Early report

Rapid production and clearance of HIV-1 and hepatitis C virus assessed by large volume plasma apheresis

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Summary

Background In chronic HIV-1 infection, dynamic equilibrium exists between viral production and clearance. The half-life of free virions can be estimated by inhibiting virion production with antiretroviral agents and modelling the resulting decline in plasma HIV-1 RNA. To define HIV-1 and hepatitis C virus (HCV) dynamics, we used plasma apheresis to increase virion clearance temporarily while leaving virion production unaffected.

Methods Plasma virus loads were measured frequently before, during, and after apheresis in four HIV-1-infected patients, two of whom were also co-infected with HCV. Rates of virion clearance were derived by non-linear least-square fitting of plasma virus load to a model of viral dynamics.

Findings Virion clearance rate constants were 0·0063/min (9·1/day) to 0·025/min (36·0/day; half-life 28–110 min) for HIV-1 and 0·0038/min (5·5/day) to 0·0069/min (9·9/day; half-life 100–182 min) for HCV. These values provided estimates of daily particle production of 9·3 $\log_{10}$–10·2 $\log_{10}$ particles for HIV-1 and 11·6 $\log_{10}$–13·0 $\log_{10}$ particles for HCV.

Interpretation Our findings confirm that HIV-1 and HCV are produced and cleared extremely rapidly. New estimates for HIV-1 clearance are up to ten times higher than previous ones, whereas HCV clearance is similar to previous estimates.

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Introduction

HIV-1 infection is characterised by dynamic equilibrium between virus production and clearance. By disturbing this equilibrium with antiviral therapy and by mathematically analysing the resulting decline in plasma viral load, researchers have gained insight into HIV-1 dynamics in vivo.[1,2] Such approaches estimate that free particles have a half-life of less than 6 h, that productively infected cells have a half-life of about 1 day, and that, on average, 10^9–10^{10} virions are produced each day.[1–5] These kinetic factors are central to our understanding of the pathogenesis of HIV-1 infection and have changed approaches towards treatment of HIV-1-infected patients by supporting early and aggressive therapy.

Effective antiviral therapy leads to a multiphasic exponential decline in HIV-1 viral load. Different segments of the decline curve represent the decay of virus in different compartments. The curvature of the initial deflection shows the decay of free virus particles, whereas the subsequent phase of exponential decline provides information on the turnover of productively infected cells.[3,4] Virion clearance rates derived from these analyses are thought of as minimum estimates because they are based on the assumption that the antiviral effect of therapy is immediate and drug penetration is complete.[3,5]

More precise information may be gained from analysing changes in plasma virus concentration after temporary interventions, such as the removal of plasma by apheresis, that alter virion clearance without affecting virion production. We used this method to estimate the rate of virion production in individuals chronically infected with HIV-1 and hepatitis C virus (HCV of genotype 1).

Methods

Four HIV-1-infected patients from the Rockefeller University Hospital (New York, USA) volunteered to undergo plasma apheresis. Plasma removed by apheresis was simultaneously replaced by an equivalent volume of isotonic normal saline containing 5% albumin. The upper rate of apheresis was set at 39 mL plasma per min with adjustments for the patency of the vein containing the outgoing catheter and the patient's tolerance of the procedure. Blood samples were taken frequently before, during, and after apheresis over 5 days.

We used a branched DNA assay (Chiron Corp, Emeryville, CA, USA) to characterise apheresis-related decline of plasma HIV-1 and HCV load and the rate of return to baseline load at the end of apheresis. Particle removal mediated by apheresis was quantified by measuring HIV-1 and HCV RNA load in the removed plasma by branched DNA assay. The lower limit of detection of the branched DNA assay was 2·7 $\log_{10}$ copies/mL for HIV-1 and 5·3 $\log_{10}$ copies/mL for HCV. Concentrations of antibody to gp120 were measured by antigen-capture ELISA.[6] The clinical protocol was approved by the Rockefeller University Hospital institutional review board, and all individuals provided informed consent.

