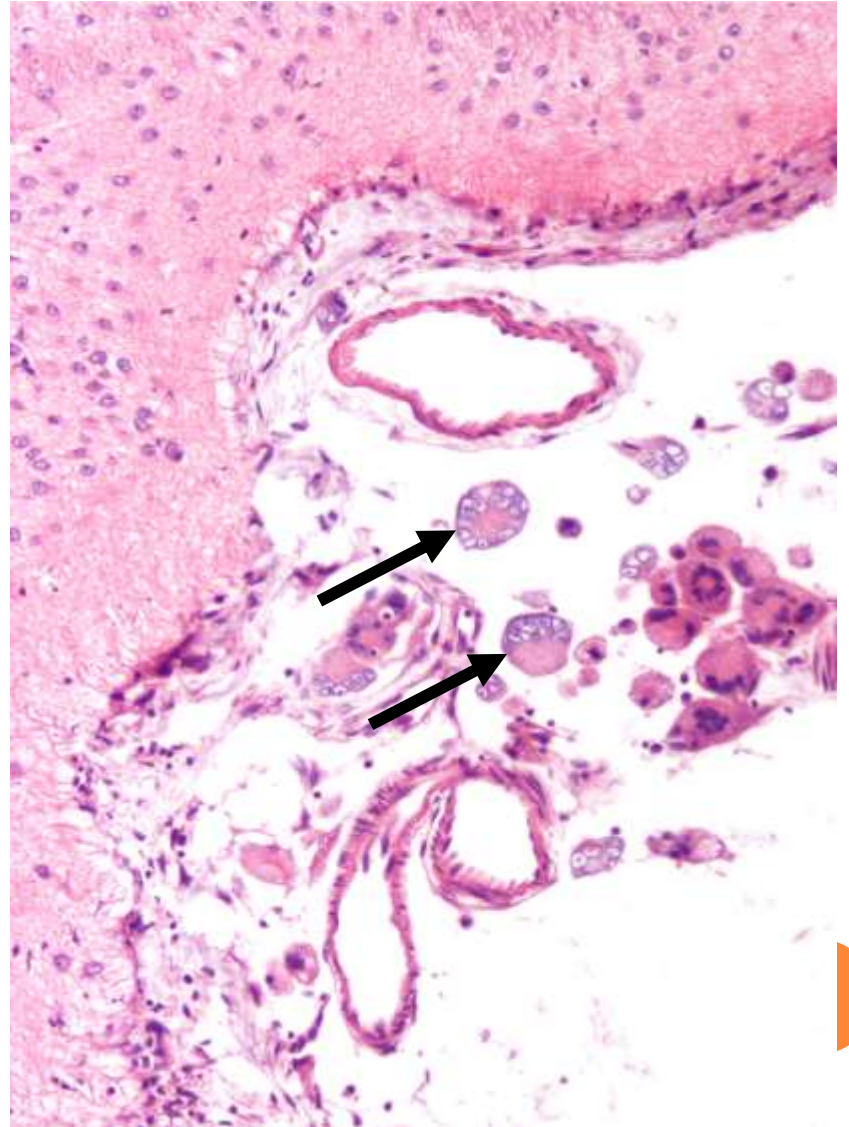
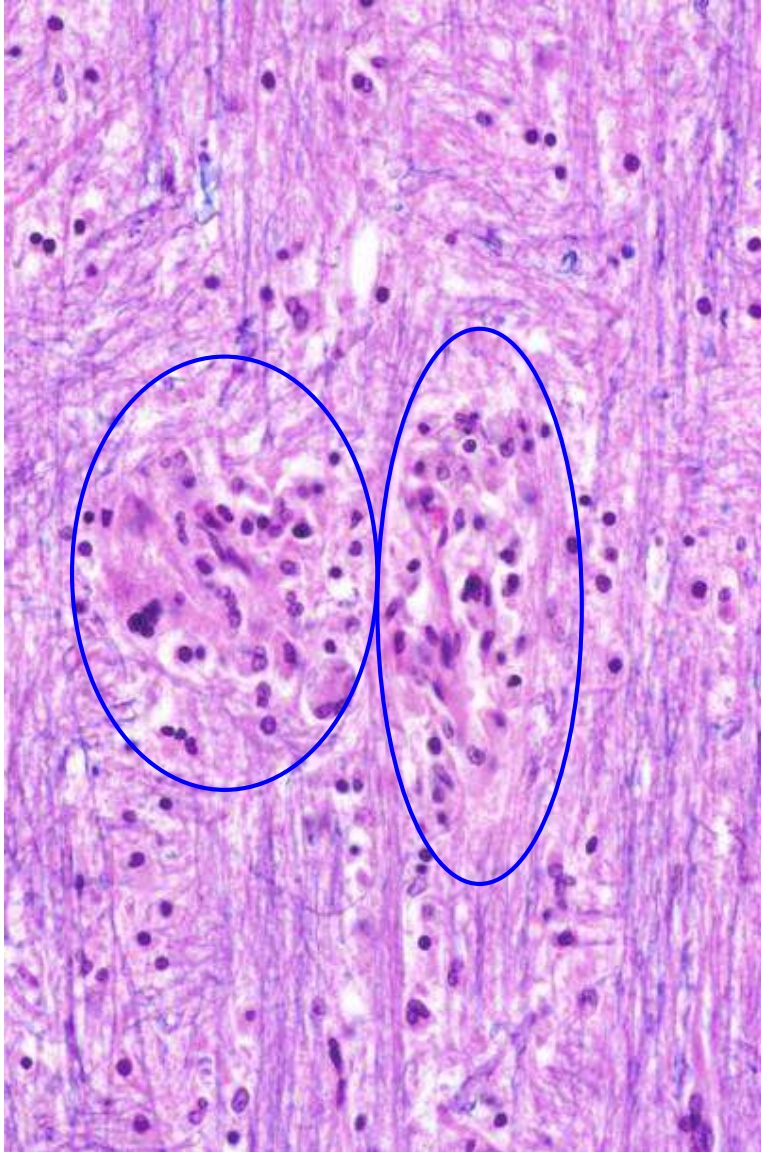
- ✓ increased numbers of activated macrophages (HIV: & lymphocytes) in neuropil
- ✓ Multinucleated giant cells, esp. perivascular
- ✓ Microglial nodules (focal collections of activated macrophages/giant cells)
- ✓ Lesions most severe at base of brain (basal ganglia, thalamus) and in subcortical white matter



