Rates of Reactivation of Latent Herpes Simplex Virus from Mouse Trigeminal Ganglia Ex Vivo Correlate Directly with Viral Load and Inversely with Number of Infiltrating CD8+ T Cells

Yo Hoshino,* Lesley Pesnicak, Jeffrey I. Cohen, and Stephen E. Straus

Medical Virology Section, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

Received 6 March 2007/Accepted 16 May 2007

Herpes simplex viruses (HSV) reactivate at rates proportional to the viral loads in latently infected ganglia. However, these rates vary substantially among infected animals. We assessed whether the numbers of HSV-specific CD8+ T cells infiltrating latently infected ganglia also affect reactivation rates and contribute to their variability. Following corneal infection of mice with HSV type 2 (HSV-2), we quantified the latent viral loads in dissociated trigeminal ganglia by real-time PCR, the numbers of infiltrating CD8+ T cells by flow cytometry, and the rates of reactivation by the detection of cell-free virus released from ganglion cells cultured in 96-well plates. The reactivation rates correlated directly with the latent viral loads (P = 0.001) but did so more strongly (P = 10^{-7}) when cultures were depleted of CD8+ T cells. Reactivation rates were reduced in a dose-dependent fashion by adding back ganglion CD8+ T cells to the cultures (P = 0.003). We related the latent viral loads, numbers of CD8+ T cells, and reactivation rates by mathematical equations. The rates of reactivation predicted from latent viral loads and numbers of infiltrating CD8+ T cells in dissociated ganglia correlated with the observed rates of reactivation (P = 0.04). The reactivation of HSV-2 from ganglia ex vivo is determined both by the latent viral load and the number of infiltrating CD8+ T cells.

The establishment of neuronal latency and periodic reactivation are definitive features of herpes simplex virus (HSV) infections in animals and humans. While the determinants of reactivation rates in humans are not well defined, the competence of the host cellular immune response is thought to be important in controlling reactivation (22, 43). In animals, the quantity of latent HSV type 1 (HSV-1) DNA in ganglia (the latent viral load) as estimated by levels of latency-associated transcripts (13, 16) and more precisely by quantitative PCR of viral DNA (29, 33–35) is an important determinant of recurrence rates ex vivo. We confirmed this association in vivo, as well, using infected guinea pigs in which genital disease due to HSV-1 or HSV-2 recurs spontaneously (17, 25).

Within individual studies, recurrence rates of HSV infection vary up to 10-fold, even among animals with comparable latent viral loads (17). Presumably, in these animals, other factors in addition to latent viral loads contribute to reactivation rates. An important clue to the nature of such factors was provided in recent seminal reports by Khanna et al. and Liu et al., who showed that HSV-specific, gamma interferon (IFN-γ)-positive, CD8+ T cells infiltrate latently infected mouse trigeminal ganglia (TG) and block the reactivation of HSV-1 from latency ex vivo in a dose-dependent manner (19, 20, 27). Neither HSV-1 nor HSV-2 undergoes spontaneous reactivation as defined by the production of infectious virus in mice; however, more sensitive molecular techniques have demonstrated low levels of spontaneous viral antigens and productive cycle transcripts in ganglia of mice latently infected with HSV-1 (14). HSV-1 and HSV-2 also do not reactivate very efficiently ex vivo in the absence of inductive stimuli, such as the treatment of mice with UV light (42), hyperthermia (36), or the addition of demethylating agents like hexamethylene bisacetamide to explanted ganglion cultures (1, 23).

We explored the hypothesis that infiltrating HSV-specific CD8+ T cells contribute to the inherently low rates and the variability of the rates of reactivation of virus from mouse TG. We tested whether the removal of CD8+ T cells from cultured, HSV-2-infected ganglia permitted virus to reactivate at higher rates that would correlate even more strongly with the latent viral loads and reduce the variability of reactivation rates compared to those for non-T-cell-depleted cultures. Further, we hypothesized and sought to confirm that infiltrating HSV-specific CD8+ T cells block HSV-2 reactivation from ganglia in a dose-dependent manner.