	Patient			
	1	2	3	4
Baseline values				
CD4-cell count (cells/μL)	493	426	89	130
HIV-1 RNA load (log_{10} copies/mL)	4·00	4·30	5·30	4·59
HCV RNA load (log_{10} copies/mL)	..	..	7·84	6·57
Apheresis conditions				
Duration (min)	78	120	136	116
Plasma volume removed (mL)	2972	2974	5319	3477
Log_{10} virions removed*				
HIV-1	7·36	7·59	8·68	7·70
HCV	..	..	11·34	10·04
Clearance and production rates				
Clearance constant (per min) (68% CI)				
HIV-1	0·018 (0·009–0·035)	0·015 (0·010–0·022)	0·006 (0·005–0·008)	0·025 (0·017–0·038)
HCV	..	..	0·007 (0·005–0·008)	0·004 (0·003–0·005)
Half-life (min)				
HIV-1	39	46	110	28
HCV	..	..	100	182
Virion production* (log_{10} particles per day)†				
HIV-1	9·30	9,54	10·18	10·18
HCV	..	..	13·00	11·60

*Each HIV-1 virion contains two RNA copies. †Calculated by plasma and extracellular fluid volumes estimated from body weights, and with the assumption that plasma and extracellular fluid compartments are in equilibrium.[1]

Baseline characteristics of patients, apheresis conditions, and apheresis-derived estimated of HIV-1 and HCV virion production and clearance

To estimate the rate of virion production and clearance, a mathematical model was designed in which the total rate of virus production (P) was assumed to be the same before, during, and after apheresis. The total rate of virion clearance was given by $c'V$, where V is the population size of free virions and c' is the rate of virion clearance. Before apheresis, c' is given by the natural rate of virion clearance, c. However, during apheresis, the rate of virion clearance is given by $c'=c+\varepsilon$, where ε is the additional rate of virus clearance due to the removal of plasma containing virus. After apheresis, we assume that clearance returns to the baseline rate ($c'=c$). The dynamics of free virus before, during, and after apheresis are given by equation 1:

$$dV/dt = P - c'V,$$

where dV/dt is the rate of change of the free virus population. If we assume that viral load is in steady state before apheresis ($P=cV_0$), the solution of equation 1 is as follows (equation 2):

$$V(t) = V_0 \text{ when } t \leq t_s$$

$$V(t) = V_0\left[\frac{c}{c+\varepsilon} - \left(\frac{c}{c+\varepsilon} - 1\right)e^{-(c+\varepsilon)(t-t_s)}\right] \text{ when } t_s < t \leq t_e$$

$$V(t) = V_0 - [V_0 - V(t_e)]\, e^{-c(t-t_e)} \text{ when } t_e < t,$$

where $V_0=P/c$, which is the steady-state load of free virus in the absence of apheresis, and t_s and t_e are the timepoints when apheresis starts and ends, respectively. V_0, c, and ε were estimated by calculating the best fit of equation 2 to the plasma virus data by use of the Levenberg-Marquardt algorithm for non-linear least-square fitting. 68% CI (corresponding to 1 SD) were obtained by the bootstrap method.[7] Estimates of virion half-life were calculated by solving the equation, half-life=(ln2)/c.[1]

Results

Before apheresis, plasma virus load ranges were 4·0 log_{10}–5·3 log_{10} HIV-1 RNA copies/mL and 6·6 log_{10}–7·8 log_{10} HCV copies/mL. During apheresis (78–136 min), 2972–5319 mL plasma were removed containing up to 8·7 log_{10} HIV-1 particles and 11·3 log_{10} HCV particles (table). The effect of apheresis on the natural clearance rate was not large because accompanying changes in plasma virus load were generally less than 50% despite the removal of more than 10^7 particles. When plasma virus loads were analysed and fitted (figure 1), they yielded an HIV-1 clearance rate constant of 0·0063/min (9·1/day) to 0·025/min (36·0/day), corresponding to a half-life of 28–110 min, and an HCV clearance rate constant of 0·0038/min (5·5/day) to 0·0069/min (9·9/day), corresponding to a half-life of 100–182 min. These values provided estimates of daily particle production of 9·3 log_{10}–10·2 log_{10} virions for HIV-1 and 11·6 log_{10}–13·0 log_{10} virions for HCV in these patients.