Classical HIV Encephalitis

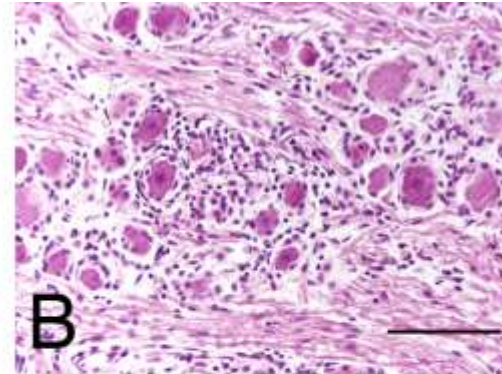
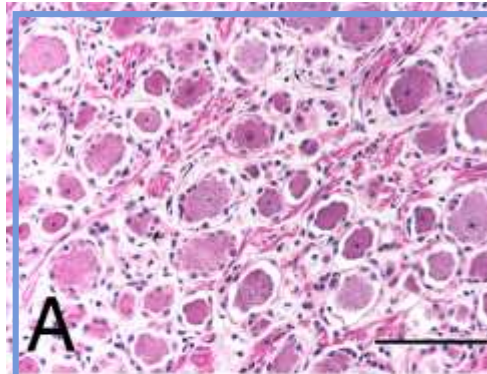


HIV Encephalitis



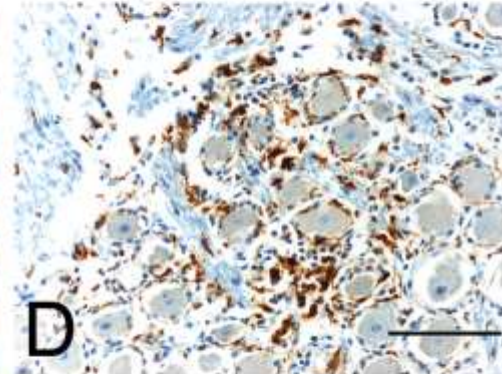
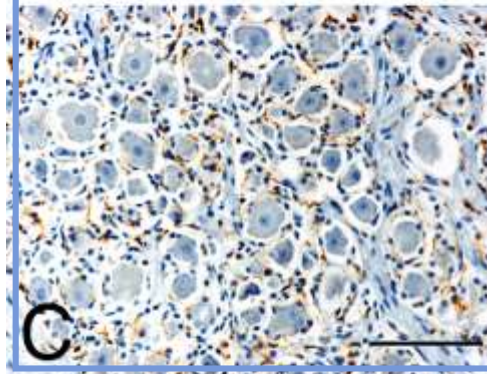
Dorsal Root Ganglia

