Prevention and Treatment of Experimental Herpes Simplex Virus Encephalitis with Human Immune Serum Globulin

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Pooled human immunoglobulin suitable for intravenous administration (IGIV) was evaluated in the prophylaxis and treatment of herpes simplex virus (HSV) type 1 encephalitis in a murine model. Four-week-old BALB/c mice received a single intraperitoneal injection of IGIV or saline 24 h before or up to 24 h after intranasal infection with 10<sup>4.6</sup> PFU of HSV type 1. Treatment with IGIV was protective against death, and the protective effects were dose and time dependent. Treatment with IGIV blocked the production of HSV antibody by infected mice and reduced the number of trigeminal ganglia containing latent virus. Removal of neutralizing antibody from the IGIV pool did not eliminate the protective effect, whereas F(ab)2 fragments of IGIV, which had virus-neutralizing activity that was identical to that of native IGIV, conferred no protection against death. Pooled human IGIV was effective for the prevention and treatment of HSV encephalitis in mice. Antibody-mediated protection required the Fc portion of the immunoglobulin molecule but did not require the direct neutralization of virus.

Disseminated herpes simplex virus (HSV) infection and encephalitis in immunocompromised hosts and neonates is associated with severe illness and death. HSV encephalitis occurs infrequently in immunologically normal adults but, if left untreated, may result in long-term neurologic deficits or death (5). Antiviral drugs such as acyclovir and vidarabine have improved the outcome of severe HSV infections, but response to therapy is often suboptimal, even if treatment is instituted early in the disease. Mortality in patients with HSV encephalitis treated with antiviral agents approaches 30%, and about 60% of survivors are left with permanent neurologic deficits (16–18).

Passive administration of antiviral antibody is effective in the prevention and treatment of HSV infections in animal models (2, 8, 12, 13). When large doses of HSV antibody are administered with acyclovir or vidarabine early in the course of murine infection, an enhanced therapeutic effect is observed compared with treatment with either agent alone (3, 4, 19). Combination therapy has not been evaluated in humans, because immune serum globulin preparations administered by intramuscular injection do not permit administration of large amounts of HSV-specific antibody over a short period of time. Pooled human immunoglobulin modified for intravenous administration (IGIV) has recently become available for clinical use, and the administration of large amounts of antibody is now permitted in a matter of hours (14); under these conditions passive immunotherapy of HSV infections may be effective, especially if combined with a specific antiviral agent. We evaluated an IGIV preparation for the prevention and treatment of HSV encephalitis in a murine model.

MATERIALS AND METHODS

Virus. HSV type 1 (HSV-1) strain BK was grown and titrated in transformed African green monkey kidney (Vero) cells. Monolayers of Vero cells were propagated in Eagle minimal essential medium supplemented with 2% fetal calf serum–1% l-glutamine–antibiotics. Stock virus was prepared by infecting the cells at a multiplicity of 0.1 PFU per cell and by incubating them in a 37°C, 5% CO<sub>2</sub> environment until an 80 to 90% cytopathic effect was achieved. The cells were then collected, washed, suspended in phosphate-buffered saline, and sonicated; and the virus suspension was divided into small volumes and stored at −70°C. Virus titers were measured by plaque formation in Vero cell monolayers with a 1% methylcellulose–media overlay.

Mice. Male BALB/c mice (age, 3 to 4 weeks; obtained from Bantin and Kingman, Fremont, Calif.) were used throughout the experiments. All mice weighed between 15 and 20 g at the time of infection.

Immune globulin. Pooled human immune serum globulin (containing 40 to 50 mg of immunoglobulin G [IgG] per ml) prepared for intravenous (i.v.) infusion by a low pH process (IGIV) was provided by Cutter Biological, Berkeley, Calif. (14). The immunoglobulin preparation used (lot 2996; Cutter) had a neutralizing titer to HSV-1 of 1:800. For selective removal of HSV antibody, the immunoglobulin pool was repeatedly incubated with HSV-infected Vero cells for 1 h at 37°C. The supernatant was removed, centrifuged to clarity, and exposed to UV irradiation for 45 min to inactivate any remaining live virus. F(ab)2 fragments (lot 2996) were prepared at Cutter Laboratories by pepsin degradation and purified on a Sephadex G-150 column, as described previously (15).

