HIV Diagnosis and Pathogenesis

Scott M. Hammer, M.D.

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

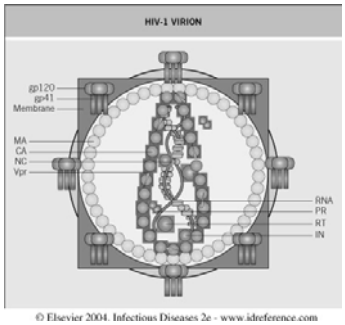
Laboratory Diagnosis of Established HIV Infection: Antibody Detection

- 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

Laboratory Diagnosis of Acute HIV-1 Infection

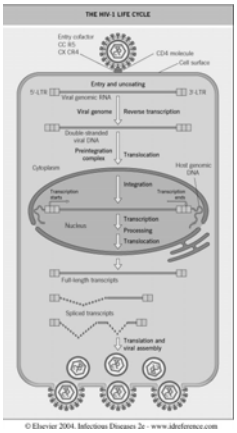
- Patients with acute HIV infection may present to a health care facility before full antibody seroconversion
  - ELISA may be negative
  - ELISA may be positive with negative or indeterminant Western blot
- Plasma HIV-1 RNA level should be done if acute HIV infection is suspected
- Follow-up antibody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

HIV-1 Virion

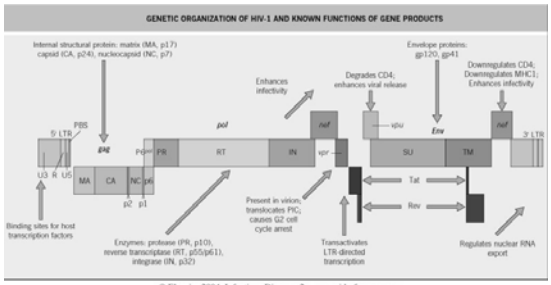


HIV Life Cycle

Tat = transcriptional activator
Rev = regulator of mRNA nuclear export



HIV-1: Genetic Organization



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

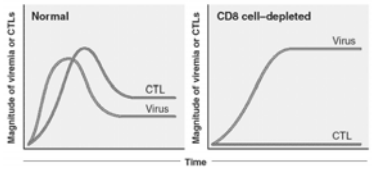
Determinants of Outcome: Selected Viral Factors

- 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

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

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

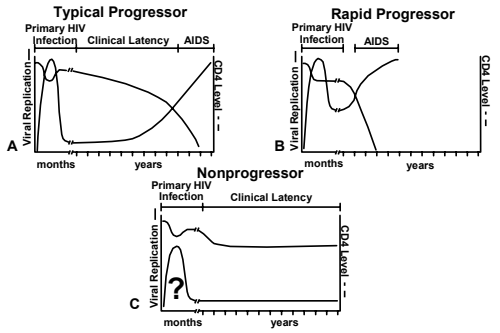
Host Factors in HIV Infection (III)

- 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

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

The Variable Course of HIV-1 Infection



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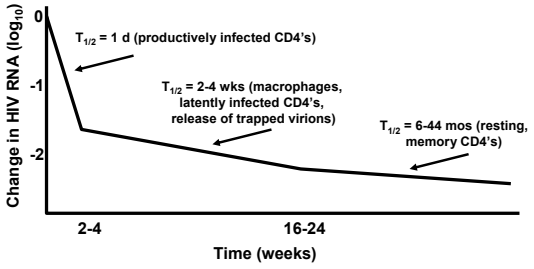
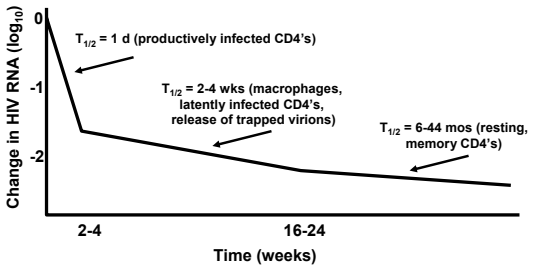
Phases of Decay Under the Influence of Potent Antiretroviral Therapy



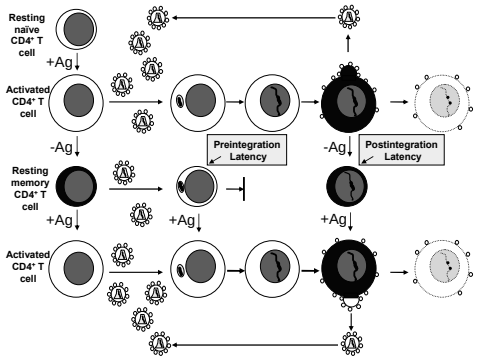
Therapeutic Implications of First and Second Phase HIV RNA Declines