Normal DRG



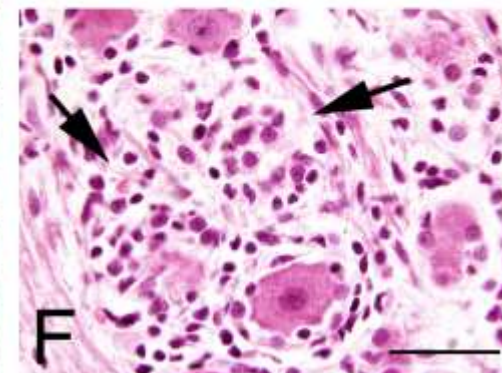
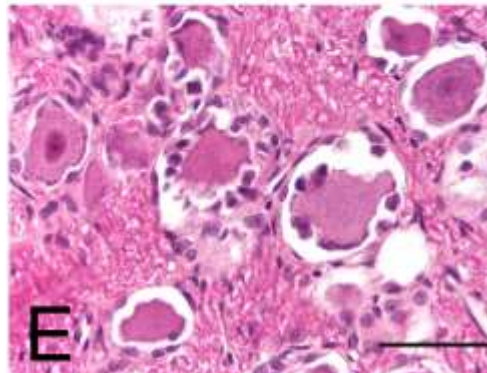
Infiltration of macrophages and lymphocytes

