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Prophylactic and therapeutic effects of human immunoglobulin on the pathobiology of HSV-1 infection, latency, and reactivation in mice

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Pooled human immunoglobulin (IgG) was evaluated as prophylaxis and treatment of HSV-1 infection in mice. We compared the effects of IgG on the course of acute infection and spread of virus through the nervous system, as well as on the establishment, maintenance, and reactivation of virus from latency. Balb/c mice received a single 3.75 mg intraperitoneal injection of IgG 24 h before or 24 h, 48 h, or 72 h after ocular infection with 10^6 pfu of HSV-1 strain McKrae. Treatment with IgG protected against death in a time-dependent manner ($P < 0.001$ for -24 h vs. $+48$ h and $+72$ h IgG treatment groups). Viral shedding from the eyes was reduced more in mice treated with IgG at -24 h or $+24$ h relative to animals treated at $+48$ h. Viral titers in the eyes were reduced in mice treated with IgG at $+24$ h, but not at $+48$ h. In ganglia, virus recovery was reduced ($P < 0.05$) in mice treated at -24 h, $+24$ h, or $+48$ h relative to untreated mice, or ones treated at $+72$ h. In brains, similar results were observed in mice treated at -24 h, $+24$ h, or $+48$ h relative to $+72$ h. Upon explantation, virus reactivated from all ganglia of all surviving mice regardless of treatment group. DNA quantitation showed that mice pretreated with IgG tended towards lower quantities of latent genome copies compared to $+24$ h treatment and $+48$ h treatment. UV irradiation induced reactivation *in vivo* in 16/40 pretreated mice, 20/29 mice treated at $+24$ h, and in 8/8 mice treated at $+48$ h ($P = 0.03$ and $P = 0.004$, for comparisons at -24 h vs. $+24$ h, and -24 h vs. $+48$ h, respectively). Histopathological studies revealed that mice pretreated and treated with IgG had milder encephalitis and reduced virus spread compared to untreated mice. Pooled human IgG attenuates the spread of, and morbidity from, HSV-1 if given before and within 2 days after ocular infection. *Journal of NeuroVirology* (2002) **8,** 35–44.

Keywords: human immunoglobulin; HSV-1 infection; reactivation; encephalitis

Introduction

Infection with herpes simplex virus types 1 and 2 (HSV-1, HSV-2) involves viral replication at the site of entry followed by centripetal transit in sensory nerves to the trigeminal or dorsal root ganglia where they establish life-long latency (Whitley, 2001). Reactivation from latency occurs when a stimulus triggers renewed replication of virus with centrifugal spread of progeny virions along neuronal axons to mucocutaneous sites at or near the initial portal of entry. In the typical cycle of HSV infection, latency, and reactivation in humans, there is little evident of spread of virus to more distant tissues. When it does occur, disseminated HSV involving the brain or other viscera is associated with severe illness and death (Corey and Spear, 1986). Fortunately, these are rare events

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in immunocompetent people. In small mammals in which HSV did not evolve to co-exist, the infection is more severe than in humans and is often a disseminated and fatal one.

Lacking proven public health strategies or vaccines for preventing HSV infection, the major clinical thrust has been the development of effective antiviral drugs. An additional, although still incompletely developed, strategy entails the use of HSV-specific immunoglobulin G (IgG) or pooled IgG containing HSV antibody for prophylaxis and treatment. Even before the development of potent nucleoside drugs and repeatedly thereafter, IgG was proven to diminish the mortality of HSV-1 in infected mice, if given before infection (Akerfeldt *et al*, 1972, 1973). These early data indicated that specific antibodies neutralize HSV infectivity in the crucial period before it binds to and enters cells.

Because HSV spreads from cell-to-cell and not via extracellular fluids it had been assumed that IgG would not attenuate HSV disease if administered after the onset of infection. Nonetheless, polyclonal and monoclonal antibodies with high titers of HSV-specific neutralizing activity (Oakes and Rosemond-Hornbeak, 1978; Davis *et al*, 1979; McKendall *et al*, 1979; Simmons and Nash, 1985) and pooled human immunoglobulin used for intravenous replacement therapy (IVIG) (Erlich *et al*, 1987) showed significant benefit, even when given 1 to 2 days after infection. These observations suggested that IgG could block HSV spread after the onset of infection and aroused some interests for considering its use not only as a prophylactic agent but also as a therapeutic one.

With this background, we sought to explore the effects of IgG in a murine model of HSV-1 infection during acute and latent phases by administering human IgG 24 h before or 24, 48, or 72 h after infection. In these studies, we compared the survival rate of infected mice, virus shedding in eyes, the titers of virus in various tissues to which it spreads after infection, the histopathology of the brain, quantified the number of genome copies that persisted in ganglia after infection (latent viral load), and determined the capacity of virus to reactivate upon exposure to ultraviolet (UV) light.

Results

Survival of animals during acute HSV-1 infection

To determine the effects of IgG on virus-induced mortality, 5 animals were mock-infected or groups of 10 animals each were infected by bilateral corneal scarification followed by inoculation with 1×10^6 p.f.u. of HSV-1 (McKrae). Mortality was scored daily for the first 17 days p.i. None of mice treated with IgG at 72-h postinfection survived beyond day 7. Survival showed a clear association with time of treatment (Figure 1). Specifically, mice given IgG 24 h before infection were more likely to survive than mice treated at 48 or 72 h after infection (both $P < 0.001$). Similarly, mice treated at +24 h were more likely to survive than those treated at +48 h ($P = 0.04$) or +72 h ($P < 0.001$).

Ocular virus shedding during acute infection

Corneal shedding of HSV-1 was monitored by taking cultures on days 1, 3, 5, 7, 9, and 11 postinfection. None of the mock-infected animals shed virus on any day, as expected (Figure 2). During the first 5 days postinfection, all infected mice shed virus. Because none of the mice treated 72 h postinfection survived beyond day 6, eye swabs could no longer be obtained in that group. However, late virus shedding could be ascertained in other groups of infected mice. Overall, for any given day, a smaller proportion of infected mice treated with IgG 24 h before and 24 h after infection shed virus than treated at 48 h after infection, but sample sizes were too small to confirm this trend.

