

Viral dynamics in hepatitis B virus infection

(lamivudine/antiviral treatment/liver/viral turnover/mathematical model)

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ABSTRACT Treatment of chronic hepatitis B virus (HBV) infections with the reverse transcriptase inhibitor lamivudine leads to a rapid decline in plasma viremia and provides estimates for crucial kinetic constants of HBV replication. We find that in persistently infected patients, HBV particles are cleared from the plasma with a half-life of ≈ 1.0 day, which implies a 50% daily turnover of the free virus population. Total viral release into the periphery is $\approx 10^{11}$ virus particles per day. Although we have no direct measurement of the infected cell mass, we can estimate the turnover rate of these cells in two ways: (i) by comparing the rate of viral production before and after therapy or (ii) from the decline of hepatitis B antigen during treatment. These two independent methods give equivalent results: we find a wide distribution of half-lives for virus-producing cells, ranging from 10 to 100 days in different patients, which may reflect differences in rates of lysis of infected cells by immune responses. Our analysis provides a quantitative understanding of HBV replication dynamics *in vivo* and has implications for the optimal timing of drug treatment and immunotherapy in chronic HBV infection. This study also represents a comparison for recent findings on the dynamics of human immunodeficiency virus (HIV) infection. The total daily production of plasma virus is, on average, higher in chronic HBV carriers than in HIV-infected patients, but the half-life of virus-producing cells is much shorter in HIV. Most strikingly, there is no indication of drug resistance in HBV-infected patients treated for up to 24 weeks.

More than 250 million people worldwide are chronically infected with hepatitis B virus (HBV), and 25–40% of these will die from liver cirrhosis or primary hepatocellular carcinoma (1, 2). Chronic HBV infection is often the result of exposure early in life, leading to viral persistence in the absence of strong antibody or cellular immune responses (3). Therapy of HBV carriers can aim to either inhibit viral replication or enhance immunological responses against the virus, or both (4).

The nucleoside analogue, (–)-2'-deoxy-3'-thiacytidine (lamivudine), originally developed as an anti-human immunodeficiency virus (HIV) drug, has potent inhibitory effects on HBV replication *in vivo* (5, 6, 31). Chronic HBV carriers were treated with various doses of lamivudine. Plasma virus load was quantified at frequent times before, during, and after treatment using quantitative methods for determining viral DNA. In the first study, 45 patients were treated for 28 days; in a subsequent study, 50 patients were treated for 24 weeks. Fig. 1A shows plasma virus changes in six patients treated for 28 days. After onset of therapy viral levels decline rapidly, but as soon as the drug is withdrawn, virus returns. Fig. 1B shows changes in plasma viremia, hepatitis B antigen (HBeAg), and serum alanine aminotransferase (ALT) in six patients treated

for 24 weeks. Again we observe rapid decline in plasma virus load, which falls below detection limit in almost all patients within 2–4 weeks, and again in most patients, virus resurges rapidly as soon as the drug is withdrawn. HBeAg is a viral protein produced by infected cells; its production is not directly inhibited by lamivudine (7), and changes in the serum concentration can therefore reflect changes in infected liver cell mass. ALT is released from damaged liver cells; thus it is an indicator of the level of cell damage and death. HBeAg and ALT decline slowly during longterm lamivudine treatment. Therapeutically induced HBeAg seroconversion, seen during successful interferon α therapy and thought to represent the lysis of infected cells by the host's immune response, is not a feature of lamivudine treatment.

For a quantitative analysis of these observations, we design a simple but natural mathematical model based on ordinary differential equations for uninfected cells, x , infected cells, y , and free virus, v :

$$dx/dt = \lambda - dx - bvx,$$

$$dy/dt = bvx - ay,$$

$$dv/dt = ky - uv. \quad [1]$$

Uninfected, susceptible cells are produced at a rate, λ , which may be constant or depend on the total population size of uninfected and infected cells. Uninfected cells die at rate dx , and become infected at rate bvx , where b is the rate constant describing the infection process. Infected cells are produced at rate bvx and die at rate ay . Free virions are produced from infected cells at rate ky and are removed at rate uv . Strictly speaking, the decay rate of free virus should also be a function of the uninfected (and infected) cell population, but we expect the leading term of viral decay to be independent of changes in the host cell population. Therefore it is a reasonable assumption to treat u as a constant. The magnitude of the parameters a , b , k , and u will be determined by antiviral immune responses. In the absence of treatment the system converges to a steady state of persistent infection, provided the basic reproductive ratio of the virus, $lbk/(adu)$ is greater than 1. This condition is likely to be fulfilled if patients have weak immune responses against free virus (low u) or against infected cells (low a). Hence, in our simple model weak immune responses predispose to carrier state.

