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AIDS Pathogenesis: From Models to Viral Dynamics in Patients

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Summary: The course of HIV infection usually follows a pattern in which the immune system is initially able to limit viral replication, but ultimately fails. It is proposed that disease progression is a consequence of viral replication and evolution within the host, the immune response providing the selection pressure for increasing diversity. When the number of mutants rises above a certain threshold level, the immune system can no longer downregulate all the viral variants simultaneously and symptomatic disease results. Recent studies of the dynamics and kinetics of HIV infection have indicated that 30% of the free virus population in the plasma is replenished each day, a very high turnover rate. After intervention with nevirapine, resistant virus was observed to have replaced wild-type virus in the plasma within 2–4 weeks. Even in late-stage patients, the immune system has the capacity to regenerate large numbers of CD4 cells, but this ability does not continue indefinitely. This implies that to reverse immunodeficiency the principal objective of treatment in the management of HIV infection should be inhibition of viral replication and thus prevention of lymphocyte destruction. **Key Words:** AIDS—Viral dynamics of HIV—Viral evolution.

A characteristic of HIV disease is the long delay in the onset of symptomatic illness after initial infection. This delay lasts for 10 years on average, but varies widely. In some individuals, disease develops after 2 years and in others only after 15 years. Among the most fundamental and interesting questions surrounding the mechanism of viral pathogenesis are: What causes the length and the variability of the asymptomatic phase, and why does the immune system initially control the virus but eventually fail? The model of HIV pathogenesis described below proposes that during the course of HIV infection the virus is constantly evolving away from immunologic pressure and finally reaches a point at which the immune system can no longer downregulate all the different viral mutants simultaneously.

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VIRAL EVOLUTION

After infection with HIV, patients initially enter the primary infection phase, which usually lasts for a few weeks and is characterized by very high viral levels and a decline in CD4 cell counts. Vigorous immune responses are then activated against the virus in the form of cytotoxic T cells and antibodies (1–3). These lead to the resolution of the initial viremia and the restoration of CD4 cell counts to almost preinfection levels. The response by cytotoxic T cells is among the most activated for any viral infection (4). These responses, and presumably also antibodies, keep the virus in check for many years. Eventually the virus replicates to very high levels, leading to the final destruction of the immune system and to clinical symptoms. An understanding of the reasons for this pattern of disease progression, in which the immune system initially controls the virus but ultimately fails, is of fundamental importance.

A model is proposed which contends that disease

progression is a consequence of viral evolution within the host (5–8). Two basic assumptions are necessary in terms of the model to explain the pathogenesis of HIV infection. First, it is presumed that the virus kills CD4 cells and thus impairs the host's immune responses. The second assumption is that HIV has the ability to mutate during the course of a given infection and to produce variants that can escape, at least to some degree, from the immune responses against the virus. Hence, the virus can evolve away from immunologic pressure. Figure 1 shows a computer simulation that assumes that rapid viral replication is taking place from the time of infection. The computer simulation begins with a heterogeneous inoculum of different viral mutants. The concentration of individual viral mutants is shown as a function of time. Initially the virus replicates at a very high level, which is characteristic of the primary phase of infection. The immune system then acts against the initial mutants and downregulates these viral strains to a low level. In the meantime, new mutants have been produced which can escape from the immune responses raised against the original mutants. They do not escape, however, from cross-reactive responses against the original mutants. This explains why subsequent mutants do not reach exactly the same abundance as original mutants. In this sequence of events, new mutants may be suppressed and other mutants may arise. The immune system is constantly providing the major selection pressure for the rise of mutants, which may not be recognized by current immunologic responses.

THE DIVERSITY THRESHOLD THEORY OF HIV PATHOGENESIS

These dynamics were predicted by the mathematical equation of the model. It was very surprising to observe that, eventually, the viral mutants suddenly begin to proliferate to very high levels. The explanation for this is that the immune system can downregulate the viral population only when viral diversity is below a certain threshold value. When the number of mutants in an individual rises above this threshold value, the immune system can no longer downregulate them all simultaneously. This is, in essence, the diversity threshold theory of HIV pathogenesis.