MATERIALS AND METHODS

Viruses and animal studies. HSV-2 strain 333 was propagated in Vero cells and the titers of virus in Vero cells were determined as previously described (17). After the administration of an anesthetic comprising 0.12 mg of xylazine and 1.2 mg of ketamine per mouse, groups of 9- to 10-week-old C57BL/6 or DBA2 mice were challenged with HSV-2 strain 333 on both of their scarified (C57BL/6) or unscarified (DBA2) corneas with a range of inocula from 1.6 × 10^6 to 1.6 × 10^8 PFU/mouse. Because of the virulence of HSV-2 strain 333 in this ocular infection model, 0.5 ml of human immunoglobulin G (a 1:8 dilution in phosphate-buffered saline; Abbott Labs, Chicag, IL) was injected intraorbitally into each mouse 24 h after infection so that most of the mice would survive the acute infection and attain substantive levels of latency (10). Twenty-seven days after immunoglobulin G treatment, 7 to 10 mice per group were sacrificed and their TG were harvested. Ganglia from each group of mice were pooled, and the pools were dispersed into single cell suspensions by using collagenase type I (3 mg/ml for 1.5 h; Sigma, St. Louis, MO). In pooled tissues, there were 4,000 to 10,000 neurons per TG as...
determined by morphology observed by microscopy. From the TG suspensions for each group, numbers of cells equivalent to those obtained from 3 to 4.5 ganglia were used for nondepleted cultures; numbers equivalent to those obtained from 8 to 10 TG were used for studies of CD8+/T-cell depletion in culture. Aliquots equivalent to one TG each were studied by real-time PCR to measure the latent viral load or subjected to flow cytometry to quantify infiltrating HSV-2-specific IFN-γ-expressing CD8+ T-cells.

Quantitative real-time PCR. Aliquots of suspension cultures to be used for viral load determinations were divided into two tubes each and stored at −80°C. DNA was isolated independently from each sample by using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN). The numbers of copies of DNA genomes of latent HSV-2 in each sample were determined by real-time PCR using the Taqman system (ABI 7700 sequence detector; PE Applied Biosystems, Foster City, CA) with primers and probes specific for HSV-2 gD, as described previously (17). Each reaction mixture included 200 ng of ganglion DNA. A standard curve for the assay was generated using known numbers of copies of a plasmid containing the HSV-2 gD coding region diluted in salmon sperm DNA. The detection limit of this PCR assay proved to be ~4 copies/reaction mixture, and the assay results showed excellent linearity (R > 0.92) over 4 logs of DNA content. The latent viral load in infected ganglia was calculated from the means of results from at least two independent reactions performed in duplicate with two independently extracted pools of dissociated TG cells from each group of mice. Thus, determinations of latent viral load for each ganglion arose from the results of at least eight PCR assays.

Flow cytometry. Flow cytometry analyses were done as previously reported (17). First, the CD8+ T cells infiltrating each TG were counted. Each aliquot of a TG suspension culture, equivalent to one entire latently infected TG, was passed through a cell strainer and stained with fluorescein isothiocyanate-conjugated anti-CD8 and peridinin chlorophyll-conjugated anti-CD45 (a panleukocyte marker) monoclonal antibodies (both from BD Pharmingen, San Diego, CA). The cells were examined promptly on a FACSCaliber cytometer (Becton Dickinson, Franklin Lakes, NJ), and data were analyzed using FlowJo software (version 4.5.9; Tree Star Inc., Ashland, OR). The numbers of CD8+ T cells among pooled TG varied somewhat, as expected given the use of two independent strains of BALB/c mice with wide ranges in the number of neurons in the starting and final cell suspensions were determined microscopically for cytopathic effects after 2 days by using crystal violet staining.

