Long-Term Administration of Valacyclovir Reduces the Number of Epstein-Barr Virus (EBV)-Infected B Cells but Not the Number of EBV DNA Copies per B Cell in Healthy Volunteers[▽][†]

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**Epstein-Barr virus (EBV) establishes a latent infection in B cells in the blood, and the latent EBV load in
healthy individuals is generally stable over time, maintaining a "set point." It is unknown if the EBV load
changes after long-term antiviral therapy in healthy individuals. We treated volunteers with either valacyclovir
(valaciclovir) or no antiviral therapy for 1 year and measured the amount of EBV DNA in B cells every 3
months with a novel, highly sensitive assay. The number of EBV-infected B cells decreased in subjects
receiving valacyclovir (half-life of 11 months; $P = 0.02$) but not in controls (half-life of 31 years; $P = 0.86$).
The difference in the slopes of the lines for the number of EBV-infected B cells over time for the
valacyclovir group versus the control group approached significance ($P = 0.054$). In contrast, the number
of EBV DNA copies per B cell remained unchanged in both groups ($P = 0.62$ and $P = 0.92$ for the control
and valacyclovir groups, respectively). Valacyclovir reduces the frequency of EBV-infected B cells when
administered over a long period and, in theory, might allow eradication of EBV from the body if reinfection
does not occur.**

Primary infection with Epstein-Barr virus (EBV) is frequently asymptomatic in infants and children, but infection of adolescents and young adults can result in infectious mononucleosis. EBV is associated with several malignancies, including Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin's disease, and lymphoproliferative disease, in immunocompromised and immunocompetent persons (6, 20).

In healthy EBV-seropositive persons, about 1 to 10 in 10^5 peripheral B cells are infected with EBV (14). The virus establishes latency in memory B cells. The level of the latent EBV load in healthy individuals remains stable over time, maintaining a "set point" for each individual (19). It is uncertain how this "set point" is maintained, but the latent EBV load is thought to reflect a balance between removal of EBV-infected cells due to the half-life of memory B cells and reinfection of new memory B cells during virus reactivation. The EBV genome replicates when B cells latently infected with EBV divide using the host DNA polymerase, which is not sensitive to the action of acyclovir. However, when the virus

reactivates in latently infected B cells, EBV replicates using the viral DNA polymerase, which is inhibited by the phosphorylated form of acyclovir. Therefore, blocking production of new virus with acyclovir should decrease the latent EBV load at a rate equivalent to the half-life of memory B cells. Patients with zoster who were treated with oral acyclovir for 28 days showed no reduction in the EBV load in the blood, despite complete inhibition of EBV shedding in the saliva (23). These results suggested that antiviral therapy for longer than 28 days is necessary to detect a reduction in the EBV load in the blood.

In this study, we administered either valacyclovir (valaciclovir) (which is absorbed more effectively than acyclovir and metabolized to acyclovir) or no antiviral to healthy volunteers for 12 months and measured the level of EBV DNA in the blood every 3 months.

MATERIALS AND METHODS

Subjects. Patients at the National Institutes of Health Clinical Center and at the University of Texas Center for Clinical Studies with a history of recurrent genital herpes with three to nine recurrences a year and either a positive culture for herpes simplex virus (HSV) from the genital area or a positive serology for HSV type 2 (HSV-2) received valacyclovir at 500 mg per day for 1 year. Patients with genital herpes were required to be off HSV-suppressive therapy for 3 months before entering the study. The control group did not have symptomatic genital herpes and received no antiviral therapy. Subjects were excluded if they were receiving immunosuppressive therapy, had malignancy, or were infected with human immunodeficiency virus. Informed consent was obtained from all subjects, and the study was approved by the institutional review boards of the National Institute of Allergy and Infectious Diseases and the University of Texas Center for Clinical Studies. Blood was obtained from the valacyclovir-treated group and the control group at day 0 (before initiating valacyclovir) and at 3, 6,

9, and 12 months. Patients seen at the National Institutes of Health Clinical Center returned their bottles of medication, and pill counts were performed. Nearly all of these patients had an excellent rate of compliance with the study medication. Of 56 subjects, 45 completed the study. Five subjects were excluded from analysis due to insufficient peripheral blood mononuclear cells (PBMCs) available for all five time points or no detectable EBV DNA in the blood at any time point throughout the study. All other subjects had measurable levels of EBV DNA for at least three different time points, including day 0.