HSV-1 titers during acute infection

To further study the time dependence of IgG treatment on HSV-1 spread in this mouse model, we quantified the virus load in the eyes, trigeminal ganglia (TG), and brains on days 2, 4, 6, 8, and 10 postinfection. These tissues were harvested from groups of three mice each per treatment arm at each time point and the mean virus titers were determined. No untreated controls survived beyond day 6 postinfection. In general, higher virus titers were detected in the untreated group compared with IgG treated groups. (Figure 3). On day 4 and to some extent on day 6 postinfection, the reduction in HSV-1 titers in ganglia and brains proceeded in a time-dependent fashion, with the least virus in animals that had been pretreated with IgG. Treatment had a less obvious impact on virus content in the eyes. Thus, the major impact of IgG was on virus spread to, and within, the peripheral and central nervous systems.

Explant cocultivation of latently infected ganglia

HSV-1 establishes and maintains a latent infection in the TG and can be reactivated *in vitro* by explant cocultivation. To detect any potential differences in relative rates of the establishment of latency and of *in vitro* reactivation, an indicator of the quantity of latent virus, HSV-1 infected, IgG-treated mice were sacrificed on day 40 postinfection, TG were harvested from 5 mock infected animals, 7 pretreated animals, 10 animals treated at 24 h, and all 4 remaining mice treated at 48 h postinfection. No animals treated at 72 h postinfection survived to develop latent infections. Explanted ganglia were cultured for 17 days, by which time ganglia from 100% of infected mice yielded virus (data not shown). There was no appreciable difference in the average time of reactivation: all reactivated by 11 to 13 days after explantation. No virus reactivated from ganglia of the

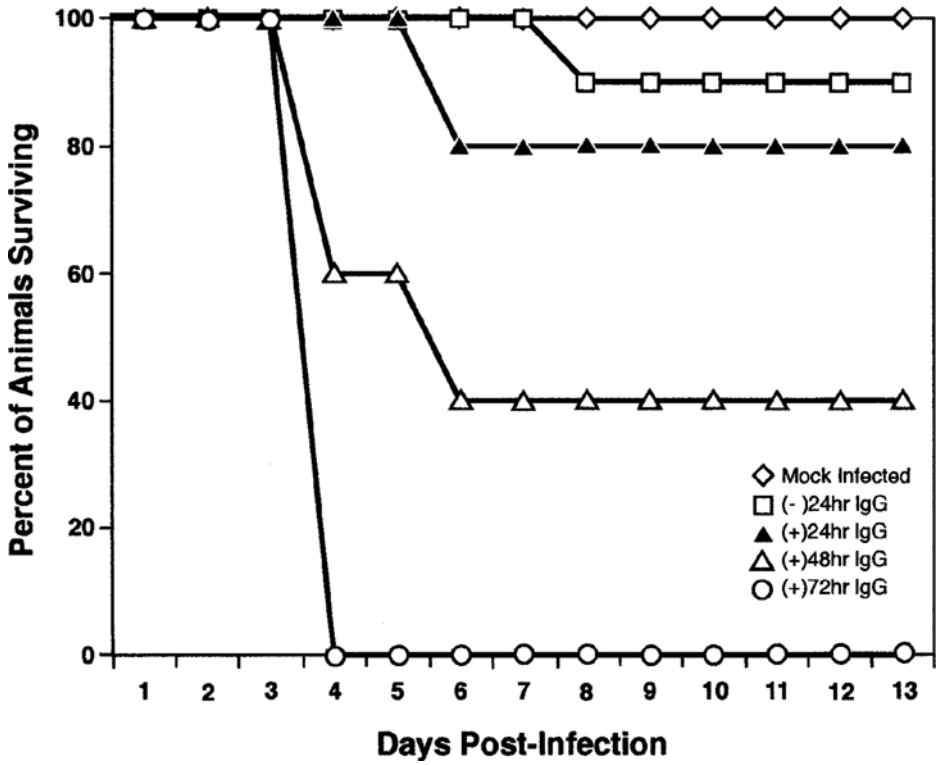

Figure 1 Survival after HSV-1 inoculation. Groups of 10 mice each were inoculated with a lethal dose (10^6 p.f.u.) of HSV-1 (McKrae) using bilateral corneal scarification. Uninfected controls (5 mice) received PBS. Animals were given a single 0.5-ml dose IgG (pooled human IVIG diluted 1:8 in PBS, 3.75 mg IgG/mouse) i.p. 24 h before or at 24 h, 48 h, or 72 h postinfection.

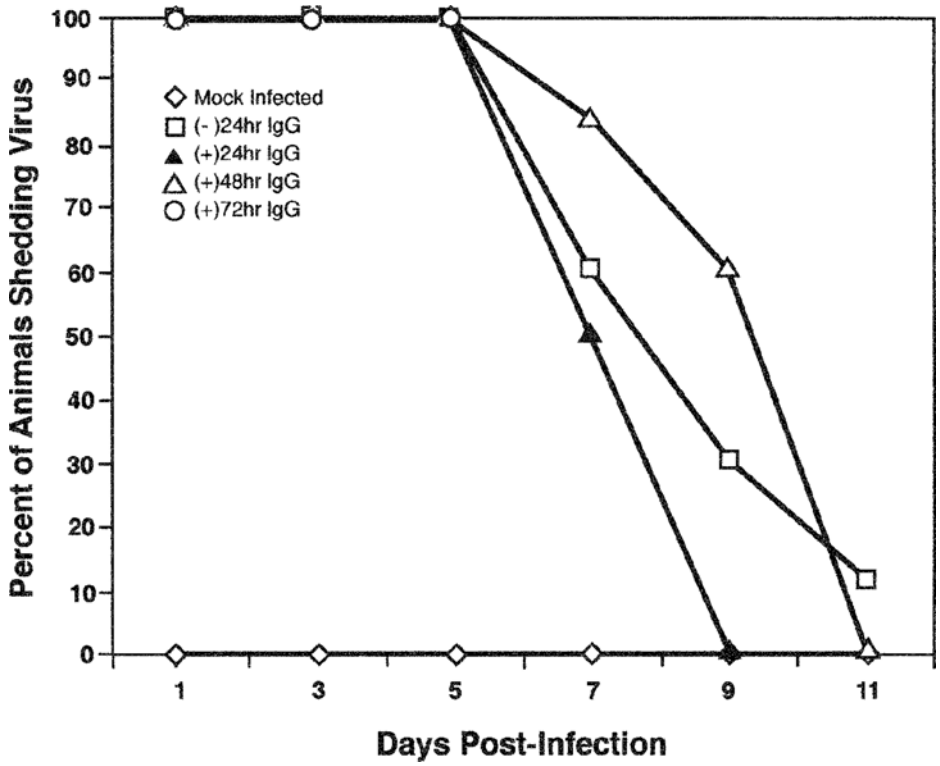

Figure 2 Ocular virus after HSV-1 infection. Groups of 5 mock-infected or 10 mice each infected with HSV-1 (McKrae) and treated with IgG i.p., as described in Figure 1. On the indicated days, corneal swabs were taken for virus isolation. Each point represents the data for 5 (mock infected) or 10 (treated groups) mice.