Unlike a previous report, we found no significant increase in plasma virus load after apheresis.[8] In all four patients, plasma virus returned to baseline concentrations at the end of apheresis, thus supporting our belief that the procedure had no effect on virion production (figure 1). However, apheresis had a pronounced effect on concentrations of antibody to gp120, which decreased to 50% and 29% of baseline values in patients 1 and 2, respectively (figure 2). Antibodies to gp120 had not returned to baseline concentrations 25 h after the end of the procedure, whereas plasma virus load returned to baseline within 42–98 min. We estimated antibody clearance and half-life by the same principles involved in the analysis of virion kinetics, and obtained an antibody clearance rate constant of 0·00005/min (0·072/day) to 0·00007/min (0·1/day), corresponding to a half-life of 6·9–9·6 days.

Discussion

HIV-1 and HCV are produced and cleared extremely rapidly. Our estimates for HIV-1 clearance are up to ten times higher than previous values,[1–3] whereas estimates of HCV clearance are within the range reported by Neumann and colleagues for patients treated with interferon-α.[9]

In HCV-infected patients, treatment with high doses of interferon-α leads to a rapid decline in plasma HCV load, with viral loads falling 1 log_{10}–2 log_{10} copies/mL during the first day of therapy. The mechanism responsible for this acute decline, which is ten times more rapid than seen in HIV-1-infected patients treated with combination antiretroviral therapy, is under debate.[9–11] Our results, based on apheresis, yield similar HCV clearance rates to

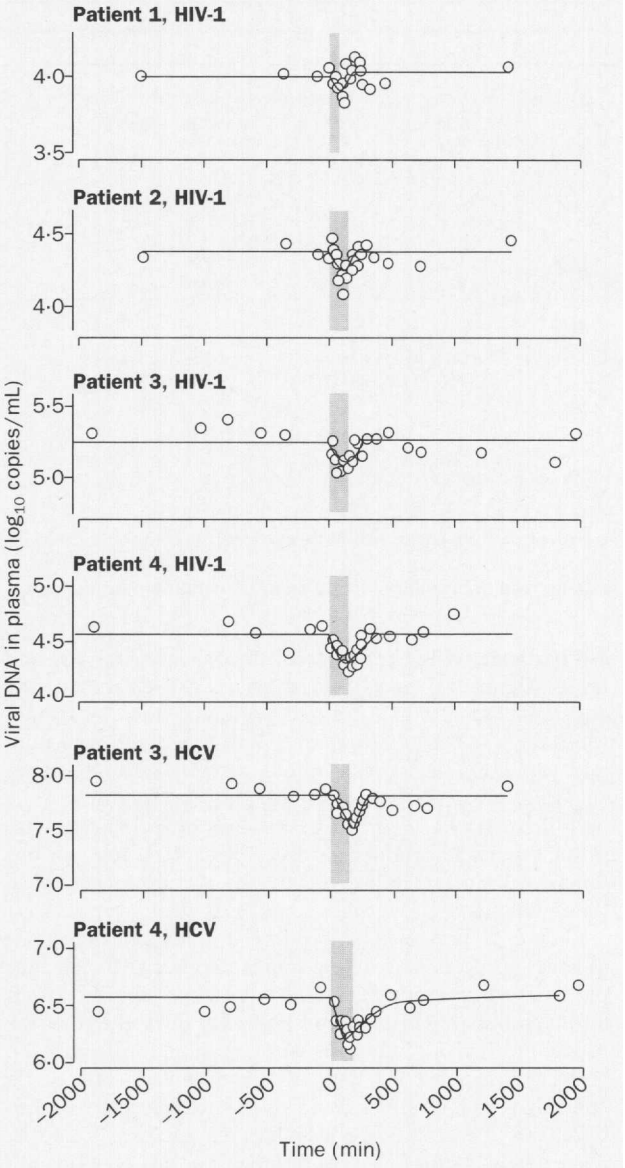

Figure 1: **Changes in plasma HIV-1 and HCV RNA during apheresis**
Shaded area indicates apheresis. Each patient had 25–30 measurements of plasma virus load.