Isolation of DNA from B cells and real-time PCR. PBMCs were isolated from whole blood and stored in liquid nitrogen. For each subject, samples from each time point were thawed and processed at the same time. B cells were isolated by negative selection (human B-cell isolation kit; Miltenyi Biotec, Germany), resuspended in 100 to 200 μl of phosphate-buffered saline, and counted. Preliminary experiments showed no notable inhibition of the real-time PCR with fewer than 10^4 cells but mild to modest inhibition with more than 5×10^4 cells/well. Therefore, the concentration of cells was adjusted to 4,000 cells per 5 μl (some experiments used 1,500 to 8,000 cells per 5 μl), and 5 μl of B cells was plated into wells of a 96-well real-time PCR plate and stored at −20°C. For all specimens, a small portion of leftover cell suspension was stained with anti-CD20 monoclonal antibody conjugated with phycoerythrin (BD Pharmingen, San Diego, CA), and purity of B cells was determined by flow cytometry. In most experiments, the fraction of B cells in each cell suspension was >80%. All plates from the same subject were thawed at the same time, and 5 μl of buffer containing proteinase K and Tween 20 was added to each well to disrupt cells as previously described (13). Quantitative real-time PCR amplification was performed by adding 15 μl of PCR master mix (Eurogentec North America, San Diego, CA) containing EBV BamHI W primers and probes (12) to each well of B-cell DNA. The limit of detection using this PCR system is ~4 copies per reaction, based on experiments using standard curves obtained with serial dilutions of a plasmid with a single copy of BamHI W. Since EBV genomes contain 5 to 12 copies of BamHI W repeats (1) and each infected cell contains at least one EBV genome, this system has the potential to detect a single EBV-infected cell per well. Since we did not know the number of BamHI W repeats for each patient's virus, we expressed the data as the number of EBV DNA copies per infected cell. We compared each patient to him- or herself over time, and the number of copies of BamHI W repeats per viral genome is not likely to change in a given patient.

Calculations to determine the number of EBV-infected cells and average EBV genome copy number per infected cell. In the real-time PCR plates, most wells were negative for EBV DNA because of the low frequency of EBV-infected cells in healthy subjects. The number of positive wells depended on the frequency of EBV-positive B cells, the purity of B cells, and the number of cells applied to each well. Since the number of EBV-positive B cells per well was very low, we used a Poisson distribution to calculate this number (as we previously described for calculating the number of HSV-positive cells per well [13]). We defined the total number of wells in the plate as n and the number of EBV-positive wells as y. We assume that the number of EBV-infected cells per well follows a Poisson distribution with parameter a. Assuming a Poisson distribution for the number of EBV-positive cells within a well, the probability that a well has at least one positive cell is $1 - e^{-a}$ (where e is the base of the natural logarithm). Thus, an estimate of a is obtained by equating the observed proportion of positive wells, or y/n, to $1 - e^{-a}$. Solving this equation for a gives $a = -\ln(1 - y/n)$. For plates without any positive wells, i.e., $y = 0$, this estimate is 0 regardless of the value for the number of total wells (n). However, plates with many wells have a more reliable 0 than plates with few wells. We thus smoothed the data to address this issue and pretended that each plate had one additional well that was 0.5 positive and estimated a as follows: $a = -\ln[1 - (y + 0.5)/(n + 1)]$. Instead of estimating the proportion of positive wells by the ratio y/n, we used $(y + 0.5)/(n + 1)$, which is the Bayesian posterior mean of the proportion of negative wells under a Jeffreys noninformative prior (5). Therefore, the estimation of the number of EBV-positive cells/well is as follows: $a = -\ln[1 - (y + 0.5)/(n + 1)]$. This can be viewed loosely as the "middle" of a confidence interval for the true proportion of positive wells. This smoothing method gives modestly higher estimates for plates with positive wells and has values closer to zero for totally negative plates with many wells compared to totally negative plates with few wells.