IHC showing normal resident macrophages



IHC showing Increased macrophages

Dead neurons being digested by phagocytes



Nageotte nodule - neuron replaced by inflam. cells

Special Stains

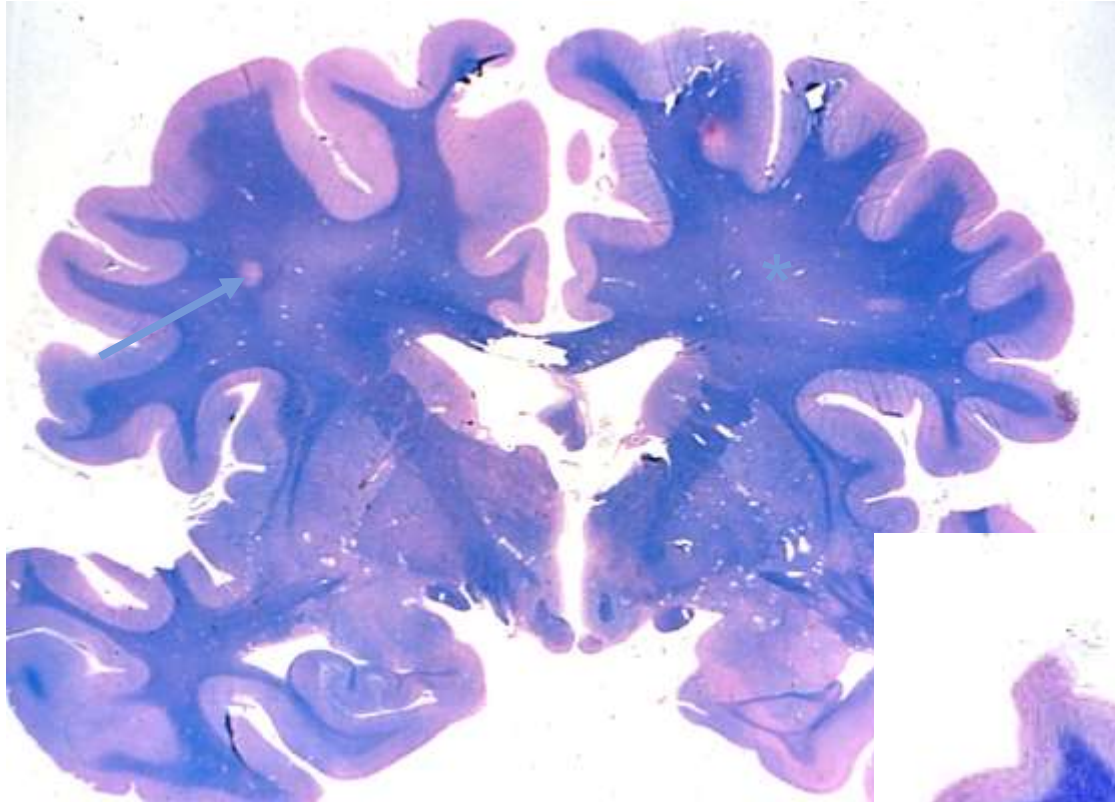
Histochemical Stains

- ✓ Luxol fast blue (LFB) - stains myelin blue*
- ✓ Periodic acid Schiff (PAS) - stains lysosomes (activated macrophages) red
