HIV Diagnosis and Pathogenesis

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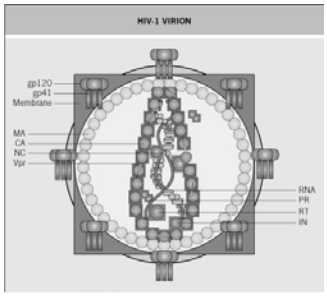
Laboratory Diagnosis of Acute HIV-1 Infection

- Patients with acute HIV infection may present to a health care facility before full antibody seroconversion
  - ELISA may be negative
  - ELISA may be positive with negative or indeterminant Western blot
- Plasma HIV-1 RNA level should be done if acute HIV infection is suspected
- Follow-up antibody testing should be performed to document full seroconversion (positive ELISA and WB)

HIV Diagnosis

- Consider in anyone presenting with symptoms and signs compatible with an HIV-related syndrome or in an asymptomatic person with a risk factor for acquisition
- Full sexual and behavioral history should be taken in all patients
  - Assumptions of risk (or lack thereof) by clinicians are unreliable

HIV-1 Virion

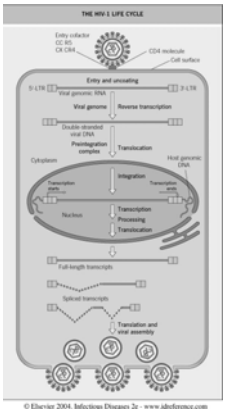


Laboratory Diagnosis of Established HIV Infection: Antibody Detection

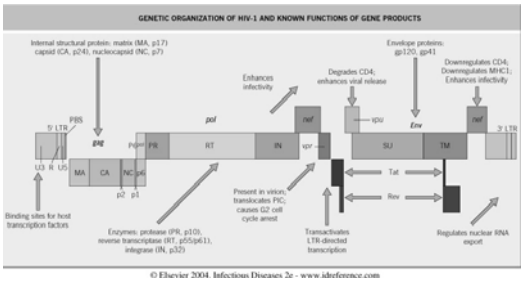
- Screening
  - Serum ELISA
  - Rapid blood or salivary Ab tests
- Confirmation
  - Western blot
- Written consent for HIV Ab testing must be obtained and be accompanied by pre- and post-test counselling

HIV Life Cycle

Tat = transcriptional activator
Rev = regulator of mRNA nuclear export



HIV-1: Genetic Organization



Determinants of Outcome: Selected Viral Factors

- **Escape from immune response**
  - **Under immune selective pressure (cellular and humoral), mutations in *gag*, *pol* and *env* may arise**
- **Attenuation**
  - ***nef* deleted viruses associated with slow or long-term nonprogression in case reports and small cohorts**
- **Tropism**
  - **R5 to X4 virus conversion associated with increased viral pathogenicity and disease progression**
- **Subtypes**
  - **Potential for varied subtypes to exhibit differential transmissibility and virulence**
    - » **Potential for greater heterosexual spread of some subtypes**

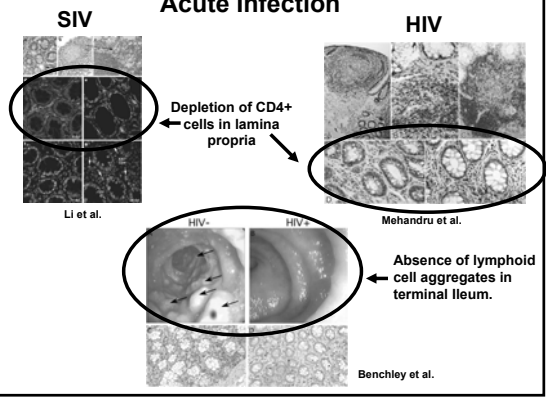
Established HIV Infection: Pathogenesis

- **Active viral replication present throughout course of disease**
- **Major reservoirs of infection exist outside of blood compartment**
  - **Lymphoreticular tissues**
  - **Central nervous system**
  - **Genital tract**
- **Virus exists as multiple quasispecies**
  - **Mixtures of viruses with differential phenotypic and genotypic characteristics may coexist**
- **At least 10 X 10^9 virions produced and destroyed each day**
- **$T_{1/2}$ of HIV in plasma is <6 h and may be as short as 30 minutes**
- **Immune response, chemokine receptor status and HLA type are important codeterminants of outcome**

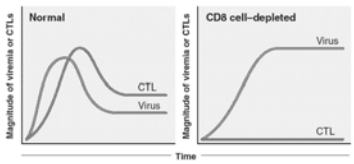
Host Factors in HIV Infection (I)