We can also estimate the frequency of a B cell being infected by EBV as follows: $q = a/(m \times p/100)$, where m is the number of cells per well and p is the purity of B cells (%). We thus estimate q as follows: $q = a/(m \times p/100) = -\ln[1 - (y + 0.5)/(n + 1)]/(m \times p/100)$. To estimate the average number of EBV DNA copies per infected cell, the sum of all EBV genomes in a plate (S) was determined directly from real-time PCR results and was divided by an estimate of the number of EBV-positive B cells in the plate ($a \times n$). For example, if a plate contained three EBV-positive wells, with 110, 58, and 152 EBV copies, S equaled 320. See Table 1 for an example.

TABLE 1. Sensitivity of real-time PCR assay

No. of X50-7 cells/well	Total no. of wells	No. of EBV-positive wells	Observed frequency of EBV-positive wells[a]	Predicted frequency of EBV-positive wells[b]	No. of EBV DNA copies/infected cell[c]
0.00	16	0	0.00	0.00	NA
0.25	24	3	0.13	0.22	19.3
1.00	24	12	0.50	0.63	28.4
4.00	24	23	0.96	0.98	18.9

[a] Observed frequency of EBV-positive wells = number of EBV-positive wells (third column) divided by total number of wells (second column).
[b] Predicted frequencies of EBV-positive wells were calculated from the Poisson distribution (see text for details).
[c] Calculation of the number of EBV DNA copies/infected cell is described in Table 2, footnote c, and in the text. NA, not applicable.

RESULTS

Development of a technique to estimate the frequency of EBV-positive cells and the average number of EBV DNA copies/B cell in healthy persons. We initially tried to determine the number of EBV DNA copies in B cells isolated from PBMCs by using real-time PCR, but we found that viral loads in healthy individuals were too low to detect in a reproducible fashion; therefore, we used a novel approach involving quantification of the EBV DNA copy number using limiting dilution of B cells and real-time PCR, similar to the approach previously described for quantifying HSV DNA in ganglion cells (13). This method allowed us to estimate both the frequency of EBV-positive B cells and the average number of copies of EBV genomes per EBV-infected cell.

We initially tested the sensitivity of the assay using an EBV cell line, X50-7, with a known number of copies of EBV DNA per cell (2). Serial fourfold dilutions of X50-7 cells (beginning at four cells per well) were added to EBV-negative BJAB cells (4,000 BJAB cells/well), and the number of EBV-positive wells and number of EBV DNA copies per infected cell were determined. The observed frequency of EBV-positive wells for each dilution was similar to the predicted frequency based on the Poisson distribution, and the average number of EBV DNA copies per EBV-positive X50-7 cell for each dilution was consistent (Table 1). Based on the Poisson distribution, the probability that any well contained two or more EBV-positive cells when EBV-positive cells were plated at a concentration of 0.25 cells per well was 2.6% $[(1 - 1.25 e^{-0.25}) \times 100]$. Therefore, we expected only 1 EBV-positive cell per well when the cells were plated at a concentration of 0.25 EBV-positive cells/well. Since we were able to detect EBV DNA in some wells with an average of 0.25 EBV-positive cells per well, our real-time PCR system was able to detect single EBV-positive cells in a well.

We next tested blood bank donors for EBV-positive B cells and EBV DNA copy numbers, using the assay. For blood bank donor A, 8 of 56 wells were positive for EBV DNA (Table 2, columns 2 and 3), and we estimated the number of EBV-positive cells/well as follows: $a = -\ln[1 - (8 + 0.5)/(56 + 1)] = 0.161$ (Table 2, column 4 and footnote a). Based on the estimate of the number of EBV-positive cells/well (0.161), the number of cells added to each well (2,500), and the purity of the B cells (87.9%) (Table 2, columns 4, 5, and 6, respectively, and footnote a), there was an estimated $(0.161 \times 10^7)/(2,500 \times 87.9) = 7.35$ EBV-positive cells/10^5 B cells (Table 2, column 7

TABLE 2. Estimation of numbers of EBV-infected B cells and EBV DNA copies per infected cell in blood bank donors