In an initial control experiment, the effect of the T-cell depletion procedure upon rates of virus reactivation was tested by comparing reactivation rates for nondepleted TG cultures with those for mock-depleted cultures in which anti-biotin-conjugated beads were used without any biotin-conjugated antibodies for five cycles of incubation with ganglion cells. We observed no substantial effects of mock depletion on virus reactivation. Specifically, virus in one well among 16 wells of nondepleted cultures reactivated, whereas virus in two wells among 16 wells of mock-depleted cultures reactivated. Based on this result, nondepleted cultures were used in all subsequent experiments.

Calculation of rates of reactivation of HSV from latency. To quantify the rates of reactivation of HSV ex vivo from depleted or nondepleted TG cell cultures, we assumed that the reactivation of HSV-2 from latency in any given TG culture is an entirely random event that takes place with the frequency r (the reactivation rate per TG per day). Thus, the probability of not reactivating is expressed as follows: 1 − r. Therefore, the probability that HSV will not reactivate in TG cell cultures during d consecutive days (PnonR) is as follows: PnonR = (1 − r)d. Taking the natural logarithm of both sides of the equation gives the following:

\[ \ln(P_{nonR}) = -d \cdot \ln(1 - r) \]

Since the reactivation rate r is reasonably assumed to be a small value, \( \ln(1 - r) \) can be approximated by −r, and therefore, we have the following expression: \( \ln(P_{nonR}) = -r \cdot d \). Thus, we can denote all cumulative reactivation events in ex vivo TG cultures (r · d) by the expression \( -\ln(P_{nonR}) \), which should be linear with respect to the number of days in culture. Therefore, when the cumulative reactivation events \( -\ln(P_{nonR}) \) for pooled TG cultures are plotted on the y axis with the number of days in culture plotted on the x axis, the slope of the linear regression line provides an estimation of r.

Statistical analysis. All statistical analysis was done using JMP 5.01 software (SAS Institute Inc., Cary, NC).

RESULTS

HSV-specific CD8+ T cells infiltrate latently infected ganglia. To address the potential effects of CD8+ T cells on HSV-2 reactivation ex vivo, we first needed to quantify these cells and characterize their phenotypes in infected ganglia. In serial, independent experiments, the eyes from two strains (CD57BL/6 and DBA2) of mice with different susceptibilities to HSV infection were infected with different titers of HSV-2. Twenty-eight days later, the TG from these mice were harvested and the numbers of infiltrating CD8+ T cells were determined using flow cytometry. Pooled TG from both strains of mice contained CD8+ CD45+ lymphocytes (Fig. 1, upper panels). Approximately 30 to 35% of these infiltrating CD8+ T cells were HSV-specific, as shown by the production of IFN-γ in response to HSV-2-infected antigen-presenting cells (Fig. 1, lower panels). In preliminary experiments, most of these infiltrating CD8+ cells were CD3+ and CD62L−, indicating that they had the effector memory T-cell phenotype; a small minority (approximately 3 to 5%, ranging from 1 to 10%) were central memory T cells (CD3+, CD62L+) (data not shown). The numbers of infiltrating CD8+ T cells were generally

Downloaded from http://jvi.asm.org/ on August 29, 2017 by guest
greater following infection with higher titers of HSV-2; however, the frequencies of HSV-specific IFN-γ-producing CD8+ T cells remained similar across the wide range of virus inocula used.

The latent viral load correlates with reactivation rates more strongly when cultures are depleted of CD8+ T cells. We performed five independent experiments using two strains of HSV-2-infected mice and calculated the reactivation rates. When the results of these experiments were pooled, the virus reactivation rates for CD8 cell-depleted cultures were significantly higher than those for nondepleted cultures (Fig. 2) \((P = 0.002\) by Wilcoxon test). Reactivation rates for both CD8 cell-depleted and nondepleted cultures correlated significantly with the titers of the inocula \((P = 10^{-3})\); however, the correlation coefficient for latent viral loads was much higher \((P < 0.03)\). Thus, reactivation rates are higher and correlate more strongly with latent viral loads when CD8+ T cells are removed from ex vivo cultures.