It is assumed that in most cases individuals are infected with a heterogeneous viral population but

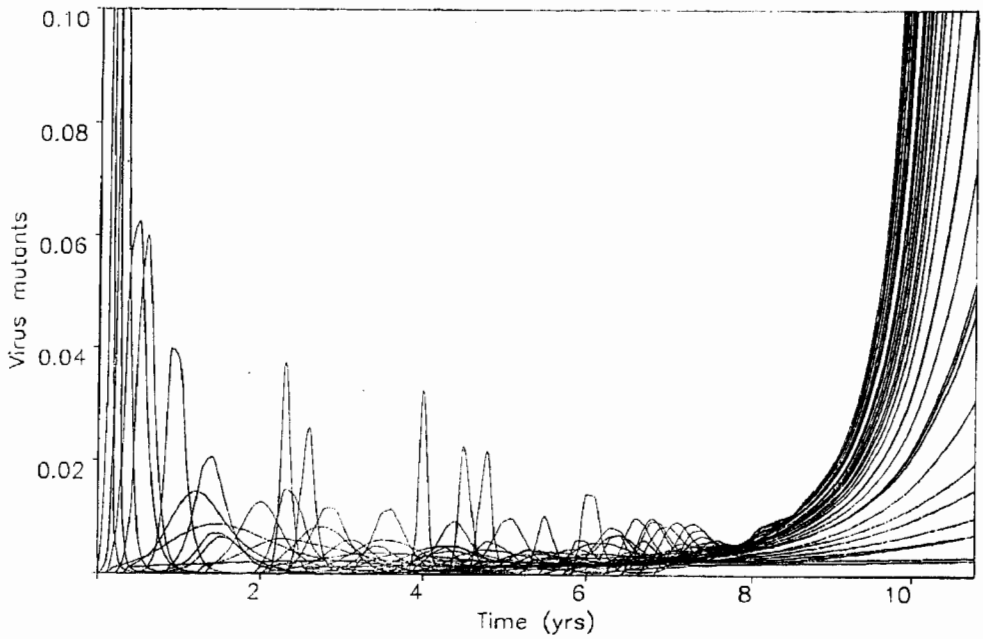

FIG. 1. Co-evolution and co-existence of many different virus strains during the time of infection. Initially the strains grow to high levels, which may cause the clinical symptoms observed during primary HIV infection. The subsequently emerging escape mutants are suppressed at a faster rate, because of the action of cross-reactive immune responses. Different virus strains grow to different levels according to their growth rates. The accumulation of viral diversity breaches the threshold after approximately 7 years in this simulation. In the final phase the fastest growing strains dominate the virus population. The y-axis indicates the relative concentration of different virus mutants. (Published with permission from Academic Press, Ltd.)

that in the initial absence of a functional immune system during the primary phase of the disease only the fastest growing strains are selected. Therefore, the viral diversity at the time of seroconversion and in the primary phase is expected to be low. Then the immune system responds and provides the selection pressure for increasing diversity and for poorly recognized variants. These and other selection pressures act on an extremely flexible HIV population and work in favor of the virus over the course of the infection. The immune system eventually loses control over the virus and the patient develops AIDS. During this final phase of advanced disease, when the immune system is completely exhausted, selection for the most rapidly replicating strains may continue, but there is no longer the drive for viral diversification.

Experimental confirmation of this theory of HIV pathogenesis has come from longitudinal studies of infected patients. One example concerns a patient who has been followed for 7 years. In the primary phase of the infection the viral population was extremely homogeneous with regard to the envelope protein. It then diversified into many different variants. These observations are therefore compatible with the diversity threshold theory.

Assuming that the immune responses against the virus are weak in the case of fast progressors, it can then be predicted that there is little selection pressure for diversification. It could be expected, therefore, that fast progressors would proceed rapidly to AIDS with little viral diversification. On the basis of this assumption, slow progressors would progress to AIDS with considerable viral diversification because a strong immune response selects for more variation.