Donor	Total no. of wells (n)	No. of EBV-positive wells (y)	Estimation of no. of EBV-positive cells/well[a] (a)	No. of cells added to well (m)	Purity of B cells (%) (p)	No. of EBV-positive cells/10^5 B cells[b] (q)	Sum of EBV DNA copies (S)	No. of EBV DNA copies/infected cell[c]
A	56	8	0.161	2,500	87.9	7.35	2,062	229
	32	9	0.340	3,000	94.5	11.98	1,432	132
B	44	2	0.057	3,500	95.6	1.71	449	179
	88	8	0.100	4,000	96.4	2.60	1,003	114
C	88	38	0.567	4,000	83.5	16.96	3,583	71.8
	38	14	0.465	4,000	76.2	15.25	2,168	123
D	44	18	0.530	3,310	94.0	17.02	3,136	135
	44	13	0.357	3,430	92.4	11.25	1,909	122
	88	34	0.491	3,570	92.4	14.87	2,354	123
	85	29	0.420	3,620	89.7	12.94	1,317	66
	88	50	0.838	4,000	94.3	22.22	3,862	114

[a] Estimation of number of EBV-positive cells/well, $a = -\ln[1 - (y + 0.5)/(n + 1)]$, where n is the total number of wells in the plate (second column) and y is the number of EBV-positive wells (third column).

[b] The number of EBV-positive cells/10^5 B cells, $q = (a \times 10^7)/(m \times p)$, where a is the estimation of the number of EBV-positive cells/well (fourth column), m is the number of cells in a well (fifth column), and p is the percent purity of B cells (sixth column). Each well contains $(m \times p)/100$ B cells.

[c] The number of EBV DNA copies/infected cell is $S/(a \times n)$, where S is the sum of all EBV genome numbers from a plate (eighth column; obtained directly from real-time PCR results), a is the estimation of the number of EBV-positive cells/well (fourth column), and n is the number of wells in the plate (second column). Therefore, $(a \times n)$ is the number of EBV-positive cells in the plate.

and footnote b). Using the total number of wells (56), the estimate of the number of EBV-positive cells/well (0.161), and the sum of EBV DNA copies in the plate (2,062) (Table 2, columns 2, 4, and 8, respectively), there was an average of $2,062/(0.161 \times 56) = 229$ EBV DNA copies/infected B cell (Table 2, column 9 and footnote c), since our primers and probe for real-time PCR detect each copy of the BamHI W repeat. In a second experiment using cells from the same donor drawn on the same day, 9 of 32 wells were positive for EBV, with an estimated 11.98 EBV-positive cells/10^5 B cells and an average of 132 EBV DNA copies/infected B cell. In experiments with cells from blood bank donors B, C, and D, the numbers of EBV-infected B cells and copies of EBV DNA per B cell were similar for a given donor from the same day in independent experiments, with different numbers of wells and purities of B cells (Table 2). The mean number ($\pm$ standard deviation) of EBV-positive cells per 10^5 B cells for donors A, B, C, and D was 9.7 ± 3.3, 2.2 ± 0.6, 16.1 ± 1.2, and 15.7 ± 4.3, respectively. The mean number ($\pm$ standard deviation) of EBV DNA copies per infected cell for donors A, B, C, and D was 181 ± 69, 147 ± 46, 97 ± 36, and 112 ± 27, respectively. Thus, this method provides an estimation of the frequency of EBV-positive cells and the average number of EBV DNA copies/B cell at low viral loads with reasonable reproducibility.

The number of EBV-infected B cells declines during valacyclovir treatment in healthy persons. Using this approach, we determined the number of EBV-infected B cells and the average number of EBV DNA copies per infected cell for patients receiving valacyclovir or no antiviral therapy in the clinical study. There were 21 subjects in the control group and 19 subjects in the valacyclovir group. We analyzed the rate of change in the number of EBV-infected cells for each subject over time, since a large variation in the number of EBV-infected B cells between subjects could underestimate the difference in the change in virus-infected cells. The number of EBV-positive cells per 10^5 B cells was plotted against time, in months, for the first three patients enrolled in each group (Fig. 1). While there was variation during the five time points, we were able to plot a linear regression line for each subject. We then determined linear regression lines and slopes for the number of EBV-positive cells per 10^5 B cells over time for all of the subjects in the study (Fig. 2; data used to generate regression lines are shown in the supplemental material). There was considerable variability in the starting value (y intercept) of each line as well as in the slope of the regression line among subjects even within the same group. The average of the regression lines (thick gray lines in Fig. 2A) was relatively flat for the control group but had a negative slope for the valacyclovir group. The mean of the slopes for the valacyclovir-treated group was $-2.8 \times 10^{-2} \pm 1.2 \times 10^{-2}$/month and was statistically different from a slope of 0 ($P = 0.02$), while the mean of the slopes for the control group was $-0.1 \times 10^{-2} \pm 0.8 \times 10^{-2}$/month, which was not statistically different from 0
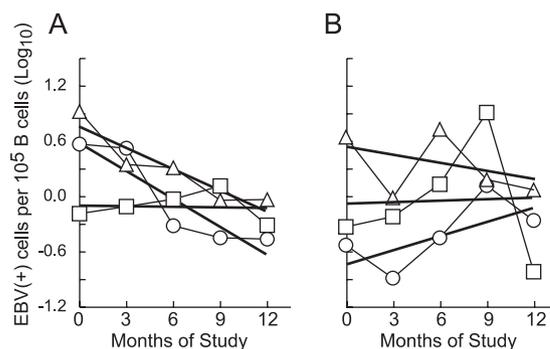

