VIRAL DYNAMICS OF HIV-1 INFECTION

Des Martin, *MB ChB, MMed, FCPath, DTM&H, DPH*
Toga Laboratories, and University of the Witwatersrand

A greater understanding of the dynamics of HIV-1 infection has resulted from the development and application of molecular biology technologies, in particular the nucleic acid technologies for the detection and quantification of virus in various tissue compartments. This has led to a greater appreciation of the interaction between the virus and the immune system and the immunopathogenesis of the disease. Based on these factors, the development of treatment guidelines has become a more rational process.

EARLY EVENTS IN HIV-1 INFECTION

The most common mode of HIV-1 infection is sexual transmission across the genital mucosa.[1] Studies in the simian animal model have provided important insights into this process.[2] The first cellular targets of the virus are Langerhans cells, which are tissue dendritic cells found in the lamina propria in the cervicovaginal epithelium. Within 2 days after infection virus can be detected in the draining internal iliac lymph nodes; thereafter dissemination of the virus occurs and virus can be cultured from the peripheral blood 5 days after infection. In the human there appears to be a variation in the time from initial infection to viraemia, with estimates varying from 4 to 11 days.[3] Clearly, breaks in the epithelium associated with trauma or genital infections will accelerate this process.

It has been shown that persons with acute HIV infection demonstrate selective infection with certain variants of HIV-1. Sexually transmitted variants are typically macrophage-tropic, which are taken up by susceptible cells bearing the CCR5 receptor. This chemokine receptor acts as a co-receptor which is a necessary requirement for HIV to enter cells. These macrophage-tropic viruses are termed R5 viruses. This co-receptor pattern explains why persons who are homozygous for a 32 base pair deletion in CCR5 (CCR5)32) are relatively resistant to the usual R5 strains.[4] After infection there is a rapid rise in plasma viraemia with widespread dissemination of the virus to many organs including the lymphoid system and central nervous system.

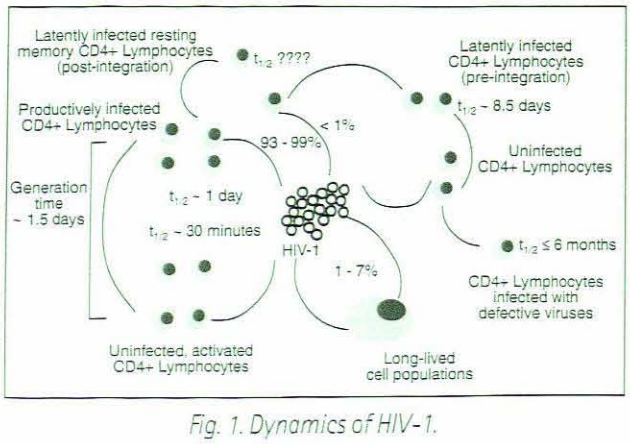

Fig. 1. Dynamics of HIV-1.

VIROLOGICAL EVENTS AT PRIMARY INFECTION

Primary infection is usually accompanied by high titres of virus, often to levels in excess of 1 million HIV-1 RNA copies/ml of plasma.[5] The plasma viraemia is curtailed by the emergence of the immune response predominantly mediated by CD8+ cytotoxic lymphocytes. It has been shown that as many as 1 in 17 CD8+ T cells in the peripheral blood may be cytotoxic T-lymphocytes specifically targeted against the virus (these may be reported on a blood count as atypical reactive lymphocytes, which are also noted in glandular fever caused by Epstein-Barr virus infections[6]). The diagnosis of acute primary infection will rely on the detection of HIV-RNA by polymerase chain reaction (PCR) or by a positive p24 antigen test in a subject who tests negative for HIV antibodies. Treatment of acute primary HIV infection (if identified) is recommended for a number of reasons: (*i*) to limit the dissemination of the virus; (*ii*) to establish a lower setpoint; and (*iii*) to preserve crucial HIV-specific immune responses, which have been shown to be lost early in infection. The duration of treatment of primary infection is currently uncertain but it should be of the order 9 - 12 months (the normal maturation period for the immune response being approximately 6 months). Thereafter treatment is deferred until the virological or immunological endpoints, according to the guidelines, are reached.

THE STEADY STATE

As can be seen in Fig. 1, the immune response is effective in reducing the viral levels to a level that is termed the virological setpoint. This setpoint varies between individuals and is influenced by a number of factors including the quality of the immune response, genetic factors and antiretroviral therapy. The level of the setpoint has prognostic significance with a survival advantage in

patients with a low(er) setpoint.[7] One important observation to emerge from measurements of plasma virus levels in HIV-1 infection is that viral replication continues throughout the disease even in the clinically latent asymptomatic phase. In untreated, asymptomatic patients the plasma HIV-1 RNA levels are typically in the range of 10^3 - 10^6 copies/ml of plasma. Titres of infectious culturable virus are several orders of magnitude lower, indicating that much of this plasma virus is defective, decayed or neutralised.[8] This steady state is reasonably stable and is a reflection that virus production equals viral clearance. Current evidence favours the idea that most of the plasma virus comes from recently infected CD4+ lymphocytes in the peripheral lymphoid tissues. It is to be remembered that only 2% of the lymphocyte population circulates at any given time. The majority of viral replication takes place in the lymph nodes and other lymphoid tissue such as the mucosa and skin-associated lymphoid tissue.[9]

