Human Cytomegalovirus (HCMV) Replication Dynamics in HCMV-Naive and -Experienced Immunocompromised Hosts

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Human cytomegalovirus (HCMV) can infect both HCMV-naive and -experienced transplant patients. In this study, the growth rate of HCMV in HCMV-naive hosts (1.82 units/day; 95% confidence interval [CI], 1.44–2.56 units/day) was shown to be significantly faster than the growth rate of virus in HCMV-experienced hosts undergoing recurrent infection (0.61 units/day; 95% CI, 0.55–0.7 units/day; $P < .0001$). The basic reproductive number (R_0) for HCMV-naive liver transplant patients was 15.1 (95% CI, 8.9–44) but was only 2.4 (95% CI, 2.35–2.8) for HCMV-experienced transplant recipients, corresponding to an anti-HCMV immune efficacy of ~84%, despite immunosuppressive therapy. The R_0 values suggest that an anti-HCMV drug or vaccine with an efficacy of >93% (95% CI, 89%–98%) is required to eliminate viral growth during infection of HCMV-naive liver transplant recipients, whereas lower efficacy levels are sufficient to reduce the R_0 value to <1 in hosts with prior HCMV immunity.

Herpesviruses, including human cytomegalovirus (HCMV), have developed elaborate cellular and immune manipulation strategies to maintain the virus-host equilibrium [1]. Thus, HCMV infection of an immunologically naive (i.e., HCMV-seronegative) immunocompetent host is usually pathologically inconsequential, and, after establishment of latency, the host suppresses HCMV replication such that reactivations (recurrent infections) are also asymptomatic. However, among T cell–immunocompromised hosts, infection of both HCMV-naive and -experienced individuals can lead to high levels of viral replication and, in many instances, results in pathological consequences [2–4]. Recent data showed that HCMV replication in vivo is a highly dynamic process [5]. In antiviral intervention studies of HCMV similar to those of human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) [6–9], the doubling time of HCMV in individuals with preexisting immunity against HCMV was ~1 day [5]. These data allowed models for the rationalization and prediction of antiviral drug resistance and virus load kinetics to be developed [10] and also can be used to identify individuals who are at risk of HCMV disease [11]. A series of studies from our laboratory and others described the natural history of HCMV replication in immunocompromised hosts [12–17]. The results show that maximum virus load attained during active infection is greatest in patients who are immunologically naive to HCMV and indicate that the natural dynamics of HCMV infection are a rapid phase of replication that reaches a peak and then, in the absence of therapy, declines spontaneously to undetectable levels.

The dynamics of HCMV replication in the context of the immune status of the host can provide insights into HCMV pathogenesis and the host's immune control of replication. For example, the basic reproductive number (R_0), a measure of the number of infected cells produced from a single HCMV-infected cell before the depletion of target cells occurs, can be estimated from the initial increase in levels of HCMV DNA. In addition, the doubling time of virus in the blood can be determined in hosts without prior immunity against HCMV and compared with those with HCMV-specific B and T cell immunity. The aim of this study was to determine the growth rate and R_0 value for HCMV and, thus, to estimate the quantitative effects of preexisting immunity against HCMV on initial replication rate and R_0 in a group of liver transplant recipients.

Subjects, Materials, and Methods

Human subjects. We selected 30 liver transplant recipients. A subset of these patients ($n = 25$) was obtained from a cohort of liver transplant described elsewhere [14]. Inclusion criteria included the availability of frequent blood samples posttransplantation to accurately estimate viral growth rates. Hence, patients had a median of 2 samples taken each week (range, 1 sample every 3.5 days to 1 sample per week). Sampling occurred during initial and subsequent hospital stays and at once-weekly outpatient clinics until 3 months posttransplantation. HCMV infection was defined as the detection of HCMV DNA by polymerase chain reaction (PCR) in the blood posttransplantation. Preexisting immunity to HCMV was defined as a pretransplant serum sample containing HCMV IgG antibodies, as determined by EIA (Biokit). Ten patients were shown to be HCMV seronegative prior to transplantation and, thus, were assigned to the HCMV-naive category for further

Received 17 July 2001; revised 8 February 2002; electronically published 31 May 2002.

Financial support: Wellcome Trust; United Kingdom Medical Research Council.