FIG. 1. EBV-positive cells per 10^5 B cells over time and linear regression lines for the first three subjects from the valacyclovir (A) and control (B) groups. Each symbol connected with a broken line represents data for one subject. Solid lines associated with symbols indicate linear regression lines of the data for each subject.
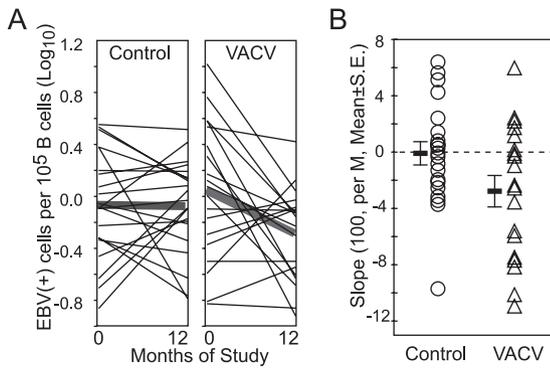
FIG. 2. Rate of change (slope) in the number of EBV-infected B cells over time. (A) Linear regression lines for individual control subjects (left) and valacyclovir (VACV) patients (right) are shown as thin lines. Thick gray lines indicate the mean slope for each group. (B) The individual slopes for each control subject (circles) and valacyclovir patient (triangles) are depicted. Short horizontal bars indicate means, and vertical bars indicate standard errors (S.E.).
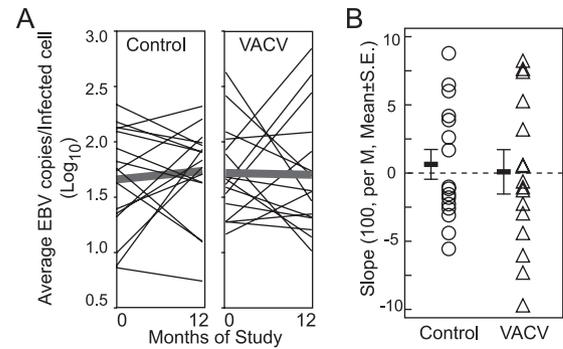

FIG. 3. Mean EBV DNA copies per B cell over time. (A) Linear regression lines for individual control subjects (left) and valacyclovir (VACV) patients (right) are shown as thin lines. Thick gray lines indicate the mean slope for each group. (B) The individual slopes for each control subject (circles) and valacyclovir patient (triangles) are depicted. Short horizontal bars indicate means, and vertical bars indicate standard errors (S.E.).

($P = 0.86$) (Fig. 2B). Comparison of the slopes of regression lines between the two treatment groups showed that the difference approached statistical significance ($P = 0.054$; Wilcoxon test). The slopes of the control and valacyclovir groups were significantly different using regression analysis, with the viral load ($\log_{10}$) as outcome and month as the covariate ($P = 0.03$ for common delta), and when analyzed using analysis of covariance, with slope as outcome and intercept as the covariate ($P = 0.04$ for common delta). These findings indicate that the number of EBV-infected B cells declined over time in the valacyclovir treatment group.