Mouse protection experiments. Mice were lightly anaesthetized and infected by intranasal inoculation of 10<sup>4.6</sup> PFU of HSV-1 in a 20-μl volume. This route of infection has been studied previously and results in an acute encephalitis by direct virus extension through the nasal turbinates (6). Dilutions of immunoglobulin, modified immunoglobulin, or normal saline were administered in a 500-μl volume by intraperitoneal (i.p.) injection at various times relative to virus infection. F(ab)2 fragments were diluted to a volume of 200 or 500 μl for i.v. or i.p. injection, respectively. Infected mice were observed twice daily for signs of illness and
mortality. Brain tissue from dead mice was ground in a tissue homogenizer and cultured quantitatively for HSV.

**Antibody titers in serum.** Serum was obtained by retroorbital puncture 24 h after treatment with IGIV and at 15 and 30 days following virus inoculation. Serum complement was inactivated by heating to 56°C for 30 min before it was tested for neutralizing antibody by a plaque reduction assay. Dilutions of sera were incubated with 30 to 50 PFU of HSV-1 for 1 h at room temperature. Fractions of the virus-serum mixture were adsorbed to Vero cell monolayers for 1 h at 37°C, and an overlay containing Eagle minimal essential media and 1% methylcellulose was added. Infected cell monolayers were incubated for 48 to 72 h, and the cell sheet was fixed and stained with alcoholic crystal violet. The plaque number was determined and plotted against the serum dilution. The antibody titer reported was the highest serum dilution that reduced the virus titer by 50%, as determined by graphic interpolation.

**Latent virus in trigeminal ganglia.** Surviving mice were sacrificed 30 to 40 days after infection. Right and left trigeminal ganglia were removed aseptically, minced into 1- to 2-mm fragments, and cocultivated with human embryonic lung cells in Eagle minimal essential media with 10% fetal calf serum. Cultures were examined weekly for 4 weeks for cytopathic effects indicative of HSV replication. Cultures with doubtful cytopathic effects were subcultured in human embryonic lung cells. Only ganglion cultures showing cytopathology after 10 days were considered to represent reactivated latent virus; cytopathology appearing before 10 days was thought to represent active, nonlatent virus.

### RESULTS

Twenty-four hours after i.p. injection, the mice that received 500 μl of IGIV had HSV-1-neutralizing activity in serum of 1:80, while the control mice that received normal saline had no detectable neutralizing activity (no reduction in plaque number at a 1:2 dilution of serum). Neutralizing activity after IGIV therapy was dose dependent, and the half-life in serum following a single 500-μl IGIV injection was approximately 7 days (data not shown).

IGIV (500 μl) prevented death when it was administered 24 h before, or up to 24 h after, HSV infection. IGIV (50 μl) was protective against death when it was administered up to 8 h after infection and prolonged survival as compared with saline-treated controls (Table 1). Treatment with IGIV (5 μl) 8 h after infection did not prevent death or prolong survival. Virus titers in the brains of the IGIV-treated mice that died of the infection were comparable to those in saline-treated controls (data not shown).

Treatment with IGIV blocked the synthesis of HSV-neutralizing antibody in serum by infected mice. All saline-treated control mice tested (six of six) had detectable neutralizing activity in serum by day 15 (mean titer, 1:40) and had no change in neutralizing titer when the test was repeated on day 30. By comparison, mice treated with 500 or 50 μl of IGIV 24 h before, or 8 h after, HSV infection had no detectable neutralizing activity 15 or 30 days after infection (five mice tested per group; \( P = 0.004 \) compared with saline-treated controls; determined by the Fisher exact test).

Although treatment with IGIV protected against death and prevented the formation of neutralizing antibody, infection with HSV was not prevented. Latent virus was found in trigeminal ganglia of the IGIV-treated mice that survived the infection, but IGIV therapy reduced the number of ganglia harboring latent virus. In 10 mice treated with 50 μl of IGIV 8 h after infection, latent virus could be demonstrated in 10 of 20 (50%) trigeminal ganglia. By comparison, the mice that received saline had latent virus demonstrated in six of six (100%) ganglia (\( P = 0.035 \); determined by the Fisher exact test).

F(ab)\(_2\) fragments prepared from IGIV had in vitro HSV-1-neutralizing activity identical to that of native IGIV. Neutralizing activity in serum was identical in the mice that received F(ab)\(_2\) fragments or IGIV shortly after treatment, but the half-life of the F(ab)\(_2\) fragments in serum was shorter than that of IGIV (3 versus 7 days; data not shown). Treatment with F(ab)\(_2\) fragments by either i.p. or i.v. injection was ineffective in the prevention or treatment of HSV infection, and repeated administration of F(ab)\(_2\) fragments to compensate for the more rapid elimination in vivo did not provide protection against death (Table 2).

