

Attenuated, Replication-Competent Herpes Simplex Virus Type 1 Mutant G207: Safety Evaluation of Intracerebral Injection in Nonhuman Primates

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This study examined the safety of intracerebral inoculation of G207, an attenuated, replication-competent herpes simplex virus type 1 (HSV-1) recombinant, in nonhuman primates. Sixteen New World owl monkeys (*Aotus nancymae* [karyotype 1, formerly believed to be *A. trivirgatus*]), known for their exquisite susceptibility to HSV-1 infection, were evaluated. Thirteen underwent intracerebral inoculation with G207 at doses of 10^7 or 10^9 PFU, two were vehicle inoculated, and one served as an infected wild-type control and received 10^3 PFU of HSV-1 strain F. HSV-1 strain F caused rapid mortality and symptoms consistent with HSV encephalitis, including fever, hemiparesis, meningitis, and hemorrhage in the basal ganglia. One year after G207 inoculation, seven of the animals were alive and exhibited no evidence of clinical complications. Three deaths resulted from nonneurologic causes unrelated to HSV infection, and three animals were sacrificed for histopathologic examination. Two animals were reinoculated with G207 (10^7 PFU) at the same stereotactic coordinates 1 year after the initial G207 inoculation. These animals were alive and healthy 2 years after the second inoculation. Cerebral magnetic resonance imaging studies performed both before and after G207 inoculation failed to reveal radiographic evidence of HSV-related sequelae. Despite the lack of outwardly observable HSV pathology, measurable increases in serum anti-HSV titers were detected. Histopathological examination of multiple organ tissues found no evidence of HSV-induced histopathology or dissemination. We conclude that intracerebral inoculation of up to 10^9 PFU of G207, well above the efficacious dose in mouse tumor studies, is safe and therefore appropriate for human clinical trials.

At present, medical therapy cannot significantly alter the poor prognosis associated with glioblastoma multiforme, the most malignant form of human astrocytic brain tumors (11, 26, 31), making it an excellent target for new therapeutic approaches. Replication-competent, cytotoxic viral vectors offer such an approach, provided that their cytopathic effect is limited to neoplastic tissues. Vectors derived from herpes simplex virus (HSV) are particularly well suited to this approach because HSV infects a broad range of cell types and causes a cytotoxic, lytic infection, yet it can also exist in a latent state within neurons without causing damage (50). However, HSV infection of the central nervous system (CNS) causes encephalitis, with substantial morbidity (~50%) even with current antiviral therapy (35, 54, 64). In addition to HSV encephalitis, HSV keratoconjunctivitis, esophagitis, pneumonitis (inflammation of the lungs), and hepatitis (inflammation of the liver) have been reported (9, 63). Therefore, it is very important that

HSV vectors, especially replication-competent vectors, are nonpathogenic in normal tissue.

A large number of HSV genes affect pathogenicity (36, 43). γ 34.5 (RL1), located in the long repeat region, enables viral replication in the CNS and is the major viral determinant of neuropathogenicity (8, 65). Mutations in γ 34.5 result in decreases in the 50% lethal dose to $>10^6$ to 10^7 PFU after intracerebral injection (8, 30, 61). A number of HSV genes involved in nucleotide metabolism also affect neurovirulence, such as ICP6 (UL39), which encodes the large subunit of ribonucleotide reductase (5, 21, 67), and genes for thymidine kinase (UL23) (15, 17, 57), dUTPase (UL50) (46), uracil DNA glycosylase (UL2) (47), and DNA polymerase (UL30) (44). Attenuated, replication-competent HSV vectors containing mutations in these genes have been shown to effectively inhibit brain tumor growth in animal models (1, 3, 7, 22, 25, 33, 34, 39, 40, 48).

We constructed a second-generation HSV vector, termed G207, containing deletions of both γ 34.5 loci (R3616 parent [8]) and an *E. coli lacZ* insertion that inactivates the ICP6 gene (hrR3 parent [16]) (40). These multiple mutations attenuate neurovirulence and make it unlikely that a pathogenic revertant could arise by recombination or by a second-site suppressor mutation (41). Other favorable properties of G207 are its easy detection due to the *lacZ* reporter gene, temperature sensitivity, and hypersensitivity to the antiviral drugs ganciclovir and acyclovir (40). As a result of its propensity for replicating in dividing cells, the cytotoxic effects of G207 are preferentially limited to neoplastic cells, resulting in efficacious treatment of established human brain tumors in athymic mice (40, 60, 68).

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TABLE 1. Summary of intracerebral inoculations of *A. nancymae*^a

Primate	First injection		Time of MRI	Death		Survival (mo)
	Virus	PFU		Time	Cause	
Group 1						
T1122	G207	10 ⁷		3 mo	Sacrifice	
T9205	G207	10 ⁷	Preop 1 mo 6 mo	6 mo	Sacrifice	
T9307	G207	10 ⁷		12 mo	Sacrifice	
T344	G207	10 ⁷				41
T317	G207	10 ⁷				41
T323	G207	10 ⁷				34
T432	G207	10 ⁷		7 mo	Inguinal hematoma	
T910805	G207	10 ⁷		4 mo	Pancreatitis	
T894	G207	10 ⁷	Preop 10 days	25 days	Fasting	
Group 2						
T558	G207	10 ⁹	6 mo	20 mo	Sacrifice	
T413	G207	10 ⁹		7 mo	Aortic aneurysm	
Group 3						
T348	G207	10 ⁷	11 mo 25 mo			41
T442	G207	10 ⁷	12 mo 15 mo			41
Group 4						
T1057	F	10 ³		5 days	Encephalitis	
T421	Mock					42
T357	Mock			30 mo	Acute heart failure	

^a Primates were inoculated with G207 (10⁷ PFU in 10 μ l or 10⁹ PFU in 180 μ l), strain F, or virus buffer (Mock). Group 3 animals were reinoculated with G207 12 months after the initial inoculation. Preop, preoperatively.