The EBV DNA copy number per infected B cell does not decline during valacyclovir treatment in healthy persons. We next examined the effect of valacyclovir treatment on the number of EBV DNA copies per infected cell. The mean number ($\pm$ standard error) of EBV DNA copies per infected B cell was 64.0 ± 17.1 for patients in the valacyclovir group and 67.9 ± 15.4 for patients in the control group. The slopes of linear regression lines for the mean number of EBV DNA copies per infected cell over time (in months) were determined for each subject (Fig. 3A), and the mean slopes for the two groups were compared (Fig. 3B). The mean regression lines had slopes of nearly 0 for both groups. The slopes of the rate of change in the number of EBV DNA copies per infected cell over time for both the control and the valacyclovir groups were indistinguishable from 0 ($P = 0.62$ and $P = 0.92$ for the control and valacyclovir groups, respectively), and the difference in mean slopes between the groups was not significant ($P = 0.66$). Thus, the mean EBV DNA copy number per infected cell ($\log_{10}$) is stable over time and is not affected by 1 year of valacyclovir therapy.

DISCUSSION

We have shown that 1 year of valacyclovir therapy in healthy EBV carriers results in a decrease in the number of EBV-infected B cells compared to an untreated control group, while valacyclovir has no effect on the mean number of EBV DNA copies per infected cell. The somewhat modest effect in the

reduction of EBV load after 1 year of valacyclovir was not unexpected. Acyclovir inhibits EBV lytic replication, which uses the viral DNA polymerase in B cells, but has no effect on EBV replication in latently infected B cells, which uses the host cell polymerase. The increase in the frequency of EBV-infected cells in five of the subjects receiving valacyclovir suggests that these persons may have had proliferation of B cells latently infected with EBV (and insensitive to valacyclovir); alternatively, they may have been noncompliant with the antiviral medication. The observation that 28 days of acyclovir had no effect on the EBV load (23) suggested that a more prolonged course of antiviral therapy was likely to have only a modest effect in reducing the viral load.

The half-life of EBV-infected cells during convalescence after infectious mononucleosis ($\sim$6 weeks from first visit) was reported to be 7.5 ± 3.7 days (11). Since EBV is latent in memory B cells, which have a half-life of 11 ± 3.3 days (17), Hadinoto et al. proposed a model for the decline of EBV-infected B cells after mononucleosis based on the kinetics of turnover of memory B cells (11). From our data, the half-life of EBV-infected B cells in the valacyclovir group based on the mean slopes ($-2.8 \times 10^{-2} \pm 1.2 \times 10^{-2}$/month) was 11 months, and the half-life calculated from median slopes was 12 months. In the control group, the half-life of EBV-infected B cells calculated from mean slopes ($-0.1 \times 10^{-2} \pm 0.8 \times 10^{-2}$/month) was 31 years, and that calculated from the median slopes was 41 years. There are several possibilities that may explain the discrepancy between our results and those of the prior study. First, Hadinoto et al. (11) measured the half-life of EBV in the blood during convalescence from mononucleosis, whereas our subjects presumably had been infected many years earlier. Balfour and colleagues (3) and Fafi-Kremer et al. (9) showed that the level of EBV in PBMCs was still elevated 6 weeks after primary infection. Second, one dose of valacyclovir each day is unlikely to completely block EBV reactivation and virus production. While the bioavailability of valacyclovir is about three to five times higher than that of acyclovir, valacyclovir is converted into acyclovir in the body, and the mean plasma elimination time of acyclovir is 1.5 to 6.3 h (4, 15). Third, it is possible that latent infection of memory B cells with

EBV prolongs the half-life of these cells or that EBV may establish latency preferentially in a subset of memory B cells that has a longer life span. Fourth, compared with EBV-infected B cells from patients convalescing after infectious mononucleosis, EBV-infected B cells in our subjects might be more heterogeneous, with some infected cells resting, others actively proliferating, and a small minority undergoing lytic replication. Since valacyclovir affects only lytic replication of EBV, the marked differences in the decline of EBV copy numbers in different valacyclovir recipients (Fig. 2) might reflect differences in the relative proportions of resting, proliferating, and lytically infected cells. Nonetheless, the general reduction of EBV-infected B cells over time in the valacyclovir group suggests that infection by EBV of previously uninfected B cells is one of the mechanisms that maintains the latent EBV pool in healthy adults.