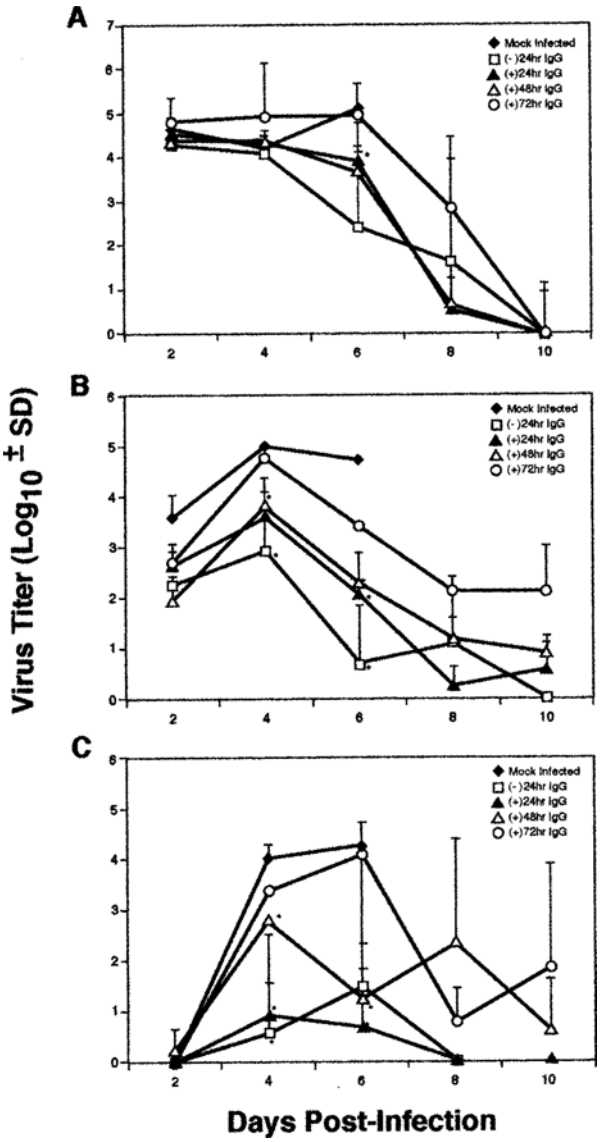

Figure 3 HSV-1 titers in the eyes, ganglia, and brains during acute infection. Mice were infected with HSV-1 (McKrae) and administered IgG as described in Figure 1. On the indicated days, the eyes (panel **A**), pooled TG (panel **B**), and brain (panel **C**) were harvested and virus titers determined by plaquing on Vero cells. Each point represents the log value of the arithmetic mean±SD for three animals. Log values shown as zero actually reflect undetectable levels of virus. Untreated controls did not survive beyond day 6 p.i. The asterisks (∗) indicate significant differences as compared with untreated animals.

5 mock-infected mice. Thus, treatment with IgG did not entirely prevent establishment of HSV-1 latent infection.

In vivo reactivation of HSV-1 after UV exposure

Typically, HSV does not reactivate spontaneously from latently infected mice. Therefore, reactivation must be induced. Using UV irradiation as a stressor (Laycock *et al*, 1991; LeBlanc *et al*, 1999), we determined whether there would be differences in rates of in vivo reactivation among the treatment groups, another indicator of the presence and quantity of latent virus. After latency was established (day 46 postinfection), the animals were exposed to UV light for 1 min per eye (360 mJ/eye). TG were harvested 48 h later, homogenized, and plated onto monolayers of Vero cells. As expected, no virus was recovered from UV-exposed, mock-infected mice. We observed significant differences in the proportion of animals reactivating virus in IgG pre- and posttreated groups (Table 1). Although 40% (16/40) of mice pretreated with IgG yielded virus, 70% and 100% of mice treated with IgG 24 h and 48 h after infection, respectively, showed reactivation ($P = 0.03$ and $P = 0.004$ for −24 h compared to +24 h and +48 h, respectively). Only 5% (2/40) of all infected mice showed HSV-1 reactivation without UV-exposure.

Table 1 UV-induced reactivation of HSV-1 in latently infected mice

Group	*UV-induced* *No. of mice reactivated/ Total no. of mice*	*Spontaneous* *No. of mice reactivated/ Total no. of mice*
Mock infected	0/10	0/10
(−) 24 h IgG	16/40	1/20
(+) 24 h IgG	20/29	0/16
(+) 48 h IgG	8/8	1/4

The latent viral DNA load in TG

To determine the quantity of latent HSV-1 genomes in the TG, we used real-time PCR (LeBlanc *et al*, 1999). Groups of 8 HSV-1 latently infected animals from each treatment arm were sacrificed on day 30 postinfection and individual ganglia were harvested for DNA extraction and subsequent PCR. By comparing the PCR results to a standard curve run simultaneously on triplicate serial dilutions of IgG-1 plasmid DNA, we estimated the genome copy number in test samples (Figure 4). Although the differences in the latent DNA copy numbers among the 3 IgG-treated groups were small and not statistically significant, there was a rise in viral DNA load with treatment after infection compared with pretreatment. The geometric mean copies of HSV-1 DNA in ganglia from animals treated at 24 h and 48 h p.i. were 2 to 3 times greater than pretreated mice.