THE DYNAMICS OF HIV INFECTION

In a recent collaboration with George Shaw of the University of Alabama, we examined the effect on viral load of introducing treatment with an inhibitor of HIV protease (9,10). Figure 2 shows the decline in the plasma viral load over time for eight representative patients after intervention with ABT-538 and L-735,524 (11,12). In each of these patients there was a very rapid decline in viral burden, as measured by plasma HIV RNA. From the rate of decline in viral load, the half-life of viral decay was estimated to be 2 days. This indicates that approximately 30% of the free virus population in the plasma is replenished every day and that there is a very high viral turnover rate, with a minimal production rate of approximately 10^9 virions/day. The plasma viral RNA load was stable before intervention with the protease inhibitor, implying that the daily production rate of virus is also 10^9 virions/day.

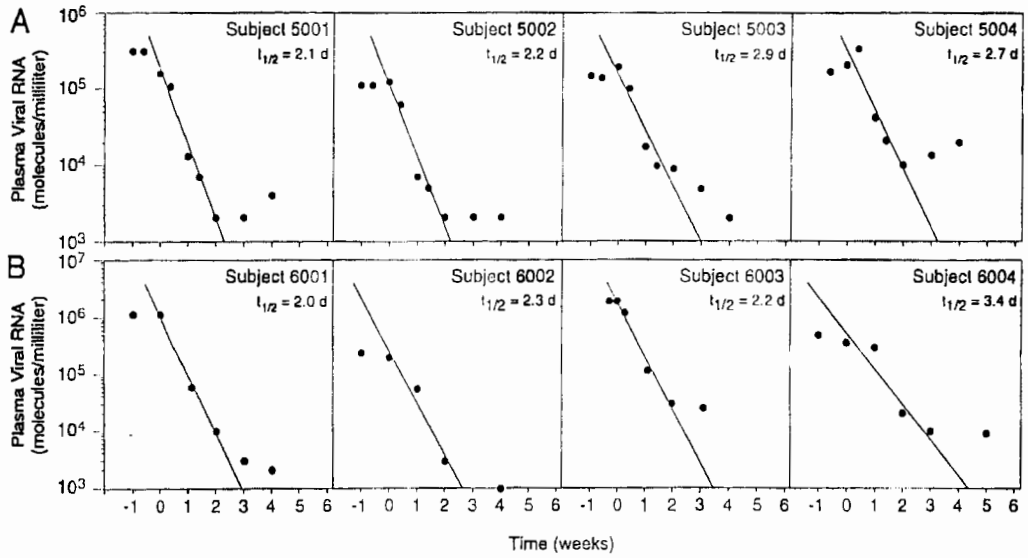

FIG. 2. Plasma viral RNA concentration in representative subjects treated with the HIV-1 protease inhibitors ABT-538 **(A)** and L-735,524 **(B).** Subjects had not received other antiretroviral agents for at least 4 weeks before therapy. Treatment was initiated at week 0 with 400–1,200 mg/day of ABT-538 or 1,600–2,400 mg/day of L-735,524 and was continued throughout the study. Viral RNA was determined by modified branched DNA (bDNA) (A) or RT-PCR (B) assay and confirmed by QC-PCR. Shown are the least-squares fit linear regression curves for data points between days 0 and 14 indicating exponential (first-order) viral elimination. (Reprinted with permission from *Nature* 1995;343:117–22. Copyright 1995 Macmillian Magazines Limited.)

Similar turnover rates can be estimated from zidovudine-treated patients (13).

The decline in plasma virus is a function of both the clearance of free virions and the rate of decay of fast virus-producing cells. The individual time constants for these two processes cannot be resolved; the decline of free virus can be measured but not the decline of virus-producing cells. It may be that the free virus decays in the plasma at a much faster rate, with a half-life of only 6 h (Dr. David Ho, Aaron Diamond AIDS Research Center, New York, personal communication), which would result in a rate of daily production and clearance of viral particles greater than 10^9. This is also only the rate for the plasma compartment and not for the lymphatic system, and therefore it may be a substantial underestimation of the total daily production of virus.