HSV-2 reactivation rates for CD8-depleted cultures and latent viral load data from DBA2 mice and C57BL/6 mice showed no notable differences between the two strains of mice \((P = 0.96\) by analysis of covariance) (Fig. 4A). The equation of the linear regression line for pooled data from five experiments with CD8 cell-depleted cultures using the log10 scale was as follows: \(y = 0.999x - 3.711\). This expression is the same as the following: \(y = \log_{10}(\text{reactivation rate/TG/day})\), and \(x = \log_{10}(\text{latent viral load/200 ng of DNA})\). Therefore, the equation becomes as follows: \(\log_{10}(\text{reactivation rate/TG/day}) = 0.998 \times \log_{10}(\text{latent viral load/200 ng of DNA}) - 3.711\). Taking the antilog of both sides of the equation gives the following: \(10^{\log_{10}(\text{reactivation rate/TG/day})} = 10^{0.998 \times \log_{10}(\text{latent viral load/200 ng of DNA}) - 3.711}\). This equation converts as follows: \(\text{reactivation rate/TG/day} = 10^{0.998 \times \log_{10}(\text{latent viral load/200 ng of DNA}) - 3.711}\). This expression is the same as the following: \(\text{reactivation rate/TG/day} = (\text{latent viral load/200 ng of DNA})^{0.998} \times 10^{-3.711}\). Since \(10^{-3.711} = 1.95 \times 10^{-4}\), and if \(v\) is the latent viral load (genome copies per 200 ng of DNA), then the following equation applies to the CD8 cell-depleted cultures: \(\text{reactivation rate/TG/day} = 1.95 \times 10^{-4} \cdot v^{0.998}\).

Dose-dependent reduction of HSV-2 reactivation rate by CD8+ T cells. We postulated that the numbers of infiltrating CD8+ T cells should correlate with the reduction in virus reactivation rates in a dose-dependent manner. Since the rates of reactivation for the CD8 cell-depleted cultures were higher (Fig. 2 and 4), we calculated the extent to which the CD8+ T cells were gated on lymphocyte populations from forward and side scatter as determined by back gating using the CD45 expression after incubation with HSV-2-infected P815 cells. The y axis indicates CD8 expression in both the upper and lower panels. For all panels, cells were gated on lymphocyte populations from forward and side scatter as determined by back gating using the CD45+ population. The numbers of CD8+ T cells per TG and the percentages of HSV-2-specific IFN-γ-producing CD8+ T cells are shown in the upper right quadrants of the upper and lower panels, respectively.
cells suppressed reactivation by subtracting the reactivation rates for the nondepleted cultures from the reactivation rates for the CD8 cell-depleted cultures. If the effect of CD8+ T cells on blocking reactivation rates is dose dependent, then there should be a positive correlation between the numbers of CD8+ T cells and the difference in reactivation rates for CD8 cell-depleted and nondepleted cultures. In three experiments (Fig. 5), the correlation was significant, while in two experiments (Fig. 5), the correlation did not reach significance. When data from the five experiments were pooled (Fig. 5), the correlation between numbers of CD8+ T cells and the difference in reactivation rates for CD8 cell-depleted and nondepleted cultures was significant ($P = 0.001$). The equation of the linear regression line is as follows: $y = (6.61 \times 10^{-3})t - 1.68 \times 10^{-3}$, where $t$ is the number of CD8+ T cells per TG and $y$ is the reduction in the reactivation rate due to CD8+ T cells. Therefore, we estimate the amount of reduction in the reactivation rate due to CD8+ T cells to be as follows: $6.61 \times 10^{-3}(t - 25)$/day for $t$ CD8+ T cells.

Next, we used a different technique to confirm the dose-dependent reduction in the reactivation rate by primary CD8+ T cells. Primary CD8+ T cells were recovered from latently infected mouse TG and added back to CD8 cell-depleted cultures in various numbers. The reactivation of HSV occurred more slowly when higher numbers of CD8+ T cells were added to the culture; when a large number (1,000) of CD8+ T cells was added, reactivation was completely blocked (Fig. 6A). The magnitude of reduction in the reactivation rate correlated significantly ($P = 0.003$) and linearly ($R = 0.96$) with the number of CD8+ T cells added to the culture (Fig. 6B).