- ✓ Mucicarmine - stains cryptococcal capsules red*
- ✓ Gomorri Methenamine Silver (GMS) - stains fungal elements black
- ✓ Ziehl-Nielson - stains Mycobacteria red
- ✓ Neuron-specific enolase - stains neurons red

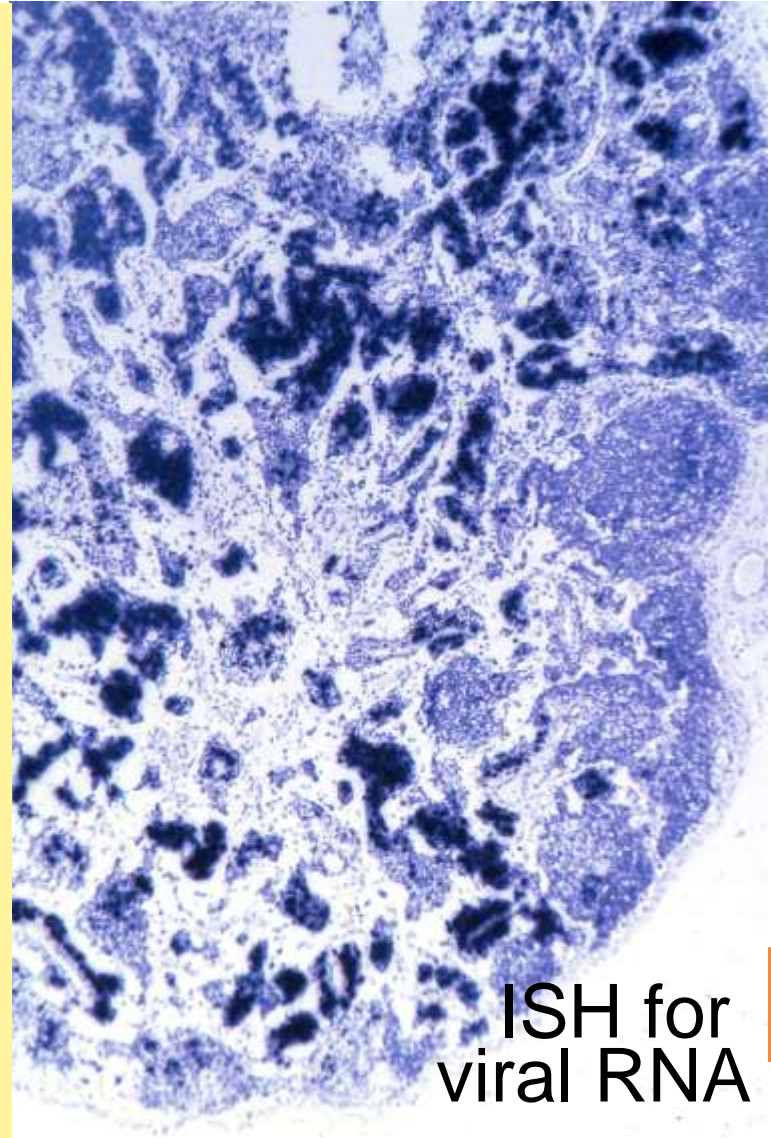
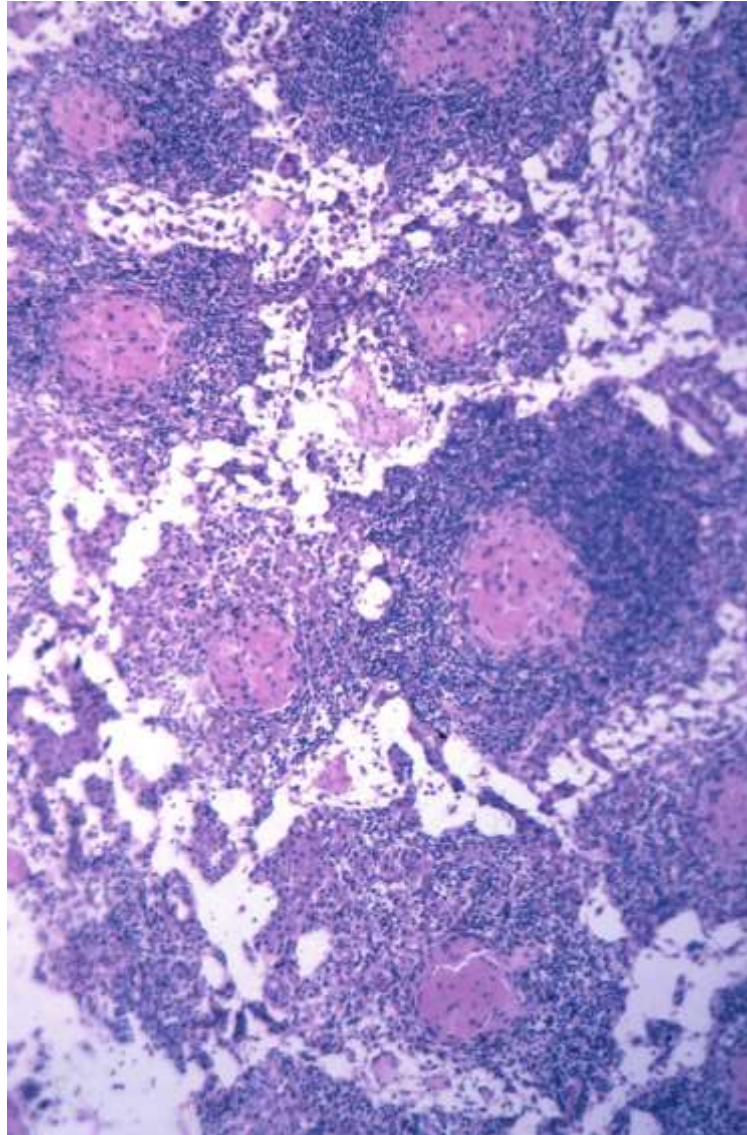
*examples provided