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The Journal of Infectious Diseases 2002;185:1723–8

0022-1899/2002/18512-0005$02.00

analyses; the remaining 20 patients were already HCMV IgG positive prior to transplantation (HCMV-experienced category).

Virologic studies. DNA was extracted from samples of whole blood (200 μL) using blood DNA extraction columns (Qiagen), as described elsewhere [12, 14, 15]. All samples were analyzed for the presence of HCMV DNA, using a qualitative PCR system, and any positive samples were quantified using a quantitative-competitive PCR assay. The detection limit of this assays is 200 genomes/mL. Details of these procedures have been described extensively elsewhere [12–17].

HCMV dynamics during primary infection. The basic model for HCMV replication dynamics is based on that described elsewhere for HIV and HCV [9–18]. In brief, the dynamics of infected cells are given by the following equation: $dI/dt = \beta_I T - \delta_I I$, where T is the target cell number, t is time, δ_I is the death rate of infected cells, β is the rate of new infections occurring within the uninfected cell population, and I is the number of infected cells.

Consistent with data from in vivo and in vitro studies of low passage clinical strains of HCMV, we assumed that most infections occur through a cell-cell route. The experimentally determined growth rate *(r)* represents the increase in HCMV load in blood per day, such that $I(t) = I(0)e^{rt}$, where *e,* is the natural base of the logarithm. To estimate the initial growth rate of HCMV (r_i), virus load levels, derived from the dynamic model when target cell numbers were relatively well maintained, were used in the exponential growth equation. The initial viral doubling time (t_d) was determined from the slope of virus load over time.

The number of new infections derived from a single infected cell when target cells are unlimited is equivalent to R_0. To estimate R_0 for HCMV, we used 2 models used previously for HIV [18]. The first model used a fixed time delay (τ) of 24 h between infection of the new cell and production of infectious virions. The model provides an estimate of R_0 according to the following equation: $R_0 = 1 + (r/\delta_I)e^{r\tau}$. The second model assumes that there is no time delay between initial and subsequent infection of susceptible target cells. Under these conditions, R_0 is given by the following equation: $R_0 = 1 + (r/\delta_I)$.

In addition to calculating R_0 from the experimentally determined viral growth rate, the corresponding values of r derived from the model (r_m) and the initial growth rate (r_i) derived from the model were also used to determine the robustness of the estimates of R_0 obtained during the later stages of HCMV growth. In the absence of reliable in vivo estimates of the target cell number for HCMV during active infection, the values of R_0 calculated from the experimental data represent minimal estimates for R_0.

HCMV dynamics during infection of HCMV-experienced hosts. Recent models describing the dynamics of viral replication and cytotoxic T cell responses [19] were adapted for HCMV. The revised model for the growth of HCMV-infected cells during infection of HCMV-experienced individuals is therefore given by the following equation: $dI/dt = \beta IT - \delta_I I - \rho IE$, where the descriptors I, β, T, and δ_I are the same as those in the basic primary infection model (see above), but the term ρIE reflects the removal of infected cells via the action of cytotoxic T lymphocytes (CTL); E is the number of HCMV-specific effector CTL, and ρ is the proportion of CTL-mediated lysis of infected target cells. The rate of increase of HCMV load in blood during recurrent infection was used to calculate the viral growth rate, as described above. Similarly, the r_i value before target cell depletion was estimated from the dynamic model. R_0 values were calculated using the fixed-delay and instantaneous models outlined above, using viral growth rates determined experimentally or from the model. In all models, multiple iterations were used, with a time interval of 0.1 day, to generate predicted virus load patterns in HCMV-naive and -experienced liver transplant recipients.

Statistical comparisons were performed between groups using the Student's t test. The correlation between the modeled virus loads and experimentally determined loads was performed using regression methods of the log-transformed data. Comparison of peak virus load levels was performed after log transformation of the data. $P < .05$ was regarded as significant.