EBV can be eliminated from the body in some patients after bone marrow transplantation (10). Based on the half-life of EBV in patients treated with valacyclovir and assuming that valacyclovir acts similarly on B cells in tissues as it does in the blood, we estimate that it would take 6 years of 500 mg of valacyclovir once each day to eradicate 99% of EBV from the B-cell compartment and 11.3 years to eliminate the virus completely from the body if persons were not reinfected during this time. Reinfection with the virus is likely, however, since multiple strains of EBV are detected in many individuals, suggesting that multiple episodes of infection occur (22). The mean peak concentration of acyclovir reaches 27.1 µM when valacyclovir is given at 1,000 mg three times a day (16), while the 50% effective inhibitory dose of acyclovir for EBV replication is 5 to 10 µM (7, 21, 24). Therefore, it might be theoretically possible to eradicate EBV from the body within several years with high-dose valacyclovir. Acyclovir or valacyclovir prophylaxis has been reported to have various rates of effectiveness in reducing the risk of EBV lymphoproliferative disease in transplant recipients (8, 18). The relatively modest effect of valacyclovir in reducing EBV DNA in B cells in the blood after a full year in persons with a healthy immune system suggests that more active drugs against EBV might be more effective for prophylaxis of EBV lymphoproliferative disease in immunocompromised persons.