Luxol Fast Blue



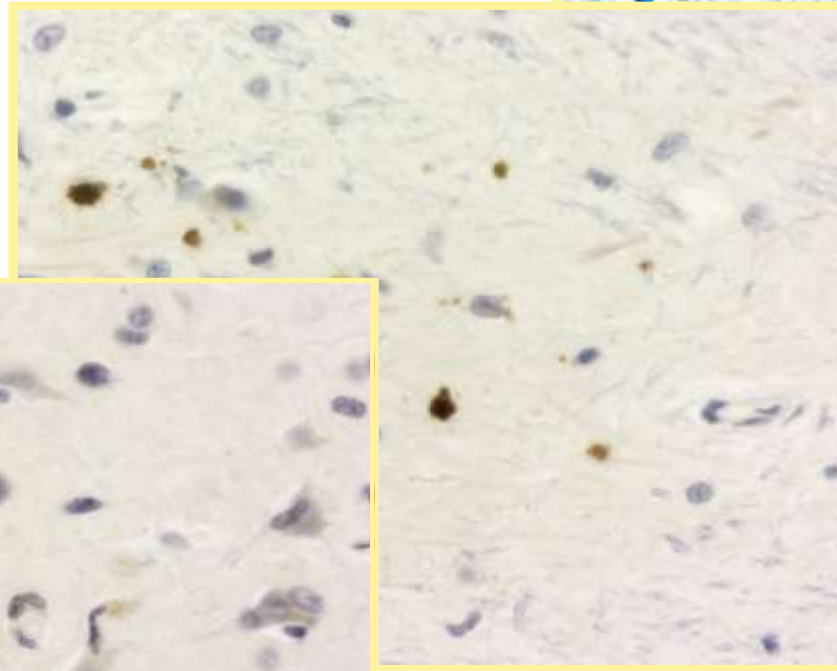
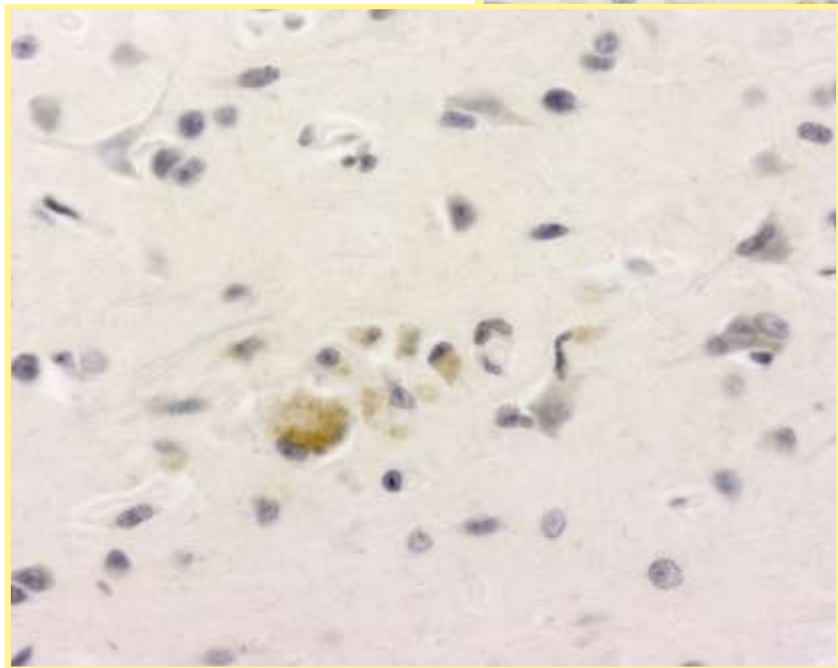
Chronic Lymphoid Depletion