Effect of IgG treatment on CNS pathology

To characterize the effect of treatment with pooled human IgG on the distribution and severity of inflammation and virally initiated cell death, brains from three groups of infected mice were evaluated histopathologically in a blinded fashion (Figure 5). In our earlier tissue studies (Figure 3C) substantive quantities of virus were first detected in the brain at day 4 postinfection, with peak levels at day 6 and resolution of the infection by day 10. Accordingly,

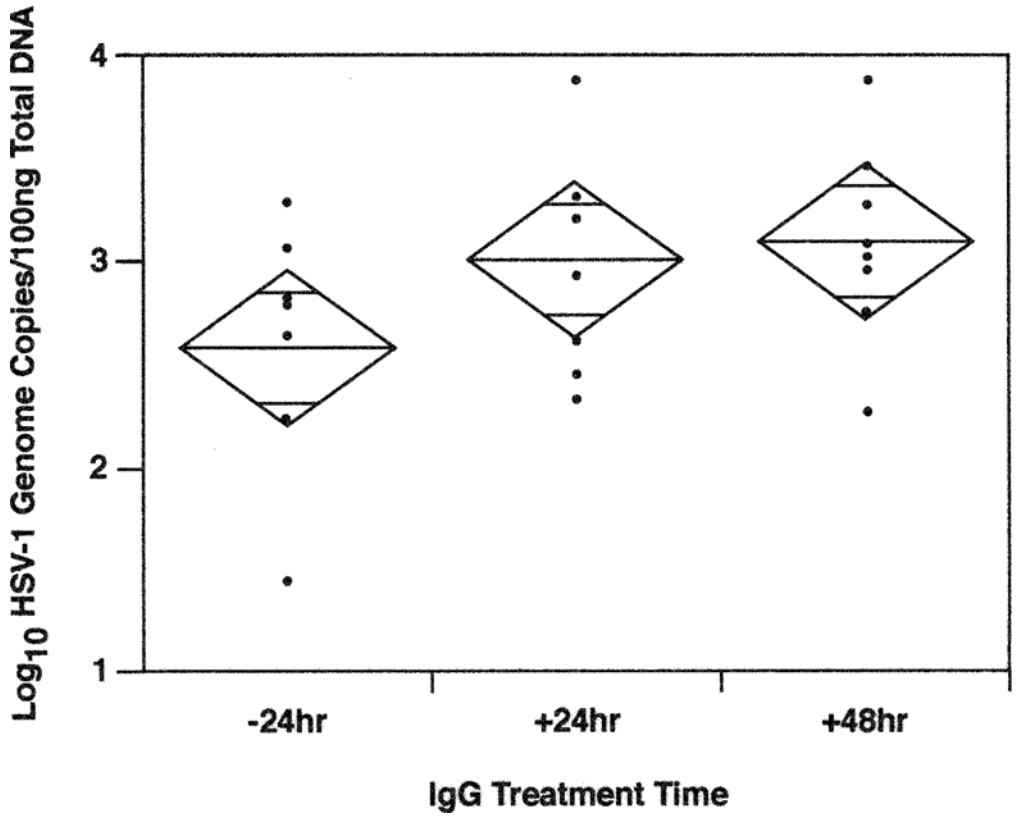

Figure 4 Quantitation of HSV-1 DNA load in the trigeminal ganglia of latently infected mice. Groups of eight mice each administered IgG at −24 h or +24 h or +48 h were sacrificed on day 30 p.i., and individual ganglia were harvested for DNA extraction. Each point represents the log value of the mean copy number for three replicate PCR determinations per pooled TG from each animal. The horizontal line along the width of each data "diamond" indicates the combined mean HSV-1 DNA copy numbers for all eight mice per group. The shorter horizontal lines above and below this line indicate the 95% confidence intervals around the mean.

we chose to accrue histopathological data at each of these three time points from infected but untreated mice, from mice that had been given IgG 24 h before infection, and from mice treated 24 h after infection.

By day 4, minimal histopathological changes were evident in the brains, with no definite differences among representatives of the untreated and treated groups. Specifically, all three untreated mice had mild acute encephalitis, minimal necrosis, and few viral intranuclear inclusion bodies, restricted to the medulla in the region of the trigeminal tracts. One mouse that had been pretreated with IgG had minimal focal encephalitis evident in the mesencephalon, whereas the other two such mice manifested minimal to mild encephalitis restricted to the medulla, with necrosis of few scattered cells. Among animals treated 24 h after infection, one showed no evidence of encephalitis, yet the other two had mild acute encephalitis restricted to the medulla, with minimal cell necrosis.

By day 6 postinfection, all untreated mice exhibited anatomically widespread areas of disease, with mild-to-severe necrosis and minimal to mild encephalitis. Mice of both treated groups had mild-to-moderate acute encephalitis. It involved the medulla in all mice, but also the hypothalamus in two pretreated and one posttreated mice, and the mesencephalon in two pretreated mice and all three posttreated mice. With the exception of one mouse treated at 24 h after infection, all treated mice had substantially less cellular necrosis than found in any of the untreated mice.

None of the untreated mice survived to day 10, in accord with our earlier data (Figures 1–3). Changes in treated mice were largely similar and included mild encephalitis involving the thalamus (one pretreated and three posttreated), the hypothalamus (two pretreated), mesencephalon (one pretreated, two posttreated), and the medulla (all six treated mice), with mild multifocal perivascular lymphocytic cuffing and limited necrosis of individual cells. Thus, the lesser degrees of necrosis and inflammation evident in the treated mice by day 10 relative to what had been observed on day 6 indicated that the infection and inflammatory responses were resolving.