Adsorption of IGIV to HSV-infected Vero cells removed all detectable neutralizing activity (neutralizing titer, <1:2), while low titers of nonneutralizing antibody could still be detected by immunofluorescence. This nonneutralizing, low-titer IGIV was still protective against death when administered to mice 24 h before, or 8 h after, HSV infection (Table 2).

### DISCUSSION

This study documents that IGIV is effective in the prevention of treatment of acute HSV encephalitis in mice. Treatment with IGIV prevented death from infection when administered by i.p. injection 24 h before, or up to 24 h after, virus infection (Table 1). The maximum amount of IGIV used in these studies (up to 500 μl per mouse) corresponds to a maximum dose of 130 mg of IgG per kg of body weight. An equivalent amount of IgG for a newborn human infant would be available in 10 ml of undiluted IGIV, a volume that could be given i.v. without fluid management difficulties.

Treatment with IGIV did not prevent HSV infection, because latent virus was recovered from trigeminal ganglia of treated mice, but treatment did reduce the number of ganglia harboring latent virus. A similar effect was observed by McKendall et al. (12), who demonstrated that hyperimmune rabbit antisera administered up to 48 h after HSV-1 footpad inoculation reduced the number of latently infected paraspinal ganglia. This observation may have clinical significance in infected humans, because a reduction in latently infected ganglion cells may result in a decreased risk of subsequent disease due to reactivation of virus.

In previous studies (2-4, 8, 12, 13) it has been demonstrated that HSV antibody is effective in the prevention and
treatment of murine HSV infections, and the results have suggested that similar effects might be observed in humans. In most previous animal studies, however, direct injection of virus suspension into the brain (3, 4), footpad (2, 8, 12, 13), or subcutaneous tissues (2, 8) has been used. These methods of inducing infection may artificially bypass important natural host defense mechanisms. In contrast to these routes of inoculation, HSV infections in humans usually occur by the ascending spread of virus along peripheral nerves following mucous membrane contact with infected droplets (5). The intranasal route of HSV infection used in this study simulates the natural route of infection, with virus spreading along peripheral nerves and establishing latent infection in ganglia which correspond to the site of inoculation (6, 7).

The mechanism by which antibody exerts its therapeutic effect is only partially understood. Direct virus neutralization could provide an explanation for activity against HSV if efficacy occurred only when antibody was present at the time of virus inoculation. Our data indicate that neutralizing activity is relatively unimportant in the beneficial effect of antibody on HSV infection. Administration of IGIV at a time when virus became cell associated and not subject to direct virus neutralization (8 or 24 h after infection) was still protective against death, and nonneutralizing HSV antibody was also protective against death. Additionally, neutralizing F(ab)_2 fragments provided no protection against death, even if they were administered repeatedly during the acute infection. These data indicate the importance of the Fc fragment of the immunoglobulin molecule in activity against HSV infection and suggest that interaction of the immunoglobulin molecule with cellular effectors is necessary. This hypothesis is supported by the results of studies by Kohl and Loo (10), who have demonstrated that intact IGIV is active in antibody-dependent, cell-mediated cytolysis (ADCC) in vitro and in vivo.

Results of studies by other investigators have also shown that neutralizing activity alone cannot account for all of the beneficial effects of passively administered antibody in HSV infections. For example, monoclonal antibodies to surface glycoproteins with no virus-neutralizing activity are protective against murine HSV infection (1). Direct data on the role of the Fc portion are inconsistent, however, because other investigators have shown that F(ab)_2 fragments do not provide either partial or no protection against infection compared with intact immunoglobulin (9, 11). It is possible that virus neutralization is important in protection against HSV infection if intact antibody or F(ab)_2 fragments are administered very early in the course of infection, before the virus becomes cell associated. Once cellular infection has occurred, however, antibody protection likely involves the binding to cellular HSV-induced glycoprotein antigens and the induction of either complement-dependent cell lysis or antibody-dependent, cell-mediated cytolysis, which are processes that require the Fc portion of the immunoglobulin molecule (10).

The therapeutic efficacy of IGIV in the mouse model suggests that passive immunotherapy for severe HSV infections may be effective in humans. Clinical trials comparing acyclovir alone to acyclovir in combination with intravenous passive immunotherapy for severe HSV infections may be warranted. Additionally, passive immunotherapy with high-titer HSV antibody may be effective in the prevention of severe HSV infection in neonates exposed to HSV at the time of birth.

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LITERATURE CITED


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