- **Cell-mediated immunity**
  - **Cytotoxic T cells**
    - » **Eliminate virus infected cells**
    - » **Play prominent role in control of viremia, slowing of disease progression and perhaps prevention of infection**
  - **T-helper response**
    - » **Vital for preservation of CTL response**
- **Humoral immunity**
  - **Role in prevention of transmission and disease progression unclear**

Acute Infection



Role of CTL's in Control of Viremia

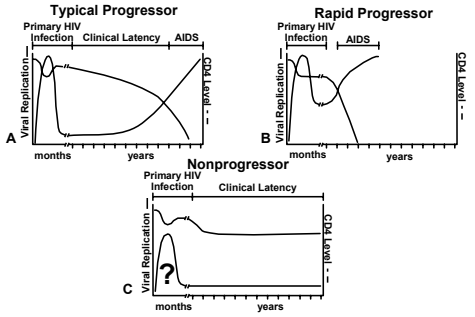


Letvin N & Walker B: Nature Med 2003;9:861-866

Host Factors in HIV Infection (II)

- **Chemokine receptors**
  - **CCR5-Δ32 deletion**
    - » **Homozygosity associated with decreased susceptibility to R5 virus infection**
    - » **Heterozygosity associated with delayed disease progression**
  - **CCR2-V64I mutation**
    - » **Heterozygosity associated with delayed disease progression**
  - **CCR5 promoter polymorphisms**
    - » **59029-G homozygosity associated with slower disease progression**
    - » **59356-T homozygosity associated with increased perinatal transmission**

The Variable Course of HIV-1 Infection

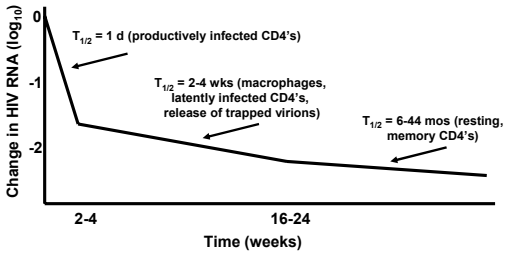


Reprinted with permission from Haynes. In: DeVita et al, eds. *AIDS: Etiology, Treatment and Prevention.* 4th ed. Lippincott-Raven Publishers; 1997:89-99.

Host Factors in HIV Infection (III)

- **Other genetic factors**
  - **Class I alleles B35 and Cω4**
    - » **Associated with accelerated disease progression**
  - **Heterozygosity at all HLA class I loci**
    - » **Appear to be protective**
  - **HLA-B57, HLA-B27, HLA-Bω4, HLA-B*5701**
    - » **Associated with long-term non-progression**
  - **HLA-B14 and HLA-C8**
    - » **?Associated with long-term nonprogression**

Phases of Decay Under the Influence of Potent Antiretroviral Therapy



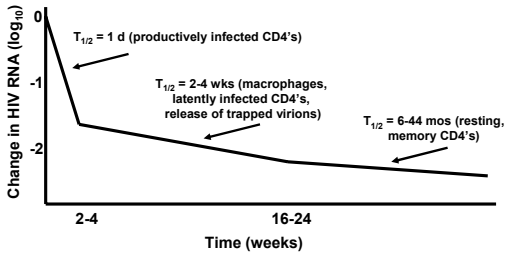
Mechanisms of CD4+ Cell Death in HIV Infection

- **HIV-infected cells**
  - **Direct cytolytic effect of HIV**
  - **Lysis by CTL's**
  - **Apoptosis**
    - » **Potentiated by viral gp120, Tat, Nef, Vpu**
- **HIV-uninfected cells**
  - **Apoptosis**
    - » **Release of gp120, Tat, Nef, Vpu by neighboring, infected cells**
  - **Activation induced cell death**

Therapeutic Implications of First and Second Phase HIV RNA Declines

- **Antiviral potency can be assessed in first 7-14 days**
  - **Should see 1-2 log declines after initiation of therapy in persons with drug susceptible virus who are adherent**
- **HIV RNA trajectory in first 1-8 weeks can be predictive of subsequent response**
  - **Durability of response translates into clinical benefit**

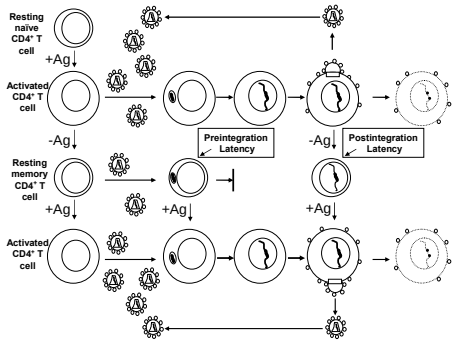
Phases of Decay Under the Influence of Potent Antiretroviral Therapy