ACKNOWLEDGMENTS

REFERENCES

1. **Allan, G. J., and D. T. Rowe.** 1989. Size and stability of the Epstein-Barr virus major internal repeat (IR-1) in Burkitt's lymphoma and lymphoblastoid cell lines. Virology **173:**489–498.
2. **Arribas, J. R., D. B. Clifford, C. J. Fichtenbaum, R. L. Roberts, W. G. Powderly, and G. A. Storch.** 1995. Detection of Epstein-Barr virus DNA in cerebrospinal fluid for diagnosis of AIDS-related central nervous system lymphoma. J. Clin. Microbiol. **33:**1580–1583.
3. **Balfour, H. H., Jr., C. J. Holman, K. M. Hokanson, M. M. Lelonek, J. E. Giesbrecht, D. R. White, D. O. Schmeling, C. H. Webb, W. Cavert, D. H. Wang, and R. C. Brundage.** 2005. A prospective clinical study of Epstein-Barr virus and host interactions during acute infectious mononucleosis. J. Infect. Dis. **192:**1505–1512.
4. **Blum, M. R., S. H. Liao, and P. de Miranda.** 1982. Overview of acyclovir pharmacokinetic disposition in adults and children. Am. J. Med. **73:**186–192.
5. **Brown, L. D., T. T. Cai, and A. DasGupta.** 2001. Interval estimation for a binomial proportion. Stat. Sci. **16:**101–133.
6. **Cohen, J. I.** 2000. Epstein-Barr virus infection. N. Engl. J. Med. **343:**481–492.
7. **Colby, B. M., J. E. Shaw, G. B. Elion, and J. S. Pagano.** 1980. Effect of acyclovir [9-(2-hydroxyethoxymethyl)guanine] on Epstein-Barr virus DNA replication. J. Virol. **34:**560–568.
8. **Darenkov, I. A., M. A. Marcarelli, G. P. Basadonna, A. L. Friedman, K. M. Lorber, J. G. Howe, J. Crouch, M. J. Bia, A. S. Kliger, and M. I. Lorber.** 1997. Reduced incidence of Epstein-Barr virus-associated posttransplant lymphoproliferative disorder using preemptive antiviral therapy. Transplantation **64:**848–852.
9. **Fafi-Kremer, S., P. Morand, J. P. Brion, P. Pavese, M. Baccard, R. Germi, O. Genoulaz, S. Nicod, M. Jolivet, R. W. Ruigrok, J. P. Stahl, and J. M. Seigneurin.** 2005. Long-term shedding of infectious Epstein-Barr virus after infectious mononucleosis. J. Infect. Dis. **191:**985–989.
10. **Gratama, J. W., M. A. Oosterveer, J. M. Lepoutre, J. J. van Rood, F. E. Zwaan, J. M. Vossen, J. G. Kapsenberg, D. Richel, G. Klein, and I. Ernberg.** 1990. Serological and molecular studies of Epstein-Barr virus infection in allogeneic marrow graft recipients. Transplantation **49:**725–730.
11. **Hadinoto, V., M. Shapiro, T. C. Greenough, J. L. Sullivan, K. Luzuriaga, and D. A. Thorley-Lawson.** 2008. On the dynamics of acute EBV infection and the pathogenesis of infectious mononucleosis. Blood **111:**1420–1427.
12. **Hoover, S. E., J. Kawada, W. Wilson, and J. I. Cohen.** 2008. Oropharyngeal shedding of Epstein-Barr virus in the absence of circulating B cells. J. Infect. Dis. **198:**318–323.
13. **Hoshino, Y., J. Qin, D. Follmann, J. I. Cohen, and S. E. Straus.** 2008. The number of herpes simplex virus-infected neurons and the number of viral genome copies per neuron correlate with the latent viral load in ganglia. Virology **372:**56–63.
14. **Khan, G., E. M. Miyashita, B. Yang, G. J. Babcock, and D. A. Thorley-Lawson.** 1996. Is EBV persistence in vivo a model for B cell homeostasis? Immunity **5:**173–179.
15. **Laskin, O. L.** 1983. Clinical pharmacokinetics of acyclovir. Clin. Pharmacokinet. **8:**187–201.
16. **Lycke, J., C. Malmestrom, and L. Stahle.** 2003. Acyclovir levels in serum and cerebrospinal fluid after oral administration of valacyclovir. Antimicrob. Agents Chemother. **47:**2438–2441.
17. **Macallan, D. C., D. L. Wallace, Y. Zhang, H. Ghattas, B. Asquith, C. de Lara, A. Worth, G. Panayiotakopoulos, G. E. Griffin, D. F. Tough, and P. C. Beverley.** 2005. B-cell kinetics in humans: rapid turnover of peripheral blood memory cells. Blood **105:**3633–3640.
18. **McDiarmid, S. V., S. Jordan, G. S. Kim, M. Toyoda, J. A. Goss, J. H. Vargas, M. G. Martin, R. Bahar, A. L. Maxfield, M. E. Ament, and R. W. Busuttil.** 1998. Prevention and preemptive therapy of postransplant lymphoproliferative disease in pediatric liver recipients. Transplantation **66:**1604–1611.
19. **Miyashita, E. M., B. Yang, K. M. Lam, D. H. Crawford, and D. A. Thorley-Lawson.** 1995. A novel form of Epstein-Barr virus latency in normal B cells in vivo. Cell **80:**593–601.
20. **Oyama, T., K. Yamamoto, N. Asano, A. Oshiro, R. Suzuki, Y. Kagami, Y. Morishima, K. Takeuchi, T. Izumo, S. Mori, K. Ohshima, J. Suzumiya, N. Nakamura, M. Abe, K. Ichimura, Y. Sato, T. Yoshino, T. Naoe, Y. Shimoyama, Y. Kamiya, T. Kinoshita, and S. Nakamura.** 2007. Age-related EBV-associated B-cell lymphoproliferative disorders constitute a distinct clinicopathologic group: a study of 96 patients. Clin. Cancer Res. **13:**5124–5132.
21. **Pagano, J. S., and A. K. Datta.** 1982. Perspectives on interactions of acyclovir with Epstein-Barr and other herpes viruses. Am. J. Med. **73:**18–26.
22. **Tierney, R. J., R. H. Edwards, D. Sitki-Green, D. Croom-Carter, S. Roy, Q. Y. Yao, N. Raab-Traub, and A. B. Rickinson.** 2006. Multiple Epstein-Barr virus strains in patients with infectious mononucleosis: comparison of ex vivo samples with in vitro isolates by use of heteroduplex tracking assays. J. Infect. Dis. **193:**287–297.
23. **Yao, Q. Y., P. Ogan, M. Rowe, M. Wood, and A. B. Rickinson.** 1989. Epstein-Barr virus-infected B cells persist in the circulation of acyclovir-treated virus carriers. Int. J. Cancer **43:**67–71.
24. **Zacny, V. L., E. Gershburg, M. G. Davis, K. K. Biron, and J. S. Pagano.** 1999. Inhibition of Epstein-Barr virus replication by a benzimidazole L-riboside: novel antiviral mechanism of 5,6-dichloro-2-(isopropylamino)-1-beta-L-ribofuranosyl-1H-benzimidazole. J. Virol. **73:**7271–7277.