FIG. 2. Latent viral loads, numbers of CD8+ T cells, and ex vivo reactivation rates for TG cultures from C57BL/6 and DBA2 mice. Reactivation rates for CD8 cell-depleted (gray bars) and nondepleted (white bars) cultures were determined. Error bars indicate standard errors. Latent viral loads (triangles) and numbers of CD8+ T cells (circles) were also determined. The y axis on the left of each panel shows the reactivation rate, and the y axis on the right of each panel shows the latent viral load and CD8+ T cell number. The strain of mice is shown at the top of each panel, and the amounts of virus (PFU) in the inocula are indicated at the bottom. * indicates that neither CD8 cell-depleted nor nondepleted cultures showed reactivation, ** indicates that nondepleted cultures did not show reactivation, and *** indicates that CD8 cell-depleted cultures did not show reactivation. Exp., experiment.
Mathematical model for ex vivo reactivation rate. Finally, we postulated that the reactivation rate should be able to be derived solely from the latent viral load and the number of infiltrating CD8+ T cells. By using the two equations given above, the predicted reactivation rate for a CD8 cell-depleted TG culture is as follows: \((1.95 \times 10^{-4})v^0.998/day\), where \(v\) is the latent viral load (genome copies per 200 ng of DNA). The reduction in the reactivation rate due to CD8+ T cells is as follows: \(6.61 \times 10^{-5}(t - 25)/day\), where \(t\) is the number of CD8+ T cells. Combining those two equations, we predicted the reactivation rate for nondepleted cultures (i.e., the reactivation rate for CD8 cell-depleted cultures minus the reduction in the reactivation rate due to CD8+ T cells), based on the latent viral load and the number of CD8+ T cells, to be as follows: \((1.95 \times 10^{-4})v^0.998 - (6.61 \times 10^{-5})(t - 25)/day\).

We performed an experiment to see if the equation would predict the reactivation rate for nondepleted cultures. Forty C57BL/6 mice were infected with three different doses of HSV-2 by the scarification of both corneas. Mice were sacrificed 28 days after infection. Twenty individual pools of TG, each containing four TG from two mice, were dispersed into single cell suspensions. The latent viral loads and the numbers of CD8+ T cells in a portion of the TG cell suspension (equivalent to one TG) were measured, and the reactivation rates for non-CD8-cell-depleted cultures were measured using the rest of the TG suspension (equivalent to three TG). From the latent viral loads and numbers of CD8+ T cells, we predicted the reactivation rates for nondepleted cultures. The predicted reactivation rates for 20 pooled TG correlated significantly with the observed reactivation rate \((P = 0.04)\) (Fig. 7).

**DISCUSSION**

We have studied mice latently infected with HSV-2 to understand the interaction between HSV-2 and virus-specific cellular immune responses during latency. We found that (i) the ex vivo HSV-2 reactivation rate correlates directly with the latent viral load in the absence of CD8+ T cells, (ii) the number of infiltrating CD8+ T cells influences the ex vivo reactivation rate, but less directly than the latent viral load, and (iii) a mathematical model derived from the measurement of the latent viral load and the number of CD8+ T cells can predict the ex vivo reactivation rate. Our results were derived from ex vivo reactivation experiments with mice, and the interactions between HSV-2 and CD8+ T cells may be different during the spontaneous reactivation of HSV-2 in humans.