The cumulative histopathological findings showed that the encephalitis in both treated and untreated animals centered first in the medulla and then spread

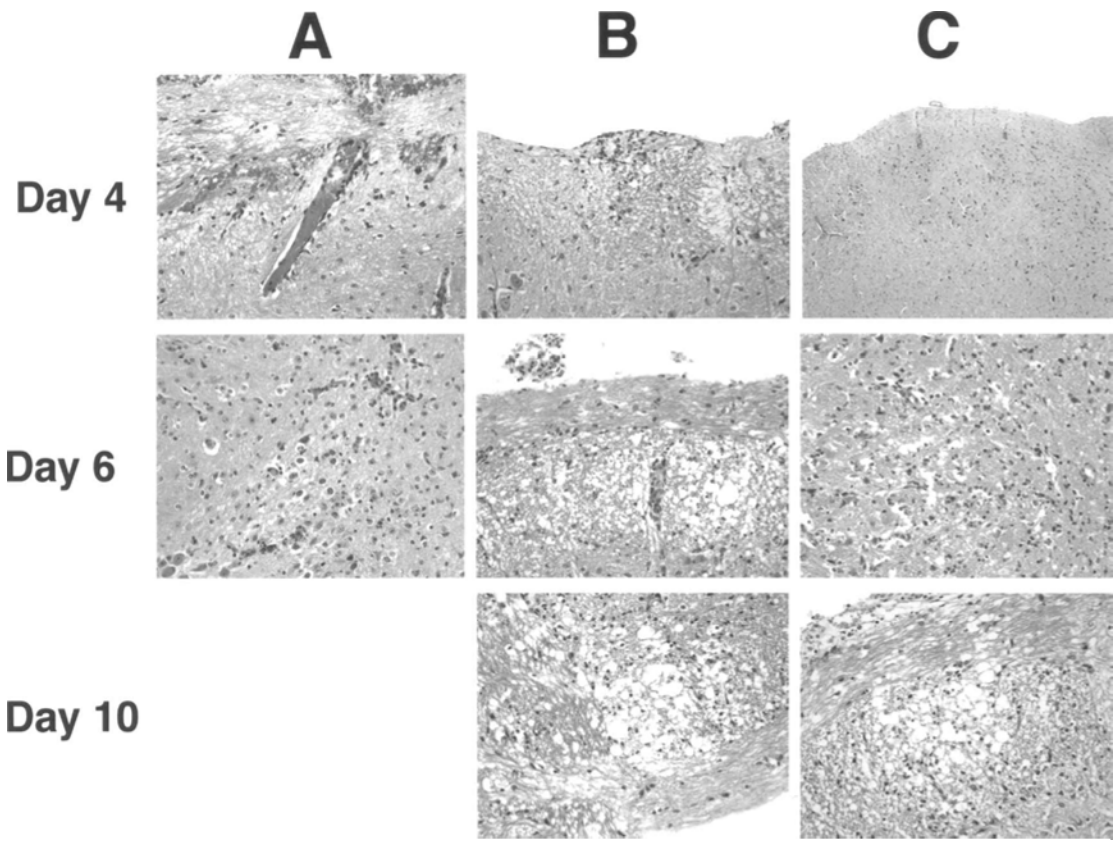

Figure 5 Hematoxylin and eosin-stained sections of mouse brain-medulla. Groups of three mice were sacrificed on the indicated days and brains were harvested, fixed, embedded, sectioned, and stained for histopathological studies. (**A**) Untreated infected mice. (**B**) Mice treated with IgG 24 h before infection. (**C**) Mice treated with IgG 24 h after infection. Original magnification, 5×.

rostrally, presumably along deep brain tracts to the cortex. These observations are consistent with earlier report (Martin *et al*, 1991). Histopathological changes in the medulla, by animal groups and study day, are shown in Figure 5. We attribute the occasional early involvement of the olfactory and frontal lobes in some animals to dissemination of the virus inoculum from the nasal mucosa, via the olfactory nerves.

Immunohistochemical staining was performed on the tissue sections from all areas of brain at each of the study days to assess the presence and spread of HSV-1 antigen. Figure 6 shows antigen staining in the medulla. Here and elsewhere in the brain, the density of antigen staining ranged widely. The heaviest concentrations of antigens were observed at day 6 in untreated mice (scored 4+ on a scale of 0 to 4). Although antigen staining in pretreated mice usually scored only 1 to 2+ in any brain segments examined, posttreated mice showed varying and occasionally intense antigen staining (Table 2).

Discussion

This study sought to explore the effects of IgG action as prophylaxis and treatment in a mouse model of HSV-1 infection using an array of virological, molecular, and histopathological tools. The cumulative data indicate that prophylaxis or early treatment with IgG attenuates early events in HSV-1 infection sufficiently to reduce virus spread, viral-induced disease, the quantity of latent virus, and its subsequent potential for reactivation. Animals receiving IgG tolerated a lethal dose of HSV-1 in a time-dependent manner (Figure 1). Although the infected mice pretreated with IgG survived best, the protective effect of IgG was observed even when its administration was delayed until day 2 postinfection. Ocular virus shedding was observed for fewer days among smaller proportions of mice treated with IgG at 24 h before and 24 h after infection, compared to mice treated at or after 48 h p.i. (Figure 2). IgG treatment was also effective in limiting HSV-1 spread to the peripheral and central nervous system in a time-dependent manner (Figure 3). IgG pre- and posttreatment had less impact on viral titers in the eye than the nervous system. This might reflect the fact that the primary inoculum was to the eyes, or that IgG may not translocate into the eye until they are inflamed, which requires a day or two in HSV infection. These observations are consistent with earlier reports on the efficacy of prophylactic IgG on HSV-1 infection (Erlich and Mills, 1986; Erlich *et al*, 1987).

Table 2 Relative intensity of HSV-1 antigen staining in anatomical segments of the of mouse brain

Day postinfection	*Animal no.*	*No treatment*	*(−) 24 h IgG*	*(+) 24 h IgG*
Day 4	1	Hypothalamus, 3+* Mesencephalon, 3+ Medulla, 3+	None detected	None detected
	2	Olfactory, 2+ Mesencephalon, 3+ Medulla, 3+	Medulla, 1+	Cerebrum, 1+ Pons, 1+ Medulla, 2+
	3	Cerebrum, 1+ Medulla, 3+	Mesencephalon, 3+ Medulla, 2+	Mesencephalon, 1+ Medulla, 2+
Day 6	1	Not done	Thalamus, 1+ Mesencephalon, 1+ Medulla, 2+	Olfactory, 4+ Cerebrum, 3+ Mesencephalon, 2+ Medulla, 3+
	2	Olfactory, 4+ Cerebrum, 3+ Mesencephalon, 2+ Medulla, 4+	Thalamus, 2+ Mesencephalon, 2+	Medulla, 2+
	3	Olfactory, 4+ Cerebrum, 3+ Thalamus, 1+ Hypothalamus, 3+ Mesencephalon, 3+ Medulla, 4+	Medulla, 2+	Olfactory, 2+ Cerebrum, 1+ Mesencephalon, 3+ Medulla, 3+
Day 10	1	Dead	Medulla, 1+	Medulla, 1+
	2	Dead	None detected	Not done
	3	Dead	Medulla, 1+	Medulla, 1+

*The antigen staining intensity was scored as none, 1+ for minimal and highly focal, 2+ for moderate and focal in distribution, 3+ for moderate and more widespread, 4+ for intense and widespread throughout the sections.

