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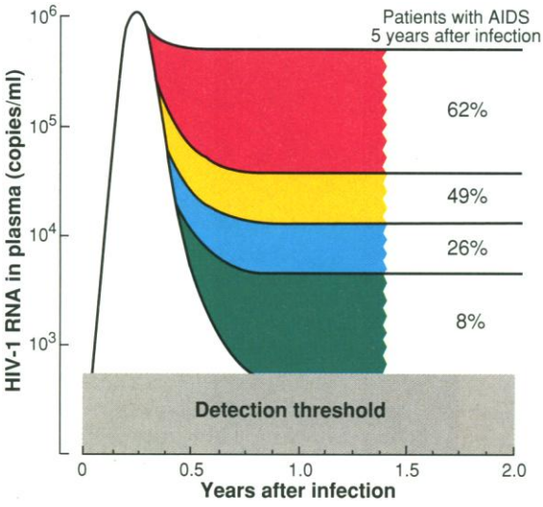
# Viral Counts Count in HIV Infection

David D. Ho

When the acquired immunodeficiency syndrome (AIDS) first appeared, its pathogenesis was frustratingly elusive because the disease does not appear immediately upon infection with the human immunodeficiency virus (HIV). There is a variable period of time during which the patient remains healthy but exhibits viremia (the virus can be detected in the patient's blood). Recent work shows that this viremia is sustained by continuous rounds of viral replication and reinfection of blood cells (*1*, *2*), dispelling the notion that HIV has a true latent period. It follows that measuring the amount of virus in the blood (viral load) should be useful in determining the prognosis of the infected individual and in monitoring the effectiveness of therapies. Among a number of reports in support of this simple view is the elegant study in this issue by Mellors *et al.* (*3*), who show that a single measurement of plasma viral load can predict the subsequent risk of AIDS or death.

Since 1989, researchers have consistently shown that patients in advanced stages of HIV-1 infection have higher concentrations of virus in their blood (*4*). Moreover, the viral load after seroconversion (appearance of antibodies to HIV in the blood) predicts the likelihood of developing AIDS later (*5*). The authors of the new study (*3*) use a commercial amplification assay (*6*) to correlate the concentration of HIV-1 RNA in plasma with the clinical course of AIDS in a cohort of infected gay men, monitored since 1984. The findings are truly striking. For example, only 8% of patients with less than 4350 copies of RNA per milliliter of blood plasma progressed to AIDS 5 years after entering the study, whereas 62% of those with viral loads greater than 36,270 copies developed AIDS (see the figure). Individuals with intermediate viral loads had progression rates of 26 to 49%. A relative hazard of death of 1.55 was noted for each threefold increase in plasma viremia. Similar results have been observed in a recent study of infected hemophiliacs (*7*). The prognostic utility of measuring plasma viral load in HIV-1 infection is now unequivocal.

When HIV-1 enters a new host, there is typically a burst of viremia, which is then inhibited by the onset of immune responses (*8*) (see the figure). The subsequent level of plasma virus is a reflection of the equilibrium reached between the virus and the host after the initial battle and is generally maintained for years. This steady-state level varies from individual to individual and is predictive of the long-term clinical outcome (*3*, *5*, *7*).




**The course of HIV infection.** Variable virologic setpoints after acute HIV-1 infection and their prognostic values. [Drawn from the data in (*3*)]

What does this virologic setpoint mean, and why is it so important prognostically? During steady state, HIV-1 clearance is balanced by its production, or $cV = \partial T^*N$ (*2*), where $c$ is the rate constant for virion clearance, $V$ is the virion concentration, $N$ is the number of virions made per infected cell (burst size), $T^*$ is the number of virus-producing cells, and $\partial$ is the rate constant for the loss of $T^*$.

Because the values for $c$ and $\partial$ do not vary significantly among patients (*2*), $V \sim NT^*$, measuring the viral load yields clear information about the total number of productively infected cells and their average burst size. A static measurement of viral load provides a kinetic view of viral production, which in turn drives a fixed rate of CD4 lymphocyte destruction. Thus, it should not be surprising that viral load is a good surrogate marker for clinical outcome in HIV-1 infection. It is, indeed, a disease marker.

If high viral loads lead to poor clinical outcome, then lowering viral loads with antiviral drugs should result in improved prognosis. Does the evidence support this assertion? In several clinical trials of relatively weak antiretroviral regimens (*9*, *10*), consistent clinical benefits were observed in conjunction with modest reductions in plasma viremia. For example, in the AIDS Clinical Trials Group Protocol 175, a tenfold reduction in HIV-1 RNA concentration in plasma was associated with an ~50% decrease in the relative hazard of death (*10*). Recently, administration of a potent protease inhibitor, ritonavir, to patients in advanced stages of HIV-1 infection reduced viral loads by a factor of 3 to 16 for 16 weeks, which corresponded to ~45% lower risk of death (*11*). However, much more impressive antiviral effects are now regularly seen with certain combination therapies. One notable example is the use of indinavir, zidovudine, and lamivudine, which together reduced the viral load to less than 1%; indeed, 85% of these subjects had undetectable plasma viremia after 24 weeks of treatment (*12*). A decline in viremia of this magnitude should result in substantial clinical improvement (*3*, *7*, *9*–*11*), but the precise benefit awaits documentation. Nevertheless, many of the patients on potent combination therapies now have viral loads below those of long-term nonprogressors (*13*). Thus, if this dramatic suppression of HIV-1 is sustainable, these patients on combination treatment will present a unique opportunity to define the viral threshold below which disease progression does not occur. Imagine, as well, the future possibilities when addi-