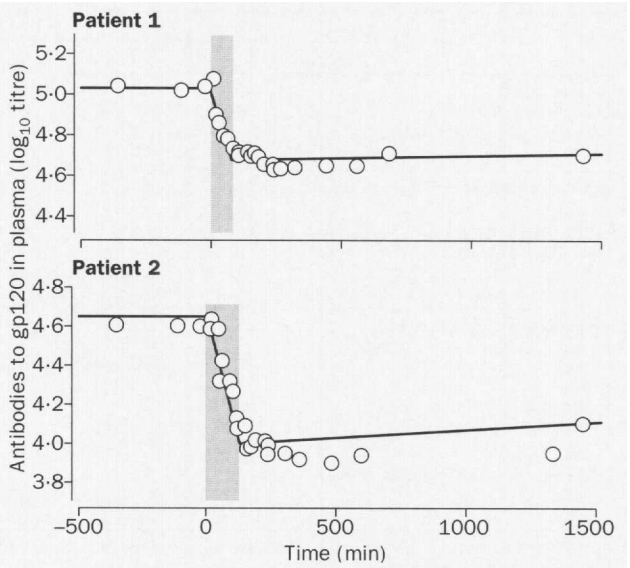

Figure 2: **Changes in anti-gp120 antibody titre during apheresis**
Shaded area indicates apheresis.

those found by Neumann and colleagues.[9] Therefore, interferon-α has little or no effect on virion clearance, and the hypothesis that interferon-α decreases the rate of HCV production is supported.

Although the apheresis-derived HIV-1 clearance rates are more rapid than previous estimates, they are still slower than those suggested by recent studies of simian immunodeficiency virus (SIV) infection in macaques. Infusion of SIV-infected or uninfected monkeys with viral particles yielded estimates of virion half-life of 3·3–4·6 min.[12] Several factors may account for this discrepancy. Clearance of endogenously produced virions may differ from that of virions that are exogenously infused. Moreover, there may be selective defects in reticuloendothelial function in HIV-1-infected patients leading to inefficient or slower clearance of viral particles.[13–15] Finally, these studies involved SIV stock that was grown in human cells. Human molecules on the virion surface may have led to xeno-specific effects that result in rapid clearance on infusion into monkeys.

Plasma virus returned and remained at baseline load shortly after the end of apheresis, which supports our hypothesis that apheresis had no effect on viral production and only a transient effect on viral clearance. By contrast, titres of antibodies to gp120 remained at about 50% of baseline for at least 1 day after the end of apheresis. Clearly virion clearance was not affected by the decline in antibody concentrations. However, if the main effect of virus-specific antibody is the prevention of new rounds of cellular infection, then we would not expect to see this effect during the study because it would only become apparent after the 1 day it takes for newly infected cells to start producing virus.

We noted that HIV-1 clearance rates were similar among patients 1, 2, and 4, despite differences in viral burden and clinical stage of disease. Clearance rates of HCV were lower than that of HIV-1. Patient 3, who had high HCV plasma viral burden, had slower clearance of HIV-1 and HCV. If we assume that the hepatic reticuloendothelial system is mainly responsible for removal of particulate matter from the circulation, as has been shown in animals, HCV-induced hepatic abnormalities may inhibit the function of the reticuloendothelial system, thereby leading to lower rates of particle clearance.[16,17] Studies that involve the selective perturbation of the reticuloendothelial system may offer insight into the precise mechanisms of virion clearance.

Interruption of combination antiretroviral therapy has been suggested as a strategy to increase HIV-1-specific immune responses in the hope that viral replication can be controlled without treatment.[18] Most individuals who stop therapy eventually experience a rebound in plasma viraemia.[18–20] Comparison of viral production and clearance rates in these individuals with our estimates may help assess whether the induced immune responses substantially alter virion production and clearance. Plasma apheresis offers a novel approach to study the kinetics of many different components of plasma, the only requirement being the ability to measure the concentration of the component of interest.

Contributors

Bharat Ramratnam and David D Ho were responsible for protocol design and data analysis. Sebastian Bonhoeffer, John E Mittler, and Alan S Perelson devised the mathematical models and did the data fitting. Linqi Zhang was responsible for plasma viral load measurements. Arlene Hurley and Martin Markowitz participated in the clinical aspects of the protocol. James Binley and John P Moore did the studies involving antibodies to gp120. Bharat Ramratnam, Sebastian Bonhoeffer, and David D Ho contributed to the writing of the paper.

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