In the replication cycle of HBV, the viral reverse transcriptase is responsible for the synthesis of new HBV DNA from the pregenomic mRNA template (8, 9). Therefore, lamivudine can prevent the production of new virus particles from already infected cells ($k = 0$). But the viral polymerase is also essential for completing the double-stranded circular

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Abbreviations: HBV, hepatitis B virus; HIV, human immunodeficiency virus; HBeAg, hepatitis B antigen; ALT, alanine aminotransferase; cccDNA, covalently closed circular DNA.

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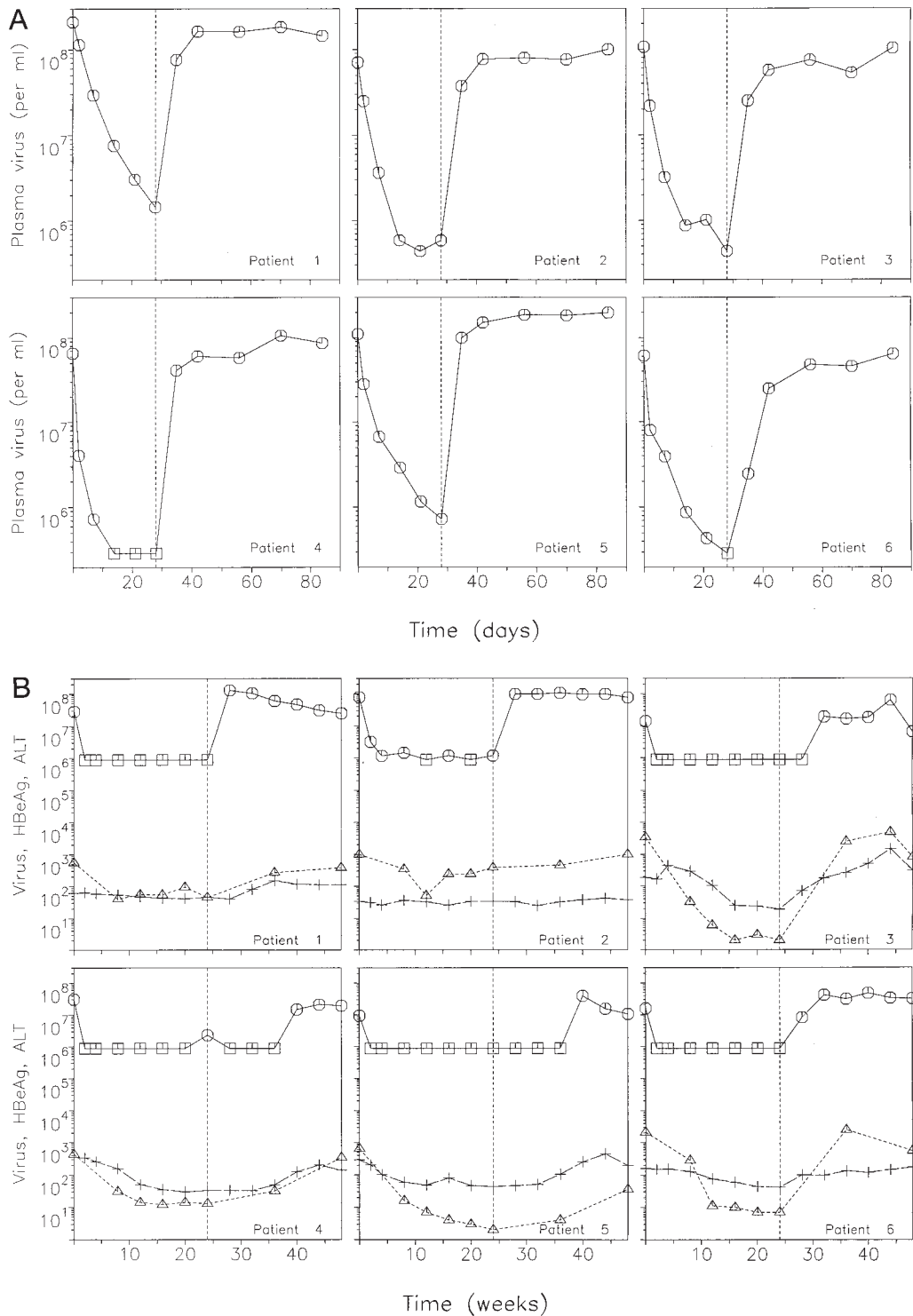