Results

HCMV replication kinetics in HCMV-naive and -experienced hosts. HCMV replication dynamics were investigated in a population of 30 liver transplant recipients with active HCMV replication. The viral doubling time of HCMV in patients with or without specific previous immunity to HCMV is shown in figure 1. The mean growth rate of virus during infection of HCMV-naive individuals was 1.82 units/day (95% confidence interval [CI], 1.44–2.56 units/day), corresponding to a viral doubling time of 0.38 units/day (~9 h; 95% CI, 0.27–0.48 units/day). Infection of patients with preexisting HCMV immunity was associated with a significantly slower viral growth rate (0.62 unit/day; 95% CI, 0.54–0.71 unit/day) and viral doubling time (1.12 days; 95% CI, 0.99–1.25 days; $P < .0001$).

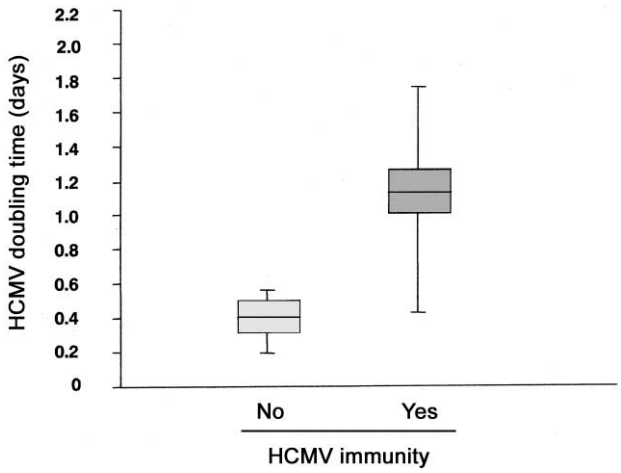

Figure 1. Doubling time of human cytomegalovirus (HCMV) during infection of liver transplant recipients in the presence ($n = 10$) or absence ($n = 20$) of preexisting HCMV immunity. The horizontal line for each group represents the mean value for the group; the shaded box indicates the 95% confidence interval of the mean. The vertical bars indicate the minimum and maximum values present within each data set.

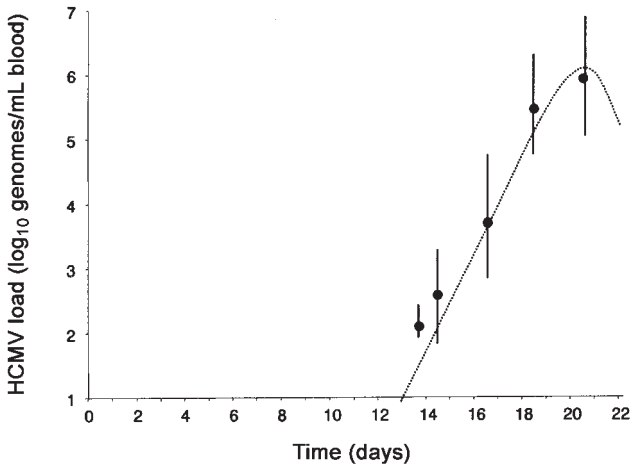

Figure 2. Correlation between experimentally determined human cytomegalovirus (HCMV) loads and the modeled virus load increase in HCMV-naive patients. In these analyses, the peak virus load attained during surveillance was used to normalize all patients, and the mean and 95% confidence interval for HCMV loads measured prior to the peak of viremia were plotted.

Differences in maximum HCMV load attained in HCMV-naive and -experienced hosts. To assess the influence of preexisting immunity on the maximum level of HCMV load attained during an active infection, we analyzed the mean difference in maximum (peak) HCMV load (ΔV_{max}) between patients with or without preexisting immunity to HCMV. As expected from our previous studies [12, 14, 15], peak virus loads were significantly lower in patients with preexisting immunity than those who were HCMV naive ($P = .015$). The mean ΔV_{max} for the liver transplant recipients was 0.97 $\log_{10}$ genomes/mL blood (95% CI, 0.4–1.54 $\log_{10}$ genomes/mL blood). Thus, in addition to reducing the rate of viral growth, preexisting immunity also suppressed the maximum virus load achieved during active infection.