Between 93% and 99% of the daily turnover of virus results from the productively infected lymphocytes (Fig. 1). Up to 10^9 virus particles are produced and cleared on a daily basis; this is accompanied by an increased daily turnover of CD4 cells. It has been estimated[10] that the average half-life of the plasma virus is short, probably less than 6 hours. The source of the remaining 1 - 7% of daily viral turnover is derived from tissue sources including macrophages, dendritic cells and to a lesser degree, the release of trapped virus and activation of latently infected cells. It has been shown that infection of resting memory CD4+ cells (post integration latency) results in a small but nonetheless important reservoir of infection. The lifespan of these cells is long and because of their resting state currently available antiretroviral drugs, which rely on attacking relevant enzymes while the virus is replicating, are thus rendered ineffective. Strategies to purge these cells of the integrated latent virus are currently under investigation, because until this reservoir is eliminated the cure of HIV infection will remain elusive.

VIRAL DECAY ON TREATMENT

FIRST-PHASE DECAY

Research in 1995 describing the effects of potent antiretroviral therapy in HIV-infected individuals not only changed the course of therapy but provided novel insights into the pathogenesis of the disease.[10,11] The critical finding was that potent antiretroviral drugs produced a rapid exponential drop in plasma virus. Typically plasma virus levels dropped by 100-fold in a period of 2 weeks (Fig. 2). This rapid exponential decline observed in treated patients was taken as evidence that, as the drugs prevent any new infection of susceptible cells, the observed decreases reflect

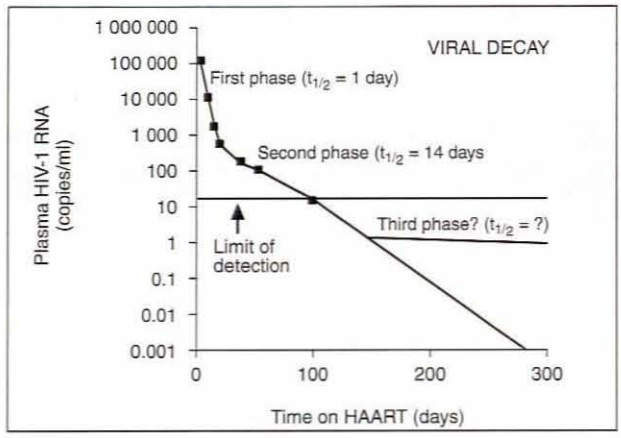

Fig. 2. Viral decay.

the intrinsic decay rates in the various cellular and extracellular compartments that harbour virus. Subsequent research[12] showed that the initial rapid decay was the result of clearance of free virions from the plasma and the loss of the productively infected cells.

SECOND-PHASE DECAY

Studies have shown that after the initial rapid decay in the first 1 - 2 weeks of treatment, plasma virus declined at a slower rate, reflecting the turnover of a longer-lived cell population or reservoir.[12] This reservoir accounts for only a small proportion of the total virus production in an untreated individual and becomes evident only when the cells that produce most of the plasma virus have decayed. The half-life of the cells responsible for this second-phase decay is estimated to be 1 - 4 weeks. In many patients the second-phase decay brings the levels of virus down below the level of detection in currently available assays (< 50 copies/ml plasma). It is assumed that provided there are no hitherto undiscovered additional reservoirs, the second-phase of decay extrapolates to zero residual cells after 2 - 3 years of completely suppressive therapy. The nature of the compartments contributing to this second-phase decay is thought to be tissue macrophages and dendritic cells.

THIRD-PHASE DECAY

It has been shown that if therapy is withdrawn in patients, who have been on long-term suppressive therapy, a vigorous viral rebound occurs 10 - 12 days after stopping therapy. This implies that there is a viral compartment in existence that in the first instance must be extremely stable, and secondly is not influenced by the antiretroviral therapy that has effectively eliminated virus from the compartments mentioned previously. It is thought that long-lived memory cells (CD45+RO+) bearing integrated provirus constitute this long-lived reservoir.[13] This form of latent infection constitutes an extreme barrier to the eradication of HIV, as research has shown that the frequency of these latently infected cells has shown no detectable decrease during the first 2 years of therapy.[14] The

decay rates of this reservoir are extremely slow, consistent with the long-term survival of resting memory cells (mean intermitotic interval 5.5 months[15]).