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tional potent agents are incorporated into these already powerful regimens.

There is little doubt that viral load determinations will become useful tools, along with CD4 lymphocyte counts, in the clinical management of HIV-1–infected patients. Both measurements provide important insights into the disease process. To borrow a crude but illustrative analogy from a prominent retrovirologist, John Coffin: The development of AIDS can be likened to an impending train wreck, where the viral load indicates the speed with which the train is headed for catastrophe and the CD4 cell count marks the distance from the site of doom. The means of slowing the train are now available, but ways of stopping and reversing the locomotive must be found.

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UPDATE

# Mammalian Cytochrome c Oxidase, a Molecular Monster Subdued

Shelagh Ferguson-Miller

The high-resolution crystal structure of mammalian cytochrome c oxidase, a key enzyme in aerobic metabloism, was recently reported in *Science* by Tsukihara *et al.* (*1*), and discussed in an accompanying Perspective (*2*). The original paper (*1*), a landmark achievement in protein structure analysis, described the structure of the six metal centers (two hemes, two copper centers, Mg, and Zn), information critical to understanding the energy-transforming activity of the enzyme. In this issue, Tsukihara *et al.* (*3*) now present the complete structure of bovine cytochrome c oxidase at ≈2.8 resolution. With a molecular weight of 200,000 and 13 different subunits, this is the largest, most complex membrane protein so far analyzed at this resolution. The atomic coordinates of the amino acids of all 13 peptides have been determined, and the refinement of the x-ray data did not alter the sites of the metal centers previously reported, which show remarkable structural identity with the bacterial version of the enzyme (*4*).

The full text of a previous Perspective on this topic (*2*) can be found at http://science-mag.aaas.org/science/scripts/display/full/269/5227/1063.htm

The number, location, and functional significance of the numerous subunits of the mammalian enzyme have been hotly debated for many years (*5*, *6*). Now, at least the number and location are resolved. As originally reported by Kadenbach and colleagues (*6*), the three largest, mitochondrially encoded subunits are associated with a total of 10 nuclear-encoded peptides. The new structural information will certainly aid efforts to define the functions of these 10 dissimilar subunits, but it is already clear that none of them directly impinges on the metal centers, supporting the idea of a regulatory or insulating role.

The dimer in the crystal structure has minimal protein contacts between monomers, with subunits VIa and VIb as the sole bridging peptides. This arrangement protects an exposed side of the active site, where the monomeric bacterial enzyme (*4*) has another subunit (IV); however, a mechanistic role for the dimer seems unlikely in view of the lack of intimate contact and the existence of functional monomeric forms of several eukaryotic enzymes (*7*, *8*).

A fundamental question regarding all membrane proteins is whether there is a requirement for specific lipids. Cytochrome oxidase has been a model system for addressing this issue, because a strong association with the anionic phospholipid, cardiolipin, has reproducibly been observed (*9*, *10*). Eight phospholipids and two cholate molecules (possibly occupying adenosine diphosphate–binding sites) are resolved in the crystal structure, but not cardiolipin. Its lack of resolution in the crystals of both mammalian and bacterial oxidases (*4*) favors the idea that cardiolipin facilitates the conformational changes necessary for activity, rather than maintaining a particular static form (*11*).

The process of energy generation in all biological systems is critically dependent on the movement of protons through proteins, yet the mechanism of this transfer is not established. In the mammalian enzyme, three proton relay pathways are postulated, for which two of the entry sites are similar to those defined in the bacterial enzyme (*4*) and by mutational analysis (*12*, *13*). However, the two proposed proton pumping pathways follow different routes than the one described by Michel and colleagues, and do not access the heme $a_3$–$Cu_B$ center where oxygen chemistry is carried out. If correct, these paths suggest an indirect coupling mechanism rather than a direct connection to the oxygen chemistry, a fundamental issue remaining to be resolved.

Pathways through the protein for water, electrons, and oxygen are also proposed, the latter taking into account the higher solubility of oxygen in hydrophobic environments and suggesting the possibility that a pool of bound lipid in subunit III could provide an oxygen reservoir. It will be a challenge for future research to determine whether specific facilitated routes are mechanistically or physiologically important for any of these key substrates and products of energy transduction. The monster is subdued, but far from tamed.

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