Figure 3A shows the initial decline in plasma viral RNA levels in four patients treated with the nonnucleoside reverse transcriptase inhibitor nevirapine (9,14). The half-life for viral decay was approximately 2 days. Immediately after therapy the CD4 lymphocyte count increased rapidly (Fig. 3B), even though these patients initially had low CD4 cell counts (range 20–200 cells/mm^3). The immune system is therefore able to regenerate large numbers of CD4 cells within a short time. Where do these CD4 cells originate? Is there a proliferation of CD4 cells in the periphery, or are they derived from the immune system through the activation of resting cells? Recent data from Dr. Ho suggest that they

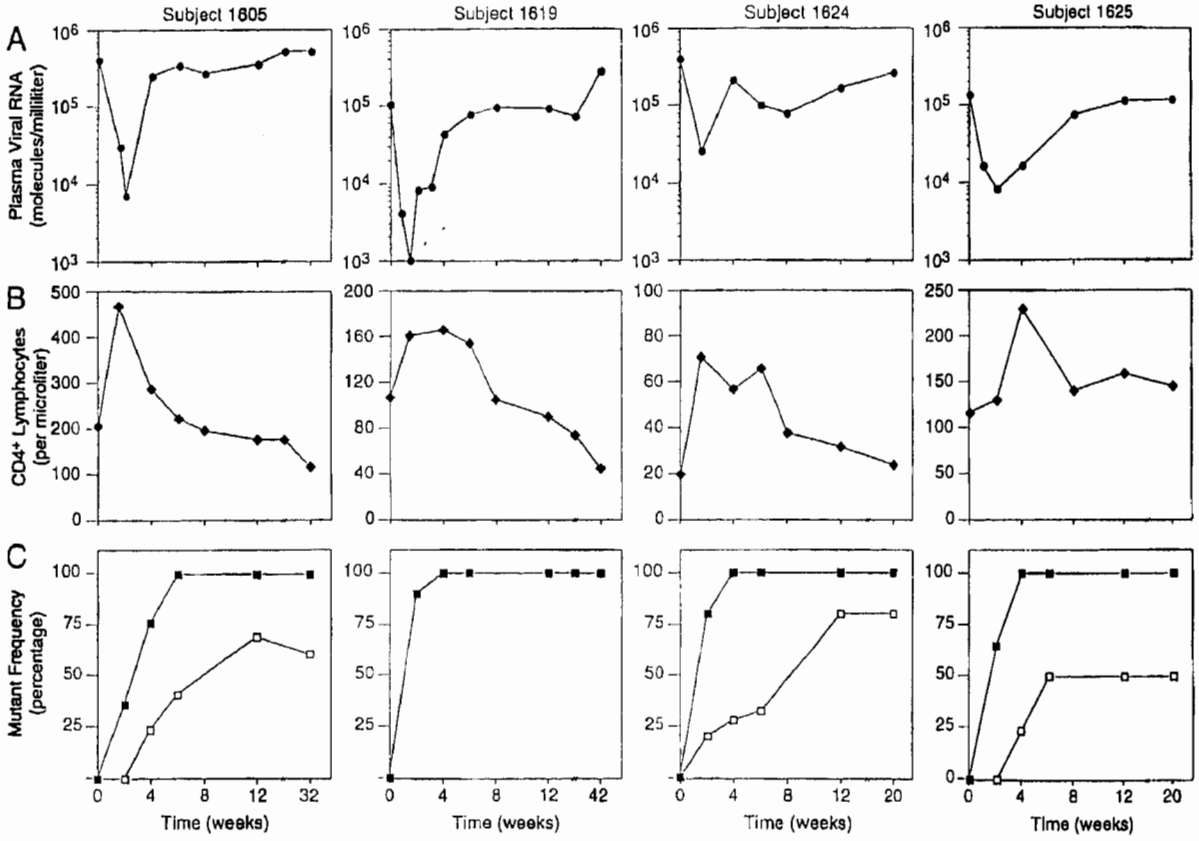

FIG. 3. Plasma viral RNA determinations **(A)**, CD4$^+$ lymphocyte counts **(B)** and percentages of mutant viral genomes in plasma and PBMCs **(C)** of subjects initiating treatment with NVP. Subjects were participants in a clinical protocol assessing the effects of nevirapine when added to existing treatment with ddI (subject 1605) or ddI plus zidovudine (subjects 1619, 1624, 1625). Treatment with nevirapine was initiated at week 0 using 200 mg/day and was increased to 400 mg/day after 2 weeks. ddI and zidovudine dosages were 400 mg/day and 300–600 mg/day, respectively. Viral RNA (●) was determined by QC-PCR assay. CD4$^+$ lymphocytes (♦) were quantified by flow cytometry. Frequencies of viral genomes containing nevirapine–resistance-associated mutations in plasma (■) and PBMCs (□) were determined by automated DNA sequence analysis with each data point representing the average of three to six independent PCR amplifications and sequence determinations. (Reprinted with permission from *Nature* 1995;343:117–22. Copyright 1995 Macmillian Magazines Limited).

arise as a result of the proliferation of CD4 cells, indicating that these cells can reproduce themselves even in late-stage-infected patients. It therefore appears that the immune system retains a high capacity to increase the CD4 cell number from a low baseline level.

At present, it is not known if this increase in CD4 cells can restore immunologic function, but the important message to be learned from these findings is that, to reverse immunodeficiency, the primary objective of treatment in HIV disease should be to interrupt viral replication rather than to increase the CD4 cell numbers in patients.