- 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



Model of Post-Integration Latency



Siliciano R et al

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

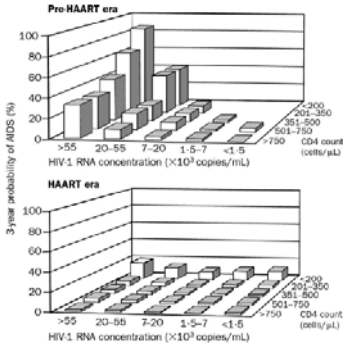
Initiation of Therapy in Established HIV Infection: Considerations

- 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

Initiation of Therapy in Asymptomatic Persons: Population Based Studies

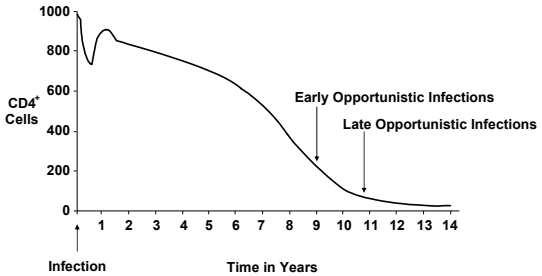
- Clinical outcome compromised if Rx begun when CD4 <200
  - Miller et al (EuroSIDA), Ann Intern Med 1999;130:570-577
  - Hogg et al (British Columbia), JAMA 2001;286:2568
  - Sterling et al (JHU), AIDS 2001;15:2251-2257
  - Pallela et al (HOPS), Ann Intern Med 2003;138:620-626
  - Sterling et al (JHU), 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

Prognosis According to CD4 and RNA: ART Cohort Collaboration

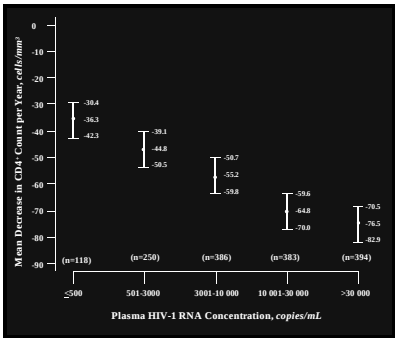


Egger M et al: Lancet 2002;360:119-129

Natural History of Untreated HIV-1 Infection



MACS: CD4 Cell Decline by HIV RNA Stratum



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

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

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

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

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

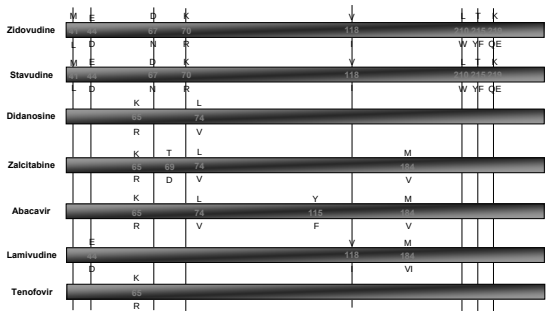
HIV Drug Resistance: Definitions

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

HIV Drug Resistance: Methodologies

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

Mutations Associated with nRTIs/ntRTIs



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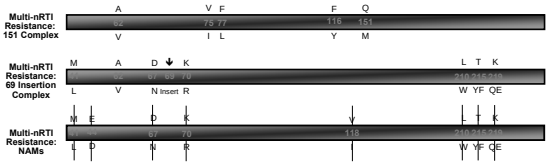
Mutations Associated with nRTIs/ntRTIs



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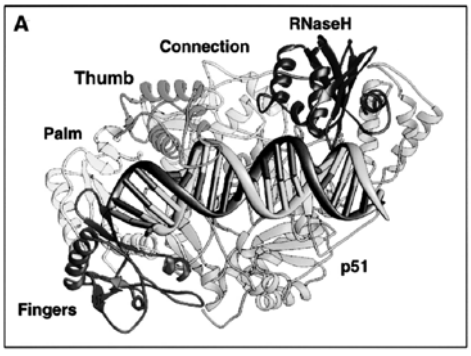

Nucleoside Analog Resistance

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

Pyrophosphorolysis

where R = AMP (ATP-dependent phosphorolysis)

or

R = H (pyrophosphorolysis)

Courtesy M. Parniak
Mellors, 9th CROI, 2002

Mutations Selected by NNRTIs

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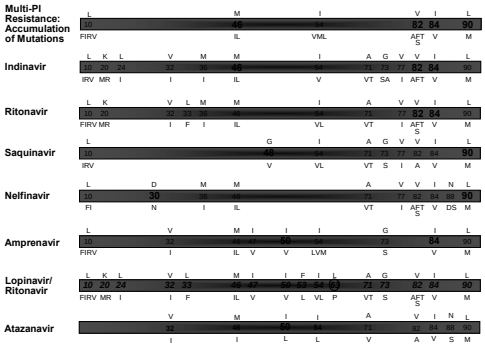
Mutations Selected by PIs



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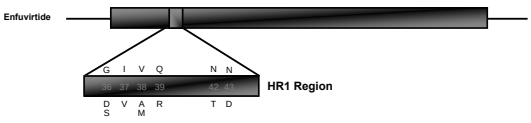
Mutations in the GP41 Envelope Gene Associated With Resistance to Entry Inhibitors



Progress in HIV Disease