Initiation of Therapy in Established HIV Infection: Considerations

- Patient's disease stage
  - Symptomatic status
  - CD4 cell count
  - Plasma HIV-1 RNA level
- Patient's commitment to therapy
- Philosophy of treatment
  - Pros and cons of 'early' intervention

Model of Post-Integration Latency



Siliciano R et al

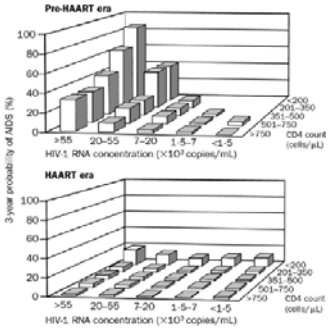
Initiation of Therapy in Asymptomatic Persons: Population Based Studies

- Clinical outcome compromised if Rx begun when CD4 <200
  - Miller et al (EuroSIDA), Ann Intern Med 1999;130:570-577
  - Hogg et al (British Columbia), JAMA 2001;286:2568
  - Sterling et al (JHU), AIDS 2001;15:2251-2257
  - Pallela et al (HOPS), Ann Intern Med 2003;138:620-626
  - Sterling et al (JHU), J Infect Dis 2003;188:1659-1665
- No virologic or immunologic advantage to starting at CD4 >350 vs. 200-350; increased rate of virologic failure when starting at CD4 <200
  - Cozzi-Lepri et al (ICONA), AIDS 2001;15:983-990
- Virologic responses comparable among groups with CD4 >200; slower decline to RNA <500 in those with RNA's >100,000 at baseline
  - Phillips et al (SHCS, EuroSIDA, Frankfurt), JAMA 2001;286:2560-2567
- Clinical outcome compromised if Rx begun when CD4 <200 or RNA >100,000
  - Egger et al (13 cohorts, >12,000 persons), Lancet 2002;360:119-129

Therapeutic Implications of Third Phase of HIV RNA Decay: Latent Cell Reservoir

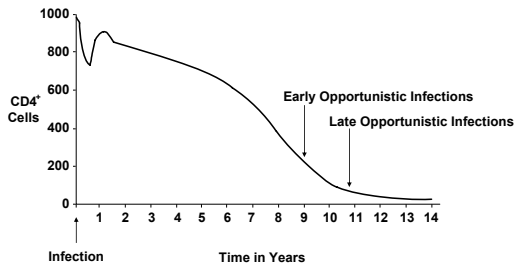
- Viral eradication not possible with current drugs
- Archive of replication competent virus history is established
  - Drug susceptible and resistant
- Despite the presence of reservoir(s), minimal degree of viral evolution observed in patients with plasma HIV RNA levels <50 c/ml suggests that current approach designed to achieve maximum virus suppression is appropriate

Prognosis According to CD4 and RNA: ART Cohort Collaboration



Egger M et al: Lancet 2002;360:119-129

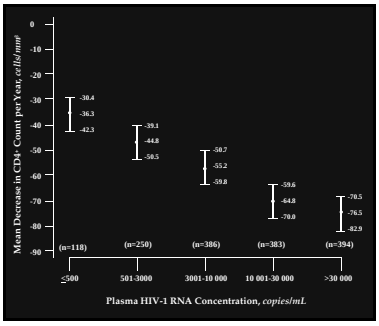
Natural History of Untreated HIV-1 Infection



CD4 and HIV-1 RNA (II)

- Good but incomplete surrogate markers
  - For both natural history and treatment effect
- Thresholds are arbitrary
  - Disease process is a biologic continuum
  - Gender specificity of HIV RNA in early-mid stage disease needs to be considered
- Treatment decisions should be individualized
  - Baseline should be established
  - Trajectory determined

MACS: CD4 Cell Decline by HIV RNA Stratum



Mellors et al: Ann Intern Med 1997;126:946-954

HIV Resistance: Underlying Concepts

- Genetic variants are continuously produced as a result of high viral turnover and inherent error rate of RT
  - Mutations at each codon site occur daily
    - » Survival depends on replication competence and presence of drug or immune selective pressure
  - Double mutations in same genome also occur but 3 or more mutations in same genome is a rare event
  - Numerous natural polymorphisms exist

CD4 and HIV-1 RNA (I)

- Independent predictors of outcome in most studies
- Near-term risk defined by CD4
- Longer-term risk defined by both CD4 and HIV-1 RNA
- Rate of CD4 decline linked to HIV RNA level in untreated persons