FIG. 1. (A) Rapid viral decline in response to lamivudine treatment in six patients. In this study, a total of 45 patients received lamivudine therapy at various doses (5, 20, 100, 300, and 600 mg per day) for 28 days. Plasma virus was determined at days 0, 2, 7, 14, 21, 28, 35, 42, 56, 70, and 84. Squares indicate virus load below detection limit. (B) Viral load (circles and squares), HBeAg (triangles), and ALT levels (crosses) in six patients treated for 24 weeks. In this study, a total of 50 patients received lamivudine therapy at 25, 100, and 300 mg per day. Plasma virus was obtained at weeks 0, 2, and 4, and subsequently every 4 weeks until week 48. For our analysis we only used patients who received at least 100 mg per day. Serum HBV DNA was quantified using the Abbott Genotics solution hybridization kit. HBeAg was quantified using the Kodak Amerlite kit.

DNA before migration to the cell nucleus (10), and hence there is some evidence that lamivudine can prevent the infection of new cells ($b = 0$) (11). This implies that during treatment free virus is decaying exponentially according to $v(t) = v_0 e^{-ut}$. Similarly, infected cells are decaying exponentially according to $y(t) = y_0 e^{-at}$. Here v_0 and y_0 indicate free virus and infected cells at the beginning of therapy. If lami-

vidine does not effectively prevent infection of new cells, then the above equations are still very good approximations, provided u is substantially larger than a (which will be shown below). Therefore our analysis does not rely on the assumption that lamivudine also blocks new infection. From the decline of free virus we can estimate the decay rate u . Before treatment, steady state of free virus implies $ky_0 = uv_0$. We know v_0 ,

hence we can estimate ky_0 , which is the total virus production per day.

From the 1-month study we can estimate the initial rate of virus decline during the first 2 days of treatment. We obtain an average of $u = 0.67$ per day ($\sigma = 0.32$, $n = 23$), which corresponds to a half-life time ($T_{1/2}$) of 1.0 day. Hence, in the absence of treatment $\approx 50\%$ of the plasma virus is replenished every day. The total serum virus load (for 3 liters of serum) before treatment varies in different patients ranging from 10^{10} to 10^{12} particles, with an average of 2.2×10^{11} ($\sigma = 2.6 \times 10^{11}$, $n = 45$). Consequently, the total amount of plasma virus production follows a wide distribution with an average of 1.3×10^{11} particles per day ($\sigma = 8.2 \times 10^{10}$, $n = 23$). These differences are likely to reflect different population sizes of virus-infected cells in individual patients. Plasma virus levels usually correlate with abundance of infected cells as determined by histological examination of the liver (12).

Virus decline during treatment is not strictly exponential (see Fig. 1). This can be explained by the assumption that the efficacy of the drug is not 100%. Assuming a certain efficacy ρ , viral decay occurs according to $v(t) = v_0(1 - \rho + \rho e^{-ut})$. Fitting this function provides an estimate for the efficacy of the drug at various doses. We find that for daily doses of 20, 100, 300, and 600 mg, viral replication is inhibited by 87, 97, 96, and 99%, respectively.

When therapy is withdrawn, virus resurges according to $dv/dt = ky - uv$. Hence, the initial virus growth rate can be approximated by

$$v(t) = v_1 e^{-ut} + (ky_1/u)(1 - e^{-ut}), \quad [2]$$

where v_1 and y_1 indicate the levels of free virus and infected cells at the end of therapy. We know v_1 by direct measurement, and we have determined the decay rate, u ; hence we can estimate ky_1 , which is the rate of virus production from infected cells at the end of therapy. Comparing ky_0 and ky_1 gives an estimate for the decay rate of infected (virus-

producing) cells, a , and consequently, their half-life. This method requires the initial growth rate of virus after therapy has stopped since y is treated as a constant. The approximation is accurate if virus load is determined early after the end of treatment. In our study, treatment was withdrawn after 28 days, and virus load was determined at days 28 and 35. We obtain an average decline of $a = 0.043$ per day ($\sigma = 0.036$, $n = 20$) corresponding to a $T_{1/2}$ of 16 days. In different patients half-lives range from about 10 to 100 days.