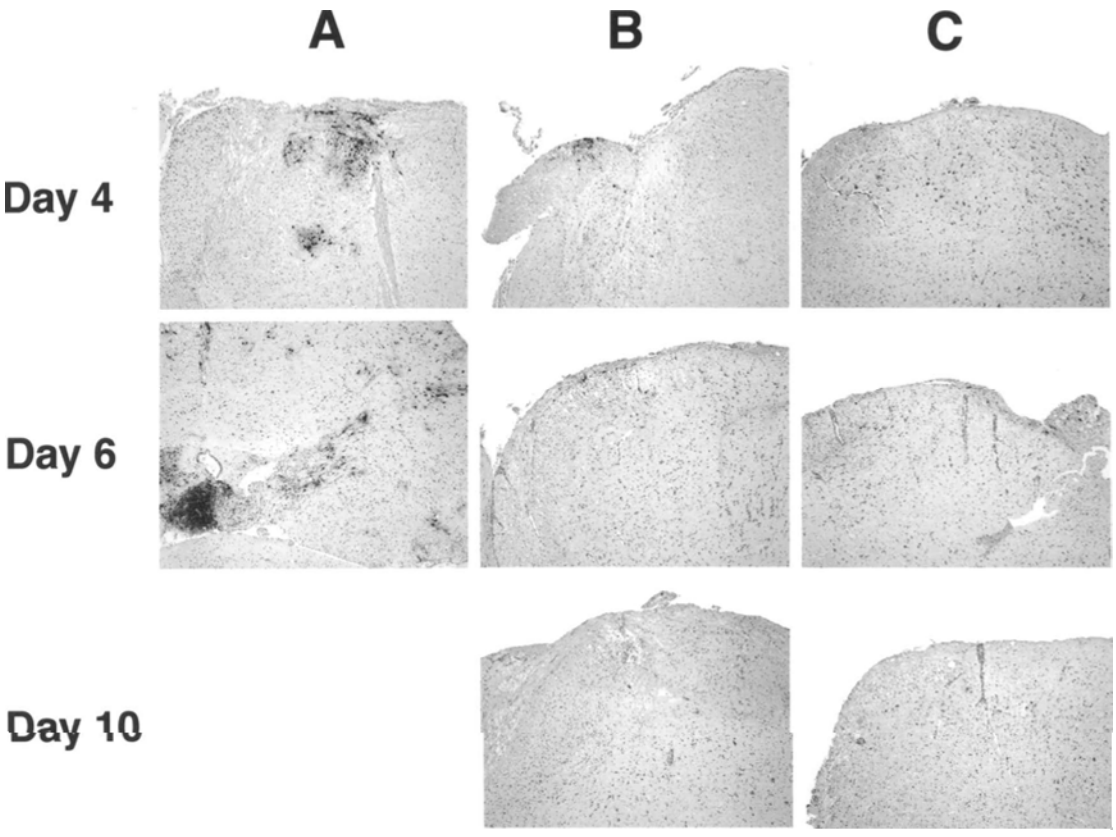

Figure 6 Immunohistochemical localization of HSV-1 antigen in sections of mouse brain medulla. The fixed brains, described in Figure 5, was also sectioned and stained for HSV-1 antigens. Original magnification, 5×.

Because the protective effect of IgG on survival and early spread of HSV-1 were observed predominantly in pretreated rather than posttreated mice, we thought that it may also result in a lower latent viral load. Explant cocultivation is fairly insensitive to viral load, so it was not surprising that virus could be recovered from all ganglia. The quantitative PCR data showed 2- to 3-fold fewer HSV-1 genome copies in pre-treated mice (Figure 4). These data contrast with those of Birmanns *et al* (1993), who reported paradoxical rises in levels of latent HSV-1 DNA in ganglia of mice pretreated with IgG. The *in vivo* induction of reactivation using UV light, however, clearly showed that mice pretreated with IgG had lower rates of reactivation compared to posttreated animals ($P < 0.05$) (Table 1). The direct correlation between rates of reactivation and latent viral DNA load is concordant with prior reports (Maggioncalda *et al*, 1996; Lekstrom-Himes *et al*, 1998; Sawtell, 1998; Sawtell *et al*, 1998). Thus, the cumulative data suggest that IgG treatment impairs spread of virus to the ganglia and results in reduced latent viral loads.

The most dramatic effects of IgG we observed were on the survival of mice from HSV-1 infection. Because HSV-1-related death in mice appears due primarily to the encephalitis, we wanted to test whether IgG treatment could reduce the extent of inflammation and necrosis in different areas of brain. The histopathological studies (Figure 5) showed that those mice that received IgG treatment 24 h before or 24 h after infection had minimal if any encephalitis, whereas untreated mice showed severe encephalitis. Similarly, HSV-1 antigen density (Figure 6, Table 2) was intense (4+ in score) in untreated mice in contrast to mice treated with IgG (scores of only 1 to 2+). These findings agree with earlier reports that antibody plays an important role in prevention of encephalitis (Davis *et al*, 1979; Beland *et al*, 1999).

That IgG attenuates the spread of HSV-1 from the initial site of infection is consistent with its ability to neutralize a cell-free inoculum. Neutralization may also limit spread within the nervous system of virus released from necrotic cells. Neutralization, however, is not sufficient to explain all of the present findings. Recognizing that HSV requires only an hour or less to bind to and enter cells, how does the administration of IgG 24 to 48 h postinfection ameliorate encephalitis and subsequent mortality? IgG has additional activities that could explain reduced virus levels in the ganglia and brain (Figure 3B, C) and, hence, attenuate encephalitis (Figure 5). Binding of IgG to free virions enhances phagocytosis by neutrophils and macrophages. Moreover, IgG participates in antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement-mediated cytolysis (ADCMC) (Simmons and Nash, 1985; Mester *et al*, 1991). Levine *et al* (1991) demonstrated that antibody mediates clearance of alphaviruses in a murine encephalomyelitis model by restricting viral gene expression within infected neurons. Sanna and his colleagues (1996) postulated that the effects of IgG on replication of virus in neurons explained, in part, the ability of a human monoclonal antibody to enhance survival when given to nude mice after HSV-1 infection.