Viruses isolated from the four patients developed drug resistance very rapidly. It was shown that a single point mutation in the viral RT was responsible for conferring complete resistance to the drug. The mutant virus first emerges in the free virus population and then in the infected cell population (Fig. 3C). In each patient, this resistant virus had substantially replaced wild-type virus in the plasma only 2–4 weeks after intervention with nevirapine. This provides an independent estimate of the fast replication rate of HIV in vivo and shows a doubling time for mutant virus of approximately 2 days, which is in accordance with the value for the elimination half-life of plasma virus. The value for the rate of increase of mutant virus in the infected cell population provides the turnover rate of the infected peripheral blood mononuclear cells (PBMCs). This shows that the half-life time of the infected PBMCs is between 50 and 100 days, the same value as the half-life of uninfected PBMCs. Turnover in the blood of infected PBMCs is therefore very slow and contributes only to a small extent to plasma viral load. Recent observations have suggested that the virus is defective in PBMCs. There is therefore a small number of infected cells that produce virus over a very short time, whereas the majority of cells in the periphery harbor defective viruses that cannot be activated and are unable to replicate (George Shaw, personal communication).

When resistance to a drug develops, it should be possible to calculate the "fitness" of different virus mutants in infected individuals on the basis of data on the rate of increase of resistant mutants and on the rate of recovery of sensitive virus if drug treatment is discontinued. The relative replication rates of sensitive and resistant virus can also be determined. The difference between these two replication rates is the maximal effect that a drug treatment can achieve when resistance has developed. In principle, such studies should be useful in the selection of optimal drug combinations.

CONCLUSIONS

The evolutionary theory of pathogenesis, in which the immune system provides the selection pressure for increasing viral diversity, may provide an explanation for the slow progression to AIDS, despite the observation that HIV infection is a very fast and dynamic process. Recent studies have demonstrated that the immune systems of individuals with HIV disease retain a high capacity to regenerate CD4 cells. However, it remains to be determined whether this replenishment of CD4 cell numbers results in the restoration of immune function. Studies designed to determine whether prolonged antiretroviral treatment causes an improvement in immune function and that measure the potential changes in cytotoxic lymphocyte responses during treatment are of high priority.

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