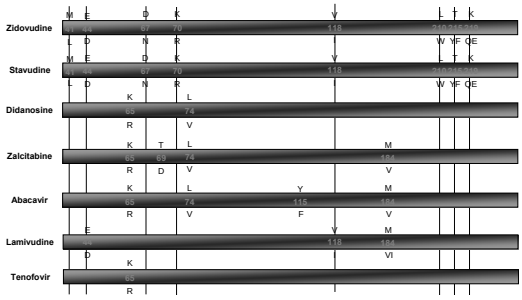
Pre-existence of Resistant Mutants

- Viral replication cycles: 10^9-10^{10}/day
- RT error rate: 10^{-4}-10^{-5}/base/cycle
- HIV genome: 10^4 bp
- Every point mutation occurs 10^4-10^5 times/day
  - In drug naïve individuals
    - » Single and double mutants pre-exist
    - » Triple and quadruple mutants would be predicted to be rare

HIV Resistance: Underlying Concepts

- **Implications**
  - **Resistance mutations may exist before drug exposure and may emerge quickly after it is introduced**
  - **Drugs which develop high level resistance with a single mutation are at greatest risk**
    - » **e.g., 3TC, NNRTI's (nevirapine, efavirenz)**
  - **Resistance to agents which require multiple mutations will evolve more slowly**
  - **Partially suppressive regimens will inevitably lead to emergence of resistance**
  - **A high 'genetic barrier' needs to be set to prevent resistance**
    - » **Potent, combination regimens**

Mutations Associated with nRTIs/ntRTIs



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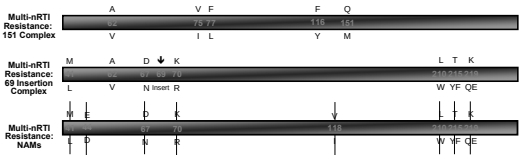
HIV Drug Resistance: Definitions

- **Genotype**
  - **Determines phenotype**
  - **Major and minor mutations for PIs**
- **Phenotype**
  - **Drug susceptibility**
- **Virtual phenotype**
  - **Result of large relational genotype and phenotype database**

Mutations Associated with nRTIs/ntRTIs



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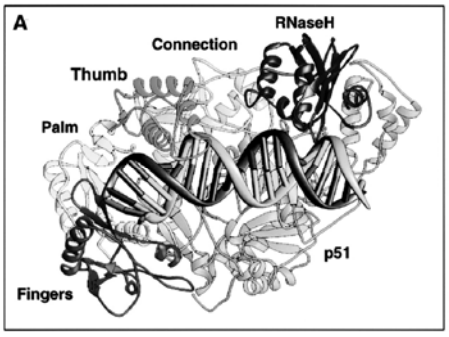
HIV Drug Resistance: Methodologies

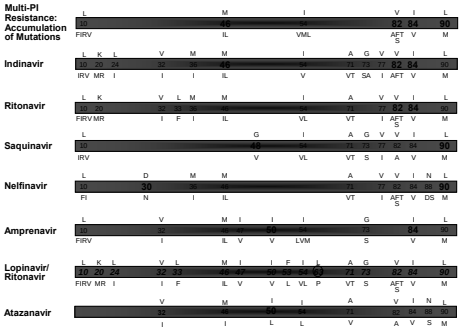
- **Genotyping**
  - **Different platforms**
    - » **Dideoxy sequencing**
    - » **Gene chip**
    - » **Point mutation assays**
- **Phenotyping**
  - **Recombinant virus assays**
- **Virtual phenotyping**
  - **Informatics**



Nucleoside Analog Resistance

TAM's (M41L, D67N, K70R, L210W, T215F/Y, K219Q/E/N)	M184V	K65R
Confer ZDV resistance thru ZDV-MP excision	Confers 3TC resistance thru decreased 3TC-TP incorporation	Confers non-ZDV NRTI resistance thru decreased analog incorporation
Antagonize K65R	Decreases ZDV resistance thru decreased ZDV-MP excision	Decreases ZDV resistance thru decreased ZDV-MP excision

Mutations Selected by PIs



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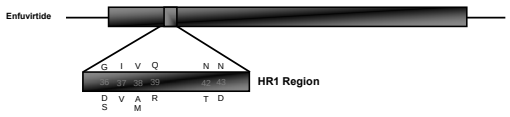
Pyrophosphorolysis

Courtesy M. Parniak
Mellors, 9th CROI, 2002

Mutations in the GP41 Envelope Gene Associated With Resistance to Entry Inhibitors



Mutations Selected by NNRTIs

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Progress in HIV Disease