Disseminated infection is not a usual finding in immunologically competent people. It is, however, a feature of severely immunocompromised patients, especially neonates. The epidemiology and morbidity of disseminated neonatal herpes, even with nucleoside analog treatment (Whitley, 2001), renders it a promising setting in which to consider IgG as an adjunctive modality for expectant or early therapeutic intervention.

Materials and methods

Cells, virus, and animals

HSV-1 strain McKrae was grown in Vero (African green monkey kidney) cells in EMEM:199 medium (Quality Biologicals, Inc., Gaithersburg, Maryland) with 10% FBS (Life Technologies Gibco BRL, Gaithersburg, Maryland) and 1% Glutamine-Streptomycin-Penicillin (Life Technologies Gibco BRL). The McKrae strain was used because of its marked neurovirulence compared with strains passaged multiple times in the laboratory that typically are attenuated. The virus dose chosen for inoculation was based on our earlier studies (LeBlanc *et al*, 1999), which showed that to study latency and reactivation, a sufficient quantity of input virus is needed. Given the virulence of HSV-1 strain McKrae, the inoculum required to reproducibly yield reactivable virus in the presence of antiviral treatment proved 100% fatal in absence of IgG treatment.

Female BALB/c mice, 4 to 6 weeks old, were obtained from HSD (Harlan Sprague-Dawley, Indianapolis, IN), housed in NIH facilities accredited by the American Association for Accreditation of Laboratory Animal Care, International, and studied under an IACUC-approved animal research protocol.

Inoculations and treatments

Mice were anesthetized with a 0.5-ml intraperitoneal (IP) injection of a mixture of ketamine/xylazine in PBS. Both corneas were scarified using a 25-gauge needle, and 5 μl of virus inoculum were applied per eye for a total of 1×10^6 plaque forming units (PFU) per mouse. Control mice received 5 μl of PBS per scarified eye. Animals assigned to IgG treatment were given a single 0.5-ml dose of pooled human IgG (prepared for intravenous administration) (Abbott Labs, Chicago, IL) diluted 1:8 in PBS (3.75 mg IgG/mouse) IP before 24 h, or at 24 h, 48 h, or 72 h after infection (LeBlanc *et al*, 1999).

Survival and ocular shedding

Mice were infected using bilateral corneal scarification and were observed daily for mortality up to day

17. Concurrent with monitoring survival, the number of animals shedding virus from the cornea was determined. On the indicated days, both eyes were swabbed with a moistened Dacron polyester-tipped applicator (Baxter Healthcare Corporation, Deerfield, Illinois). The swab collection and observation of cytopathic effects (CPE) were done as per our earlier study (LeBlanc *et al*, 1999).

Virus titers in tissues
On days 2, 4, 6, 8, and 10 postinfection, the eyes, brain, and trigeminal ganglia (TG) were removed from animals using aseptic techniques and placed into separate tubes containing 1 ml of medium each. Organs were ground using a Tekmar Tissue homogenizer (VWR Scientific, McGaw Park, Illinois) and the homogenates were diluted serially and then plated in duplicate on Vero cell monolayers. After 1 h incubation at 37°C on a rocking platform, cells were overlaid with media containing 0.5% pooled human IgG (Abbott Labs) and then placed in a humidified 37°C CO_2 incubator for 48 h. Then, the dishes were stained with crystal violet and plaques were counted (LeBlanc *et al*, 1999).

Explant cocultivation
Animals that survived the acute HSV-1 infection were housed for more than 30 days postinfection. Whole trigeminal ganglia (TG) from different groups of latently infected mice were aseptically harvested, and each pair of TG was placed onto separate Vero cell monolayers with media containing 0.1% N,N′-hexamethylene-bis-acetamide (Sigma, St. Louis, Missouri) and 2% FBS. Explant cultures were kept in a 37°C humidified CO_2 incubator. The explants were checked daily for CPE, and they were carefully transferred onto fresh monolayers weekly, if necessary.

In vivo *reactivation*
The protocol used for inducing HSV reactivation *in vivo* was as reported (LeBlanc *et al*, 1999). Briefly, animals were anesthetized and placed on top of a TM-20 transilluminator (UVP Inc., Upland, CA) so that one side of the head was exposed to UV light for 1 min. Then, they were turned to expose the contralateral eye. Some infected animals were not exposed to serve as "unstressed" controls. Forty-eight h post-UV exposure, the pair of TG from each animal was harvested, ground in 1 ml of media, and plated on Vero cells. Monolayers were incubated at 37°C and checked daily for CPE.

Quantitative real-time PCR
The protocol used to quantitate the latent viral genome copy number in ganglia was as reported (LeBlanc *et al*, 1999). Briefly, TG pairs from latently infected mice were dissected, separated, and rinsed three times each in PBS. DNA was isolated separately from each ganglion using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, Minnesota). After extraction, the DNA from both ganglia from an animal was pooled. The number of copies of latent HSV-1 DNA was quantified by real-time fluorescence PCR using the Taqman System, ABI Prism 7700 Sequence Detector (PE Applied Biosystems, Perkin-Elmer, Foster City, California) with primers and probe specific for glycoprotein G of HSV-1 (gG1) (LeBlanc *et al*, 1999). Each reaction of 100 ng TG DNA was done in triplicate. A positive-control plasmid containing the gG1 segment (cloned into PCR™ 2.1, Invitrogen) was used to develop a standard curve ranging from 3×10^0 to 3×10^5 copies.

Histopathologic evaluation of the brain
To characterize the effects of the pre- and postexposure IgG treatment on the inflammatory response and dissemination of the virus within the brain, we infected three groups of mice each with 1×10^6 p.f.u. HSV-1 (McKrae) via corneal scarification. Group A mice received no prophylactic treatment. Group B mice received pooled human IgG 24 h prior to inoculation, yet group C mice received IgG 24 h postinfection. Mice were sacrificed by carbon dioxide inhalation at 2, 4, 5, 6, 7, 8, and 10 days postinfection. The brains were harvested, fixed with Z-Fix (Anatech Ltd.), trimmed, routinely processed, and embedded in paraffin. Five-micron sections were cut and stained with hematoxylin and eosin. Sections of olfactory lobes, cerebrum, thalamus, mesencephalon, medulla, and cerebellum were coded and evaluated histopathologically without knowledge of the animal group or date of sacrifice. The inflammatory response was characterized as none, minimal, mild, moderate, or severe, based on the relative number of acute inflammatory cells and the extent of the brain parenchyma involved. Likewise, necrosis was characterized based on the relative number of necrotic cells observed and the extent of the parenchyma involved.