Calculation of R_0 *for HCMV during primary and recurrent infection.* The experimentally determined growth rate during infection of HCMV-naive liver transplant recipients was used to calibrate a basic dynamic model of HCMV replication. The death rate of HCMV-infected cells derived from our previous studies, coupled with values of β comparable to other viral systems, provided a viral dynamic model that closely paralleled the experimental data when virus loads increased to >200 genomes/mL blood ($r^2 = 0.94$; $P = .0002$; figure 2). The model developed for the replication dynamics in patients with preexisting HCMV immunity included an additional term corresponding to the death rate of infected cells due to CTL lysis. This model produced the appropriate decrease in both viral growth rate and peak virus load and provided a good fit to the experimental data ($r^2 = 0.90$; $P = .007$). The virus load kinetics derived from the dynamic models for the liver transplant recipients are shown in figure 3. It should be noted that these models need to satisfy multiple constraints, namely differences in the replication rate in the HCMV-naive and -experienced hosts and differences in peak virus load attained during infection. The close agreement between the experimental and modeled values for viral growth rate, viral doubling time, and change in peak virus load are shown in table 1.

Estimates of R_0 values for HCMV during infection of HCMV-naive or -experienced hosts were determined from the experimentally determined initial viral growth rate *(r)* and also from the initial viral growth rate (r_i) estimated from the model when target cells were not depleted (i.e., when virus loads were <200 genomes/mL blood). These results are shown in table 2. In the fixed-delay model, mean R_0 values derived from the r of HCMV were 15.1 and 2.4 for infection of HCMV-naive and -experienced liver transplant recipients, respectively. When r_i values determined from the dynamic models were used, very similar values for R_0 were obtained (table 2). The dynamic models indicated that, when HCMV loads are 200–5000 genomes/mL blood, target cells have not been substantially depleted; hence, $r \approx r_i$.

The differences in R_0 values between HCMV-naive and -experienced hosts show that preexisting immunity against HCMV in liver transplant recipients has an antiviral efficacy of 84%. In addition, these estimates for R_0 show that an anti-HCMV drug or vaccine must be ⩾93.3% (95% CI, 89%–98%) effective to reduce R_0 to <1 in HCMV-naive liver transplant recipients and ⩾58.3% (95% CI, 57.5%–64%) to reduce R_0 to <1 in HCMV-experienced patients.

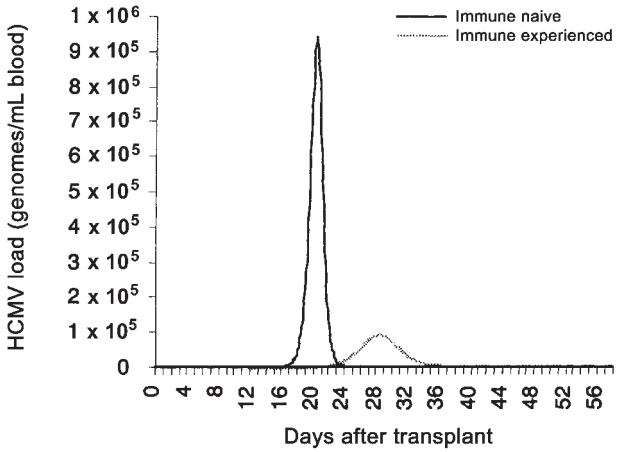

Figure 3. Dynamic models of the human cytomegalovirus (HCMV) load patterns present during infection of HCMV-naive (immune naive) or -experienced (immune experienced) liver transplant recipients. Basic parameter values were as follows: death rate of infected cells, 0.77 cells/day; rate of new infections occurring within the uninfected cell population, 0.0035 infection/day; and target cell number, 800 cells. In the case of the HCMV dynamics during infection of HCMV-experienced hosts, the effective $CD8^+$ cytotoxic T lymphocyte (CTL) population was assumed to be 1% of the total $CD8^+$ cell population [20–23], with the proportion of CTL-mediated lysis of infected target cells equal to 0.28 cells/day.

Table 1. Comparison of experimental and modeled values for human cytomegalovirus (HCMV) growth *(r)* and doubling time (t_d) in liver transplant recipients with (HCMV experienced) or without (HCMV naive) infection and difference in peak virus load (ΔV_{max}) between these 2 populations.

Group, parameter	Experimental value (95% CI)	Modeled value
HCMV naive		
r/day	1.82 (1.44–2.56)	1.84
t_d, days	0.38 (0.27–0.48)	0.37
HCMV experienced		
r/day	0.61 (0.55–0.70)	0.60
t_d, days	1.12 (0.99–1.25)	1.15
ΔV_{max}, $\log_{10}$ genomes/mL blood	0.97 (0.4–1.54)	0.94

NOTE. CI, confidence interval.