We have shown that the total numbers of CD8+ T cells in latently infected mouse ganglia correlate with the titers of the inocula used to infect the animals; however, the frequencies of HSV-2-specific CD8+ T cells in the ganglia are independent of the titers of the inocula. These results imply that assessing both virus-specific and nonspecific CD8+ T cells, rather than solely HSV-specific CD8+ T cells, gives a more accurate measure of the immune response induced by the latent infection of neurons with HSV. Most (more than 90%) of the CD8+ T cells in the ganglia were effector memory cells (CD62L-), indicating...
that the CD8\(^+\) T cells in latently infected ganglia are persistently stimulated rather than simply remaining in the ganglia after acute HSV infection. Our results agree with previous observations that activated memory CD8\(^+\) T cells accumulate in mouse TG latently infected with HSV (19) and that latent HSV infection provides antigenic stimulation to CD8\(^+\) T cells (40).

We found that 30 to 35% of the total CD8\(^+\) T cells in the ganglia of infected mice were HSV-2 specific and produced IFN-\(\gamma\) in response to virus. Khanna et al. showed that more than 60% of infiltrating CD8\(^+\) T cells in mouse ganglia recognize a single glycoprotein B peptide incorporated into a tetramer and that most of these CD8\(^+\) T cells are activated and express CD69 and CD44 at 1 month after infection (19). In addition to CD8\(^+\) T cells, other types of cells such as NK cells, CD4\(^+\) T cells, macrophages, and microglial cells may also affect the rate of reactivation. Some of these cells have been shown to control acute HSV infection (2, 5, 9, 18, 30, 31, 37), but the function of individual cell types other than CD8\(^+\) T cells during HSV reactivation from latently infected neurons has not been studied.

Infiltrating T cells in latently infected mouse TG secrete IFN-\(\gamma\), tumor necrosis factor alpha, and other cytokines (e.g., IL-2, IL-10, IL-4, and RANTES) (4, 7, 15, 28). After the induction of reactivation by various stimuli, cytokine-producing lymphocytes accumulate around HSV antigen-positive neurons. Changes in levels of transcripts of immune response genes in latently infected ganglia have also been detected previously. A comparison of mRNAs in mouse TG latently infected with HSV with those in mock-infected TG showed that multiple immune response genes are significantly upregulated during latency, including genes for IFN-\(\gamma\), tumor necrosis factor alpha, macrophage inflammatory protein 1\(\alpha\), macrophage inflammatory protein 1\(\beta\), RANTES, CCR6, and CXCR3 (6, 8).

The importance of the innate immune response in latency and the control of reactivation is evident from the results of experiments showing that latent HSV-1 reactivates at higher frequencies in IFN-\(\gamma\) or IFN-\(\gamma\) receptor knockout mice than in wild-type mice and that IFN-\(\gamma\) can block the in vitro reactivation of HSV-1 even after the expression of late viral gene products (3, 12, 24, 26).
We found that primary HSV-specific CD8\(^+\) T cells, obtained from the ganglia of HSV-2-infected mice, can block the reactivation of HSV from latency ex vivo in a dose-dependent manner. We demonstrated this both (i) by comparing reactivation rates for cultures depleted of CD8\(^+\) T cells with those for cultures not depleted of these cells (Fig. 5) and (ii) by adding increasing numbers of CD8\(^+\) T cells to HSV-2-infected TG cell cultures (Fig. 6). The results of our experiments using primary infiltrating CD8\(^+\) T cells are consistent with those of studies by Khanna et al. in which an HSV-specific CD8\(^+\)-T-cell clone, specific for glycoprotein B, inhibited the reactivation of HSV-1 from latently infected mouse TG cultures ex vivo in a dose-dependent manner (19, 21) and with those of studies by Noisakran and Carr using lymphocytes from immune mice (32). One important difference between the findings of our study and those of Khanna et al. is the number of CD8\(^+\) T cells needed to block reactivation. Khanna et al. found that 10\(^6\) HSV-specific CD8\(^+\) T cells derived from the T-cell clone did not block reactivation completely when added to TG cell cultures; however, we found that 10\(^3\) HSV-specific primary CD8\(^+\) T cells completely blocked reactivation (Fig. 6). The difference in our findings may be due to differences in culture systems or strains of animals or viruses but also may indicate that the primary infiltrating CD8\(^+\) T cells are more effective in blocking reactivation than an HSV-specific CD8\(^+\)-T-cell clone.