The broad distribution of turnover rates of infected cells may be a consequence of heterogeneity of the immune response against infected cells in different patients (13, 14). Damaged liver cells release ALT, and hence plasma ALT levels should provide some crude estimate for the amount of cell death in the liver. If most cell damage is caused by immune responses directed to infected cells (15), then ALT levels provide some estimate for the strength of the immunological response against HBV. We find a positive correlation between the decay rate of infected cells and the pretreatment ALT level among different patients (Fig. 2). This supports our hypothesis that the variability of cell decay rates reflects different strengths of anticellular immune responses and is not simply caused by fluctuations in measurement or inaccurate approximation.

In the subsequent study, treatment continued for 24 weeks, but sampling was less frequent (the first time points after 2 and 4 weeks, then every 4 weeks), and we cannot obtain accurate estimates of the viral or cellular decay rates with the above methods. However, we can obtain an independent estimate of the turnover of infected cells from the initial decay of HBeAg. Reverse transcriptase inhibitors such as lamivudine have no direct effect on the synthesis and secretion of HBeAg, which is derived from mRNA transcribed from existing covalently closed circular DNA (cccDNA) molecules of HBV within infected cells. The capacity of the infected host to synthesize this protein is dependent on the rate of infection of cells (inhibited by lamivudine) and the rate of lysis of infected cells (not inhibited by lamivudine) and dependent on immune

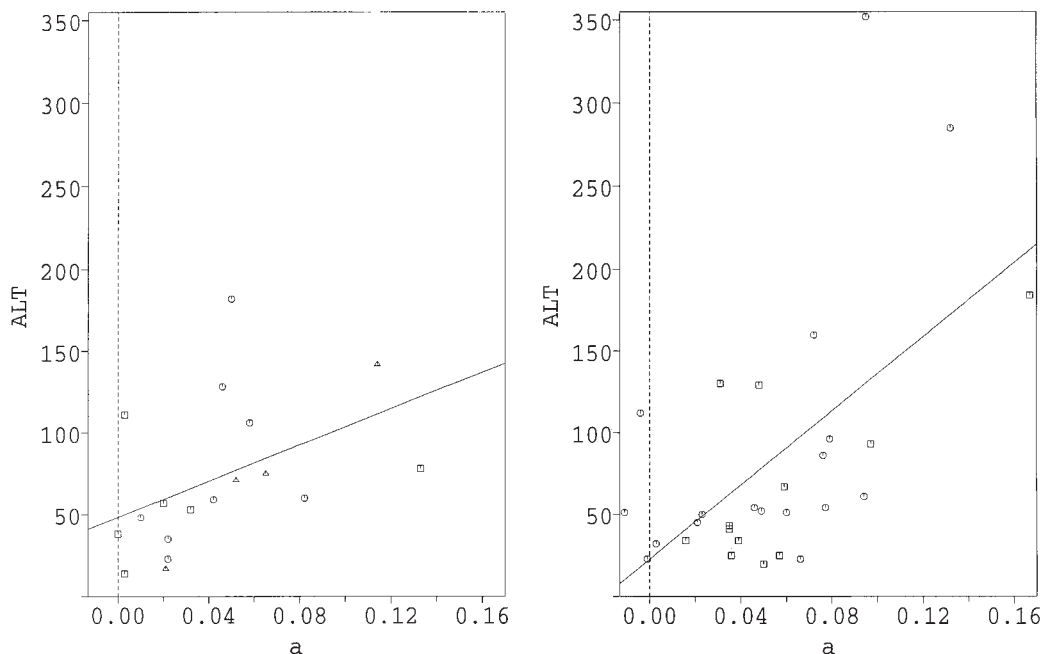


FIG. 2. Correlation between initial serum ALT level before treatment and estimated decay rate of virus-infected cells, a , during treatment. For the 28-day study the decay rate, a , was estimated by comparing the rate of virus production before and after treatment (at days 0 and 28). For the 24-week study a was estimated from the initial decline of HBeAg over the first 2 weeks. A Spearman rank correlation gives a correlation coefficient of $r = 0.61$ ($P = 0.007$) for the 28-day study, and $r = 0.50$ ($P = 0.005$) for the 24-week study. The correlation between ALT levels and decay rates a suggests that the wide distribution of calculated a values does reflect biological heterogeneity (rather than measurement uncertainty); different patients appear to have different immune responses against infected cells. The symbols reflect patients with different daily dosage of lamivudine. Squares, 100 mg; circles, 300 mg; triangles, 600 mg.

recognition of these cells. Hence the initial decay of HBeAg in patients taking lamivudine should reflect the decay of infected hepatocytes. During the first 8 weeks of treatment we observe an average decline of $a = 0.053$ per day ($\sigma = 0.039, n = 29$), which corresponds to a $T_{1/2}$ of 13 days. This is in agreement with our previous estimate. Again there is a wide distribution of half-lives and we find a strong correlation between decay of infected cells and ALT levels among different patients (Fig. 2).