Discussion

The present study of the HCMV growth rate in the early phases of viral replication in immunocompromised hosts has allowed for the first estimates of R_0 for HCMV to be obtained. These results demonstrate that preexisting immunity against HCMV reduces both the replication rate (thus increasing viral doubling time) and the peak virus load attained during active infection and thus extend our previous studies in this area. Importantly, these data illustrate that, despite the immunocompromised state of these solid-organ recipients following transplantation, the residual immune system still has functional capacity to modulate HCMV replication.

Dynamic models that paralleled the experimentally determined growth rate were generated. Modification of the HCMV-naive model by incorporating removal of infected cells through a cytotoxic T cell–mediated mechanism produced good agreement with the lower rate of viral growth and reduction in peak virus load observed during infection of patients with preexisting HCMV immunity. These models should facilitate the development of more-sophisticated models incorporating our improved knowledge of $CD4^+$ and $CD8^+$ T cell responses against HCMV in vivo [20–22]. The calculation of R_0 allows for an assessment of the number of newly infected cells arising from a single infected cell during the course of an infection. The natural dynamics of HCMV show that, in the early stages, the growth rate of virus approximates to R_0, whereas, at much later stages, target cell depletion results in a decrease in the viral growth rate and underestimates the R_0 value. For most patients studied here, we calculated the growth rate of the virus at the earliest stages of active HCMV replication, and estimates of R_0 thus should accurately reflect its true value. Consistent with this assumption was the use of the dynamic model to provide estimates of r_i and, hence, R_0 when no target cell depletion had occurred. These growth rate values produced R_0 values which were very similar to those obtained using *r*.

Knowledge of R_0 values for HCMV in different contexts can be used to estimate the immune system's efficacy in inhibiting HCMV replication in HCMV-experienced, compared with HCMV-naive, hosts and the efficacy of anti-HCMV therapy required to reduce R_0 to < 1 and, hence, to eliminate active HCMV infection. In the case of the former, using a biologically plausible fixed-delay model for estimating R_0, the immune system reduces R_0 from 15.1 to 2.4 among liver transplant recipients, corresponding to an immune efficacy of 84%. Although these residual R_0 values are still above the critical R_0 level of 1, they demonstrate that the immune system makes a substantial contribution toward controlling HCMV replication, even in immunocompromised hosts. Indeed, this result explains why HCMV disease is predominantly found in patients undergoing primary infection. To completely control HCMV replication, the R_0 values indicate that an anti-HCMV drug or vaccine administered during the early stages of infection in an HCMV-naive host has to be $\geq$93.3% effective to fully inhibit viral growth. However, in HCMV-experienced hosts, this efficacy level is reduced to $\geq$58.3%. Previous work from our laboratory has shown that the efficacy of intravenous (iv) ganciclovir (Gcv) at a dose of 5 mg/kg 2×/day is ~91.5% (95% CI, 89%–94%). Hence, this dose of Gcv would be expected to reduce growth substantially in HCMV-naive liver transplant recipients ($R_0 = 1.28$; efficacy, 91.5%). Nevertheless, during infection of HCMV-naive liver transplant recipients, a growth rate at the upper end of the confidence interval would yield R_0 values > 15.1, and, in some cases, an apparent initial inability of iv Gcv to control HCMV replication thus could be observed after initiating therapy, illustrating that antiviral resistance is not the only explanation for apparent therapeutic failure [24].

The alternate therapeutic management strategy for HCMV in immunocompromised hosts is via antiviral prophylaxis [25, 26]. The dose of Gcv used for prophylaxis (1 g by mouth 3×/day)

Table 2. Estimates of the basic reproductive number (R_0) for human cytomegalovirus (HCMV) in naive or immune patients, based on experimental and modeled viral growth rates.