TURNOVER OF CD4+ LYMPHOCYTES

A hallmark of HIV-1 disease is the loss of CD4+ lymphocytes (Table I). The rate of loss of these cells varies between 20 and 80 cells per year but will vary according to the factors such as viral load, with increased loss of cells occurring in patients with higher viral loads. Up to 10^9 cells are destroyed and replenished in a quasi-steady state in early disease. A variety of mechanisms contribute to the loss of CD4+ cells, including direct effects of the virus and indirect immunological effects. Recent research has implicated a failure of regenerative thymopoesis as an important mechanism, resulting both from infection of stem-cell precursors and failure of programming of naïve T cells in the thymus or thymic equivalents.[16] There is an impressive increase in both CD4+ and CD8+ lymphocytes following the institution of antiretroviral therapy. The early rise in lymphocytes is due to cells bearing the memory phenotype (CD45RO+) and comes about as a result of the redistribution of these cells from peripheral lymphoid tissues. There follows a more gradual increase over months of cells bearing the naïve phenotype (CD454RA+) as part of the immune reconstitution process.

TABLE I. CD4 LYMPHOCYTE DEPLETION

- Hallmark of HIV disease
- 2 billion CD4 cells destroyed each day
- CD4 destruction by many means
- Failure of homeostasis related to regenerative capacity
- Role of the thymus

VIRAL DIVERSITY AND RESISTANCE

The high rate of viral replication of HIV-1 promotes the development of different but related strains within the blood and lymphoid tissues of the patient. This heterogeneity of viral strains leads to a 'quasispecies' of viral strains within individual patients, which broadens as the infection progresses over time. The factors associated with viral variability are listed in Table II.

The replication dynamics in various anatomical compartments differ leading to the generation of distinct quasispecies within the central nervous system, genital tract and other tissue sites. As the penetration of drugs in these sites varies, the generation of distinct resistant species within these sites may occur. There is always the possibility that viruses possessing resistant patterns, distinct from the populations within the blood, can traffic back into the blood.

TABLE II. VARIABILITY OF HIV

- RNA viruses lack proof-reading mechanism
- High replicative rate
- RT enzyme is error prone
- Selection pressure
- Recombination events

Leads to the generation of viral 'quasispecies'

It is also important to understand that a virus that has such a high replicative capacity can generate mutations associated with resistance **prior** to the introduction of specific antiretroviral drugs. It has been estimated that there is a mutation rate of one nucleotide base per replication cycle. Against a background HIV replication rate of 10^9 viral particles per day, this leads to the generation of every possible mutation 10^3 per day.[17] Single mutations associated with drug resistance are therefore likely to exist in the untreated patient. The replicative capacity of these variants is often impaired (viral 'fitness' is reduced) and they exist in the quasispecies as a minority population, but have the capability to become a major population when a specific drug is introduced.

REFERENCES

1. Royce RA, Šena A, Cates W jun, Cohen MS. Sexual transmission of HIV. *N Engl J Med* 1997; **336:** 1072-1078.
2. Spira AI, Marx PA, Patterson BK, *et al.* Cellular targets of infection and route of viral dissemination after an intravaginal inoculation of simian immunodeficiency virus into rhesus macaques. *J Exp Med* 1996; **183:** 215-225.
3. Nui MT, Jermano JA, Reichelderfer P, Schnittman SM. Summary of the National Institutes of Health workshop on primary human immunodeficiency virus type 1 infection. *AIDS Res Hum Retroviruses* 1993; **9:** 913-924.
4. Paxton WA, Martin SR, Tse D, *et al.* Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposure. *Nat Med* 1996; **2:** 412-417.
5. Bollinger RC, Brookmeyer RS, Mehendale SAM, *et al.* Risk factors and clinical presentation of acute primary HIV infection in India. *JAMA* 1997; **278:** 2085-2089.
6. Borrow P, Lewicki H, Wei X, *et al.* Antiviral pressure exerted by HIV-1-specifiic cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. *Nat Med* 1997; **3:** 205-211.
7. Mellors JW, Rinaldo CR jun, Gupta P, *et al.* Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 1996; **272:** 1167-1170.
8. Finzi D, Siliciano RF. Viral dynamics in HIV-1 infection. *Cell* 1998; **93:** 665-671.
9. Pantaleo G, Graziosi C, Demarest JF, *et al.* HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature* 1993; **362:** 355-358.
10. Ho DD, Moudgil T, Alam M. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *N Engl J Med* 1989; **321:** 1621-1625.
11. Weu X, Ghosh SK, Taylor ME, *et al.* Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 1995; **373:** 117-122.
12. Perelson AS, Essunger P, Cao Y, *et al.* Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature* 1997; **387:** 188-191.
13. Chun TW, Stuyver L, Mizell SB, *et al.*. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci USA* 1997; **94:** 13193-13197.
14. Finzi D, Hermankova M, Pierson T, *et al.* Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 1997; **278:** 1295-1300.
15. McLean AR and Michie CA. *In vivo* estimates of division and death rates of human T lymphocytes. *Proc Natl Acad Sci USA* 1995; **92:** 3707-3711.
16. Hellerstein MK, McCune JM. T cell turnover in HIV-1 disease. *Immunity* 1997; **7:** 583-589.
17. Omrani AS, Pillay D. Multi-drug resistant HIV-1. *J Infect* 2000; **41:** 5-11.