After 24 weeks of treatment, patients were followed for another 24 weeks. Interestingly, there is a strong capacity in individual patients to return to the pretreatment steady-state level after these 48 weeks. Fig. 3 shows plasma virus load, ALT levels, and HBeAg levels for each patient at time 0 and after 48 weeks. Whatever factors—e.g., efficacy of antiviral and anticellular immune responses—determine the particular pretreatment steady-state level of plasma virus, HBeAg, and ALT in individual patients, it is interesting to note that these factors have apparently not changed over the time course of 48 weeks in most patients.

We also analyzed data from a cohort of Japanese patients, where treatment continued for 28 days and serum virus load

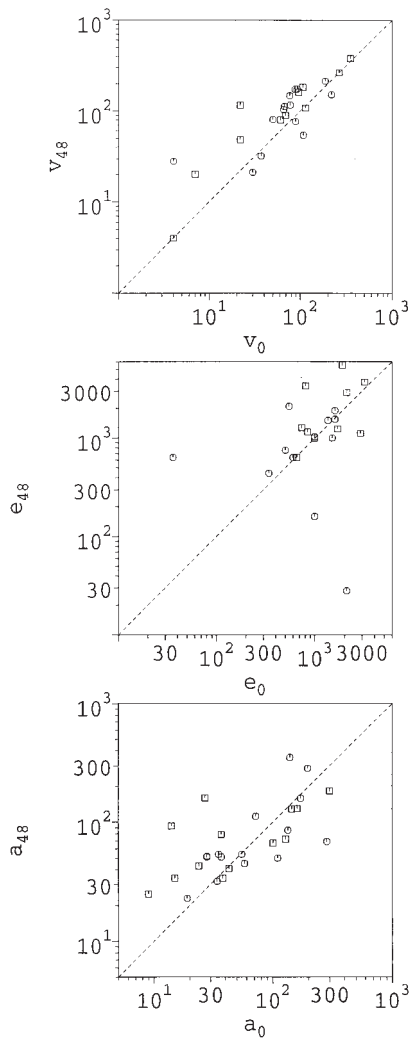


FIG. 3. Capacity to return to pretreatment steady-state levels in chronic HBV infection. Patients were treated for 24 weeks and were subsequently followed for another 24 weeks. The figure indicates virus load, HBeAg, and ALT before therapy (x axes) versus after 48 weeks (y axes). In each diagram, each patient is indicated by a point; different symbols reflect different daily dosages of lamivudine (squares, 100 mg and circles, 300 mg). Most points lie close to the diagonal indicating the tendency to return to the pretreatment level. Units: virus load in particles/ml, HBeAg in units/ml, ALT levels in units/liter.

and ALT levels were determined at days 0, 7, and 14, and subsequently every 14 days. For the virus decline as measured from the decay during the first week of treatment, we obtain $u = 0.56$ ($\sigma = 0.18, n = 67$) which corresponds to a $T_{1/2}$ of 1.2 days. Average plasma virus production is about 3×10^{12} ($\sigma = 5 \times 10^{12}, n = 57$) particles per day. For the cellular decay rate (determined by comparing virus production at day 0 and 28) we obtain a wide distribution with an average of $a = 0.053$ per day ($\sigma = 0.067, n = 46$) corresponding to a $T_{1/2}$ of 13 days. There is, however, only a weak (not significant) correlation between a and ALT levels in these patients, probably because the estimate of a is problematic in some patients, which is because viral measurements are often below detection limit and viral increase after therapy is determined from virus titer at days 28 and 42.

It is likely that HBV infects and replicates at different rates in a number of cell types (16, 17). Therefore the estimated viral production rates and turnover rates of infected cells have to be interpreted as average values. Similarly, the rate of production of viral particles and HBeAg from an infected cell may depend on the number of cccDNA molecules of HBV in the nucleus of an infected cell. During drug treatment the cccDNA copy number per infected cell may decline, which could lead to less viral and HBeAg production independent of death of infected cells. Therefore the decline of viral production and HBeAg could overestimate the actual rate of clearance of infected cells and rather reflect the decay of cccDNA. But for practical purposes the most important figure is the decay of potential virus “production units” during treatment, independent of whether this reflects cell death or decay of viral cccDNA. However, the observed correlation between ALT and the death rate of infected cells (as estimated by HBeAg decline) argues in favor of clearance of infected cells as the major mechanism. Patients with high ALT levels appear to clear virus-producing units faster.