HCMV immune status	HCMV growth rate, units/day		R_0, no delay		R_0, 24-h delay	
	Observed (95% CI)	Modeled[a]	Observed (95% CI)	Modeled	Observed (95% CI)	Modeled[a]
Naive	1.82 (1.44–2.56)	1.85	3.3 (2.9–4.3)	3.4	15.1 (8.9–44.0)	16.3
Experienced	0.61 (0.55–0.70)	0.60	1.8 (1.7–1.9)	1.8	2.4 (2.35–2.8)	2.46

NOTE. For an explanation of the difference between "R_0, no delay" and "R_0, 24-h delay," see Subjects, Materials, and Methods. CI, confidence interval.

[a] Determined when target cells were not depleted.

Table 3. Comparison of parameter values for acute human immunodeficiency virus (HIV), hepatitis B virus (HBV), and human cytomegalovirus (HCMV) infection dynamics.

Parameter	Acute HIV	Acute HBV	HCMV naive	HCMV experienced
Early increase in virus load, units/day	2.0 (1.4–3.5)	0.19 (0.13–0.28)	1.8 (1.5–2.6)	0.6 (0.55–0.70)
Doubling time, days	0.3 (0.2–0.5)	3.7 (2.2–5.2)	0.38 (0.27–0.48)	1.12 (0.99–1.25)
Peak virus load, $\log_{10}$ genomes/mL	6.9 (6.4–7.3)	9.5 (9.0–10.0)	5.8 (4.9–6.7)	4.8 (4.5–5.1)
R_0, 24-h delay	19.3 (7.4–34.0)	5.0 (3.1–7.5)[a]	15.1 (8.9–44.0)	2.4 (2.35–2.8)
Efficacy required to reduce R_0 to <1, %	95 (86–97)	80 (68–87)[a]	93 (89–98)	58 (57.5–64)

NOTE. Data are mean (95% confidence interval). The data for acute HIV infection are taken from Little et al. [18], and the data for acute HBV infection are taken from Whalley et al. [28]. R_0, basic reproductive number. For an explanation of "R_0, 24-h delay," see Subjects, Materials, and Methods.

[a] Only 3 patients were available for analysis.

has an efficacy of ~46.5% (95% CI, 45%–47.5%) against wild-type strains of HCMV [10] and, therefore, will reduce the viral growth rate in both HCMV-naive and -experienced hosts. However, the resultant R_0 values after therapy in the liver transplant recipients would be 8.1 (HCMV-naive) and 1.31 (HCMV-experienced); therefore, at this dose of Gcv, viral growth in the HCMV-experienced host will be substantially inhibited. However, because R_0 in the naive host remains >1, viral growth will continue throughout the period of prophylaxis, such that, after prophylaxis is stopped, a late resurgence in viral growth will occur predominantly in HCMV-naive patients. Late HCMV infection and disease has indeed been described as an emerging clinical problem among liver transplant recipients after the cessation of oral Gcv prophylaxis [26]. The different R_0 values in the liver transplant recipients helps explain why acyclovir, a drug with only moderate potency against HCMV, did not significantly reduce HCMV disease in these patients, compared with preemptive Gcv therapy [27].

These are a paucity of estimates of R_0 for acute viral infections in the human host. Recently, the primary dynamic parameters for HIV and HBV replication during acute infection have been published [18, 28], and it is interesting to compare these values with those obtained in the present study of HCMV dynamics (table 3). The early increase in HCMV load in liver transplant recipients is comparable to acute HIV infection, although peak virus load is lower during primary HCMV infection. Using the fixed-delay model, the R_0 value of HCMV in HCMV-naive individuals is ~22% lower than the R_0 value of acute HIV infection but substantially higher than the corresponding value for HBV.

In HCMV-experienced hosts, the doubling time of HCMV (~1.1 days) is substantially slower than that in acute HIV infection. However, it is faster than the doubling time observed for HIV in patients with preexisting immunity to HIV (~1.7 days) and the doubling time during acute HBV infection (~3.7 days).

In conclusion, we have defined, for the first time, R_0 values for HCMV replication in immunocompromised hosts with or without specific prior immunity to HCMV. These data further emphasize the rapid replication rate of HCMV in vivo and illustrate that HCMV is more similar to HIV than to HBV during infection of HCMV-naive individuals. These results also provide insight into the quantitative effects of the antiviral immune response and the efficacy levels of anti-HCMV therapy required to inhibit viral replication in different contexts. In addition, they define correlates of immune protection useful for successful vaccine development against HCMV.

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