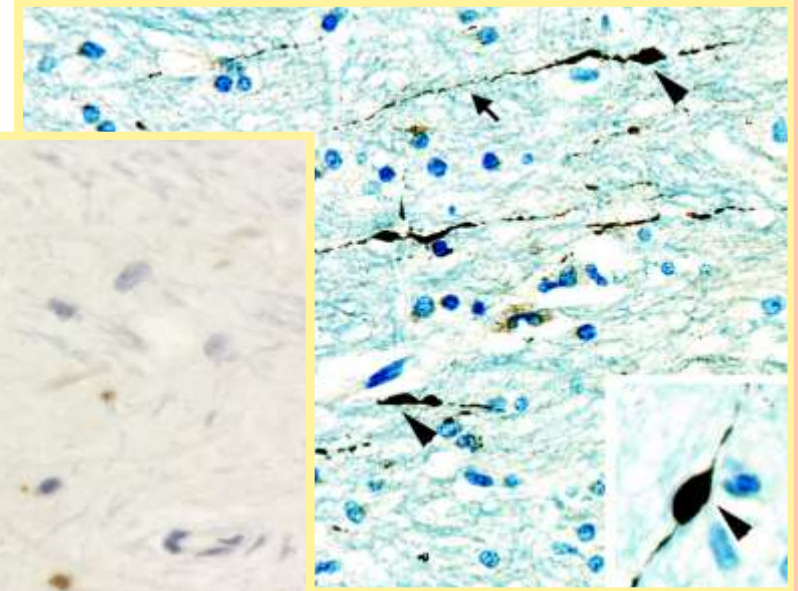
ISH for
viral RNA

β -APP and Amyloid- β in SIV Encephalitis

Amyloid- β in macrophages



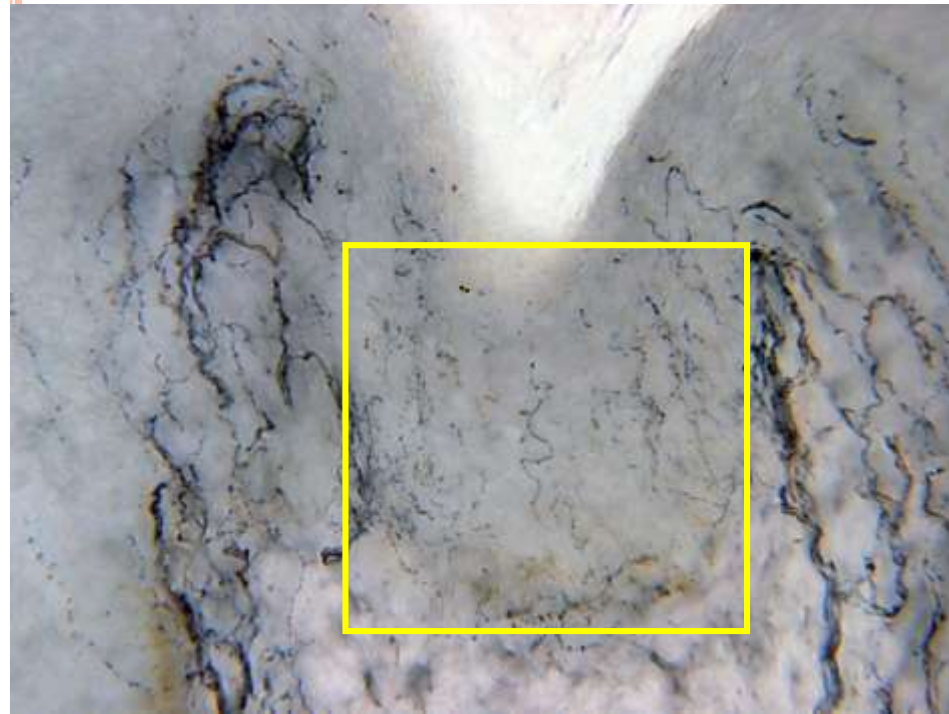
Amyloid- β in neurons



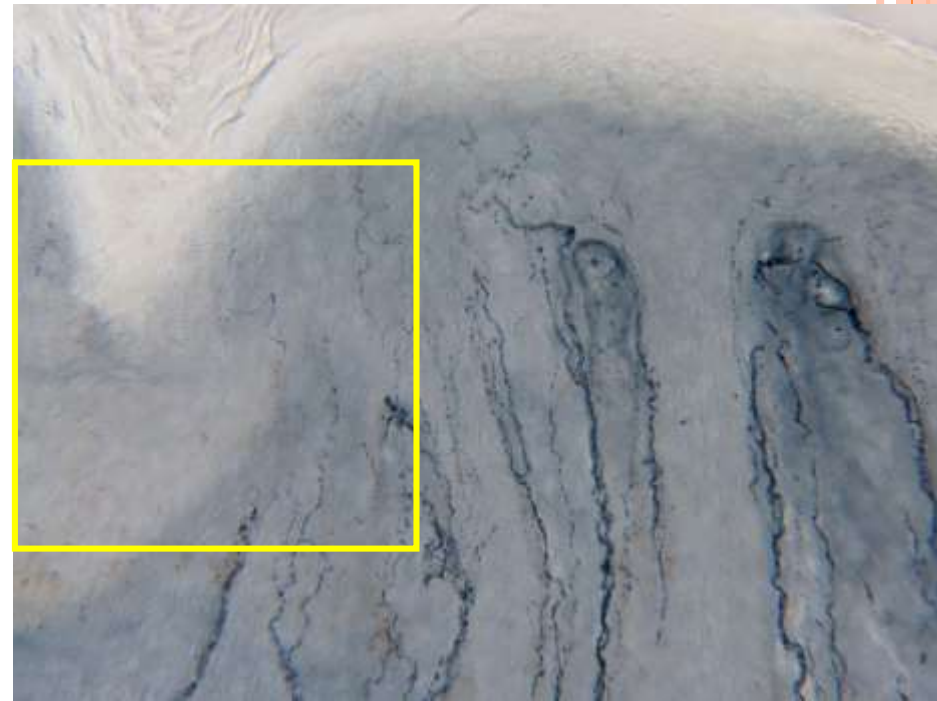
β -APP in axons



Quantitation of IHC-Staining



Uninfected Control



SIV-infected



In Situ Hybridization

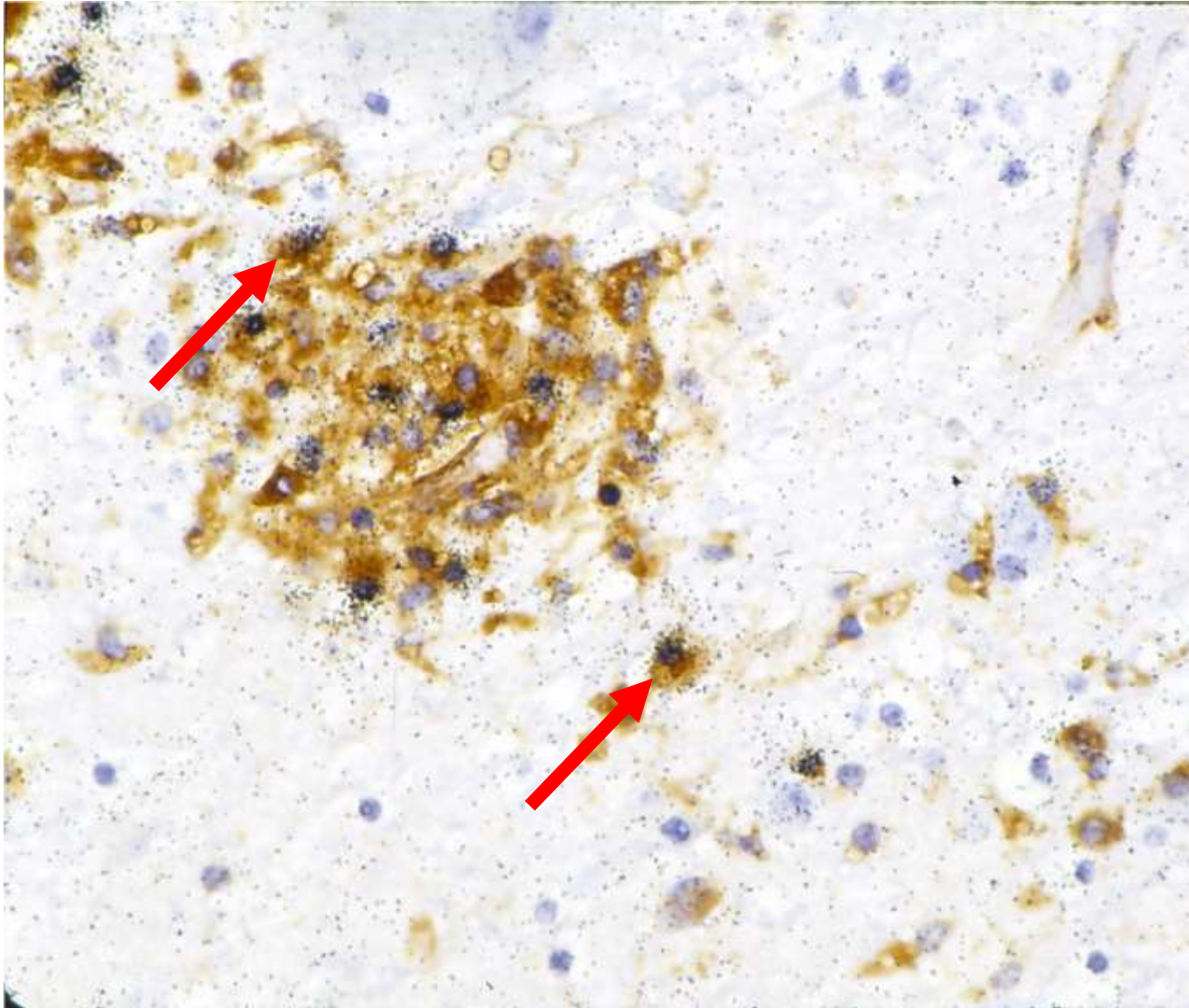
Complementary antisense probe
bound to nucleic acid

Usually used to detect RNA (viral or cellular)

- ✓ Viral *gag*, *gp120*, *nef*, *etc.*
- ✓ Upregulated cellular RNAs such as cytokines, chemokines, *etc.*
- ✓ Fluorescent or light microscopic detection methods



Viral *gag* RNA in Macrophages



BUT REMEMBER IT IS NOT JUST INFECTIONS

○ Neoplasias

- Invasive cervical cancer
- Kaposi's sarcoma
- Cervical cancer
- Lymphoma (Burkitt's, immunoblastic, primary CNS)

○ Other

- HIV dementia
- PML
- HIV wasting

○ Suggestive of HIV infection

- Bacillary angiomatosis
- Recurrent mucosal candidiasis
- Cervical dysplasia or carcinoma in situ
- Constitutional symptoms
- Hairy leukoplakia
- Herpes zoster
- ITP
- Listeriosis
- PID
- Peripheral neuropathy