A half-life of 10 days for infected, virus-producing hepatocytes implies that $\approx 7\%$ of this cell population is lost per day. In a chronic HBV infection, between 5% and 40% of all hepatocytes can be infected and produce virus (18). Therefore, between 0.3% and 3% of all hepatocytes are killed and must be replenished every day to maintain a stable liver cell mass. Since the liver contains $\approx 2 \times 10^{11}$ hepatocytes (19), this comes to 10^9 cells per day. This enormous activity of cell death and regeneration is likely to be a major driving force for development of hepatocellular carcinoma (17, 20).

Our data provide an interesting comparison with HIV dynamics (Table 1). In HIV infections, most patients have a turnover rate of infected cells of ≈ 2 days and free virus is cleared faster than this (21–24). In HBV infections, decay of plasma virus occurs with a half-life of ≈ 1 day in patients that generally seem to have no antibody against free virus. Interestingly, there is a much wider distribution of half-lives of virus-producing cells in HBV-infected patients ranging from

Table 1. HBV versus HIV dynamics

	HBV	HIV
Plasma virus		
Half-life	24 hr	6 hr
Daily turn-over	50%	90%
Total production (periphery)	10^{11}	10^9
Load	2×10^{11}	10^9
Infected cell		
Half-life	10–100 days	2 days
Daily-turnover	1–7%	30%

A comparison of HBV and HIV *in vivo* dynamics. Both viruses have a rapid turnover and a massive production of plasma virus. Perhaps the most important difference is in the half-life of virus-producing cells, which is much shorter for HIV. The HIV data are from refs. 21, 22, and 30.

about 10 to 100 days. HBV is believed to be largely noncytopathic, and therefore the turnover rates of infected cells can be due to different anticellular immune responses. HIV, on the other hand, can probably kill an infected cell within a few days, and the rather uniform turnover rate of productively infected cells could be the consequence of a tight struggle between viral cytopathicity and the strong cytotoxic T-lymphocyte response found in most HIV patients. The large turnover of infected CD4 cells in HIV infection [$\approx 10^9$ CD4 cells are estimated to be killed by the virus and regenerated by the immune system every day (21, 22)] is matched by an equivalent number of hepatocytes in HBV infection. The total amount of plasma virus production is larger for HBV; on average, $\approx 10^9$ HIV particles are generated every day compared with $\approx 10^{11}$ HBV particles. Thus chronic HBV infection emerges also as a rapid dynamic process with vast amounts of virus and infected cells produced and killed every day.

With respect to accumulation of genetic diversity and escape from drug treatment and immune responses, the relevant figure is the viral generation time, which is largely determined by the turnover rate of virus-infected cells and is therefore much shorter in HIV. Another important factor is that the genome length of HBV is only ≈ 3200 bp compared to $\approx 10,000$ bp for HIV and that multiple overlapping reading frames may impose more constraints against variation on HBV than HIV. While there is rapid emergence of drug-resistant strains in HIV (21, 25), we do not find any indication of resistance in HBV; in our studies HBV virus loads do not increase as long as the drug is given.

Treatment of chronic HBV infections with lamivudine leads to a rapid and sustained decline of plasma virus levels, but clinical benefit with a reduced risk of cirrhosis and development of liver cancer will greatly depend on the decline of infected cells (26–28). In patients where infected cells decline with a half-life of 10 days, treatment for 1 year could potentially reduce the number of infected cells to $\approx 10^{-11}$ of its initial value (with a 100% effective drug). Eradication of the virus infection depends on whether the efficacy of the drug is sufficiently high to reduce the basic reproductive ratio of the virus below unity [in analogy to epidemiological theory (29)]. In patients with an infected cell half-life of 100 days, 1 year of treatment could reduce the number of infected cells to $\approx 8\%$ of its initial value. Thus lamivudine could be used over a prolonged period as single-agent therapy or to reduce the number of infected cells before immunotherapy designed to eradicate infected cells. Immunotherapy without antiviral treatment could be problematic because of the very large number of infected liver cells in the typical HBV carrier. The quantitative understanding of HBV dynamics derived here will make it possible to devise optimal treatment strategies for individual patients.

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