Immunohistochemical staining for HSV antigen
To detect HSV-1 antigen in the brain, tissue sections from uninfected mice and from each of the three groups of mice (A, B, C, as before) were stained. To do so, the sections were quenched for endogenous peroxidase activity with 0.3% hydrogen peroxide for 30 min followed by several PBS washes. Then, normal goat serum (1:50) was applied for 30 min as a blocking reagent, followed by the use of an avidin/biotin blocking kit (Vector). Following further washes with PBS, primary rabbit anti-HSV-1 antibody (a gift from Dr Gary Cohen, University of Pennsylvania, Pennsylvania) was applied for 1 hr at a dilution of 1:1,000. After further PBS washes, a biotinylated goat anti-rabbit secondary antibody (Vector) was applied to the tissue sections for 30 min. Next, an avidin-HRP kit (Vector) and a DAB substrate kit (Vector) were used to detect the viral antigen.

Finally, the sections were counterstained with hematoxylin and covered with glass, and evaluated in a blinded manner.

Statistical analysis

Analyses were done using JMP software from the SAS Institute (Cary, North Carolina). Comparisons between the Kaplan-Meier survival estimates were done using the log-rank test. Nonparametric methods were done to analyze the data. Arithmetic means and one-way analysis of variance with log-transformed numbers were used to analyze results from acute phase tissue virus titers and quantitative PCR. The proportions of samples reactivating by UV stress were compared by the two-tailed Fisher exact test.

References

Akerfeldt S, Geijer S, Holubars E, Fuchs G, Brundin M (1972). Prophylactic and therapeutic antiviral effect of human gamma globulin. *Biochem Pharmacol* **21:** 503–509.

Akerfeldt S, Lofberg E, Fuchs G (1973). Antiviral effect of human gamma globulin in mice. Comparison between the efficacy of local and systemic administration. *Biochem Pharmacol* **22:** 2911–2917.

Beland JL, Sobel RA, Adler H, Del-pan NC, Rimm IJ (1999). B cell-deficient mice have increased susceptibility to HSV-1 encephalomyelitis and mortality. *J Neuroimmunol* **94:** 122–126.

Birmanns B, Reibstein I, Steiner I (1993). Characterization of an *in vivo* reactivation model of herpes simplex from mouse trigeminal ganglia. *J Gen Virol* **74:** 2487–2491.

Corey L, Spear PG (1986). Infection with herpes simplex virus. *N Eng J Med* **314:** 686–691.

Davis WB, Taylor J, Oakes JE (1979). Ocular infection with herpes simplex virus type 1: prevention of acute herpetic encephalitis by systemic administration of virus-specific antibody. *J Infect Dis* **140:** 534–540.

Erlich KS, Dix RD, Mills J (1987). Prevention and treatment of experimental herpes simplex virus encephalitis with human immune serum globulin. *Antimicrob Agents Chemother* **31:** 1006–1009.

Erlich KS, Mills J (1986). Passive immunotherapy for encephalitis caused by herpes simplex virus. *Rev Infect Dis* **8:** S439–S445.

Laycock KA, Lee SF, Brady RH, Pepose JS (1991). Characterization of a murine model of recurrent herpes simplex viral keratitis induced by ultraviolet B radiation. *Invest Opthalmol Vis Sci* **32(10):** 2741–2746.

LeBlanc RA, Pesnicak L, Godleski M, Straus SE (1999). Treatment of HSV-1 infection with immunoglobulin or acyclovir: comparison of their effects on viral spread, latency, and reactivation. *Virology* **262:** 230–236.

Lekstrom-Himes JA, Pesnicak L, Straus SE (1998). The quantity of latent viral DNA load correlates with the relative rates at which herpes simplex virus type 1 and 2 cause recurrent genital herpes outbreaks. *J Virol* **71:** 2760–2764.

Levine B, Hardwick JM, Trapp BD, Crawford TO, Bollinger RC, Griffin DE (1991). Antibody-mediated clearance of alphavirus infection from neurons. *Science* **254:** 856–860.

Maggioncalda J, Mehta A, Su YH, Fraser NW, Block TM (1996). Correlation between herpes simplex virus type 1 rate of reactivation from latent infection and the number of infected neurons in trigeminal ganglia. *Virology* **225:** 72–81.

Martin JR, Jenkins FJ, Henken DB (1991). Targets of herpes simplex virus type 1 infection in a mouse model. *Acta Neuropathol (Berl)* **82:** 353–363.

McKendall RR, Klassen T, Baringer JR (1979). Host defences in herpes simplex infections of the nervous system: effect of antibody on disease and viral spread. *Infect Immun* **23:** 305–311.

Mester JC, Glorioso JC, Rouse BT (1991). Protection against zosteriform spread of herpes simplex virus by monoclonal antibodies. *J Infect Dis* **163:** 263–269.

Oakes JE, Rosemond-Hornbeak H (1978). Antibody-mediated recovery from subcutaneous herpes simplex virus type 2 infection. *Infect Immun* **21:** 489–495.

Sanna PP, De Logu A, Williamson RA, Hom YL, Straus SE, Bloom FE (1996). Protection of nude mice by passive immunization with a type-common human recombinant monoclonal antibody against HSV. *Virology* **215:** 101–106.

Sawtell N (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.

Sawtell NM, Poon D, Tansky CS, Thompson RL (1998). The latent herpes simplex virus type 1 genome copy number in individual neurons is virus strain specific and correlates with reactivation. *J Virol* **72:** 5343–5350.

Simmons A, Nash A (1985). Role of antibody in primary and recurrent herpes simplex virus infection. *J Virol* **53:** 944–948.

Whitley RJ (2001). Herpes simplex viruses. In: *Fields Virology*. Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE, (eds). Lippincott, Williams & Wilkins Publishers: Philadelphia, pp 2461–2509.