We developed a mathematical model, based on the latent viral load and the number of infiltrating CD8\(^+\) T cells in ganglia, to predict the ex vivo reactivation rate and validated the model prospectively. The difference in the predicted reactivation rate and the observed rate may be due to biological variation, other types of cells that contribute to reactivation (e.g., CD4\(^+\) T cells and NK cells), or local concentrations of cytokines or chemokines. The numbers of infiltrating CD8\(^+\) T cells and the latent viral loads correlated significantly (\(P = 10^{-4}\)) when data from five experiments were pooled (data not shown). An increased latent viral load may increase the probability of virus reactivation from neurons; however, a higher viral load may also increase the number of CD8\(^+\) T cells infiltrating the ganglia. Both of these effects likely contribute to the observed rate of reactivation. We speculate that low-level, subclinical reactivation of latent HSV in mouse TG, as proposed by Feldman et al. (14), maintains CD8\(^+\) T cells in TG long after acute infection is over. In the present study, we used TG infected with HSV-2. While most HSV-2 infections result in genital disease, HSV-2 does cause ocular and oral disease. We have found the ocular model of HSV-2 infection to be useful for studies of immunity, latency, and the reactivation of HSV-2 (17, 42). Nonetheless, the rates of reactivation of HSV-2 from the TG differ from those from the lumbosacral ganglia.

Our findings have implications for the development of both therapeutic and prophylactic HSV-2 vaccines. CD8\(^+\) T cells in human TG from HSV-seropositive subjects have been detected previously (39), a finding similar to our observations with mice. An immunotherapeutic vaccine which seeks to reduce HSV-2 reactivation rates should aim to increase the number of infiltrating virus-specific CD8\(^+\) T cells in the ganglia. Therapeutic vaccines have shown efficacy in guinea pig models of HSV infection when given shortly after primary infection (17, 41); however, a therapeutic vaccine for humans would need to be effective in persons who were infected several months or years prior to vaccination. Large-scale clinical trials of therapeutic vaccines using recombinant glycoprotein D or virus with the deletion of glycoprotein H have not been successful in reducing recurrence rates in HSV-2-seropositive persons with recurrent genital herpes (11, 38). Our studies suggest that the quantification of virus-specific CD8\(^+\) T cells in the ganglia of animals, especially over time, may provide a useful surrogate marker to predict the ability of a vaccine to reduce the rate of reactivation.

A prophylactic vaccine should similarly increase the level of virus-specific cells in the ganglia; if the vaccine contains live virus that can reactivate, it should yield a low or absent latent viral load in the ganglia. Our data indicate that a low latent viral load is the most critical factor to prevent reactivation. While a number of vaccines have been effective in animal studies, a vaccine that effectively prevents HSV-2 disease in humans does not yet exist. Our studies provide a mathematical model for the relationship of the latent viral load, the HSV-specific CD8\(^+\)-T-cell response, and the rate of reactivation that may be useful in the development of future vaccines and the testing of these vaccines in animals.

ACKNOWLEDGMENTS

This study was supported by the intramural research program of the National Institute of Allergy and Infectious Diseases. Part of Yo Hoshino’s work was supported by the Japan Herpes Virus Infection Forum (JHVF).

We thank Kennichi Dowdell, Kening Wang, Anthony Nicola, and Kozaburo Hayashi for suggestions and assistance and Jonathan Yewdell for the P815 cell line.

REFERENCES

5. Carr, D. J., and S. Noisakran. 2002. The antiviral efficacy of the murine alpha-1 interferon transgene against ocular herpes simplex virus type 1 requires the presence of CD4\(^+\), CD8\(^+\) T-cell receptor-positive T lymphocytes with the capacity to produce gamma interferon. J. Virol. 76:9398–9406.
34. Sawtell, N. M. 1998. The probability of in vivo reactivation of herpes simplex virus type 1 increases with the number of latently infected neurons in the ganglia. J. Virol. 72:6888–6892.