The use of potentially pathogenic, replication-competent viruses in humans for tumor therapy is dependent upon the demonstration that the mutant viruses will not cause additional disease. The New World owl monkey, *Aotus nancymae* (karyotype 1, formerly believed to be *Aotus trivirgatus* [18]), was selected as a nonhuman primate model to test the safety of G207 because of its exquisite sensitivity to HSV (20, 24, 38). Peripheral inoculation of 100 PFU of wild-type HSV-1 or -2 is lethal (37). Owl monkeys have previously been used to test the safety of live, attenuated HSV vaccine strains (37). Using this model, we have now demonstrated the safety of intracerebral inoculation of G207 at doses of up to 10⁹ PFU.

MATERIALS AND METHODS

Virus. HSV-1 strain F was provided by J. Chou and B. Roizman (University of Chicago) and G207 was constructed as previously described (40). Stocks of G207 were grown in African green monkey kidney (Vero) cells infected at a multiplicity of infection of 0.01 and cultured at 34°C in Dulbecco's minimal essential medium supplemented with 5% heat-inactivated fetal calf serum (HyClone). Virus was isolated from infected cells when the complete cytopathic effect was observed after a freeze-thaw/sonication regimen and low-speed centrifugation (2,000 \times g for 10 min at 4°C) to remove cell debris. Virus was concentrated by high-speed centrifugation (45,000 \times g for 150 min at 4°C) and resuspended in virus buffer (150 mM NaCl and 20 mM Tris, pH 7.5), for a final G207 titer of 6 \times 10⁹ PFU/ml. The titer of viral stocks was determined by a plaque assay on the Vero cells. Following each surgery, the titer of the inoculum was confirmed.

Animals. Sixteen New World primates (*A. nancymae*), 12 males and four females ranging in age from 1 to 6 years and in weight from 500 to 1,000 g, were obtained through the Veterinary Resources Program, National Center for Research Resources, National Institutes of Health (Poolesville, Md.). Seven of the animals were involved in prior experimental studies (T9205 and the others below), two of which had previously undergone splenectomy (T894 and T558). Four of the animals (T1122, T910805, T9307, and T1057) (Table 1) were briefly described in a previous publication (40). The animals were housed separately

prior to and after inoculation. Animal procedures were approved by the Georgetown Animal Care and Use Committee and the Institutional Biosafety Committee.

Stereotactic intracerebral inoculations. The intracerebral inoculations and the surgical time frames for the 16 inoculated animals (13 with G207, 1 with strain F, and 2 with virus buffer) are listed in Table 1. Monkeys were given general anesthesia (propofol drip of 50 to 100 mg/kg of body weight/h with saline in the lateral saphenous vein), and by using a Kopf stereotactic head frame, inoculations were made into the left frontal lobe, 2 mm anterior to the coronal suture and 7 mm lateral to the midline. After manual placement of a burr hole in the calvarium, a sample was stereotactically injected with a Hamilton syringe into the cortex at a depth of 5 mm. Ten microliters of diluted G207 (10⁷ PFU), F, or virus buffer was injected over 5 min, or 180 μ l of G207 (10⁹ PFU) was injected over 40 min. Following injection, the burr hole was covered with bone wax and the incision was sutured. No perioperative antibiotics were used. Postoperatively, all animals underwent serial neurologic assessment, ear temperature measurement, and monitoring of food and water intake.

Physiological evaluations. All animals were screened every 3 months (pre- and postoperatively) with a physical examination, a tuberculin test, and serologic analyses, including blood count with differential, protein measurement, chemistry, and lipase and amylase measurements. Additionally, anti-HSV-1 antibody testing was performed (MA BioServices, Inc., Rockville, Md.) on some blood samples with enzyme-linked immunosorbent assay (ELISA). Since one of the cardinal signs of HSV encephalitis is fever, aural tympanic body temperatures were measured twice daily for the first postoperative week, then daily for 3 weeks, and then at a further reduced frequency. The normal aural tympanic body temperature of the primates ranged from 96.9 to 101.5°F. Primates attempting to elude capture for these temperature measurements experienced a temperature elevation of approximately 2.5°F on average, as determined with a subcutaneously implanted temperature transponder (BioMedic Data Systems Inc., Maywood, N.J.).

MRI. High-resolution cerebral magnetic resonance imaging (MRI) studies were performed with a Vision 1.5-T MRI unit (Siemens, Erlangen, Germany). Each study consisted of precontrast T1-weighted images in the axial and sagittal planes (time to repeat [TR] = 500, time to echo [TE] = 20, excitations [E] = 4), proton density (TR = 2500, TE = 30, E = 4) and T2-weighted images in the sagittal plane (TR = 3500, TE = 39, E = 2), and postcontrast T1-weighted images in the axial and coronal planes after intravenous administration of ga-

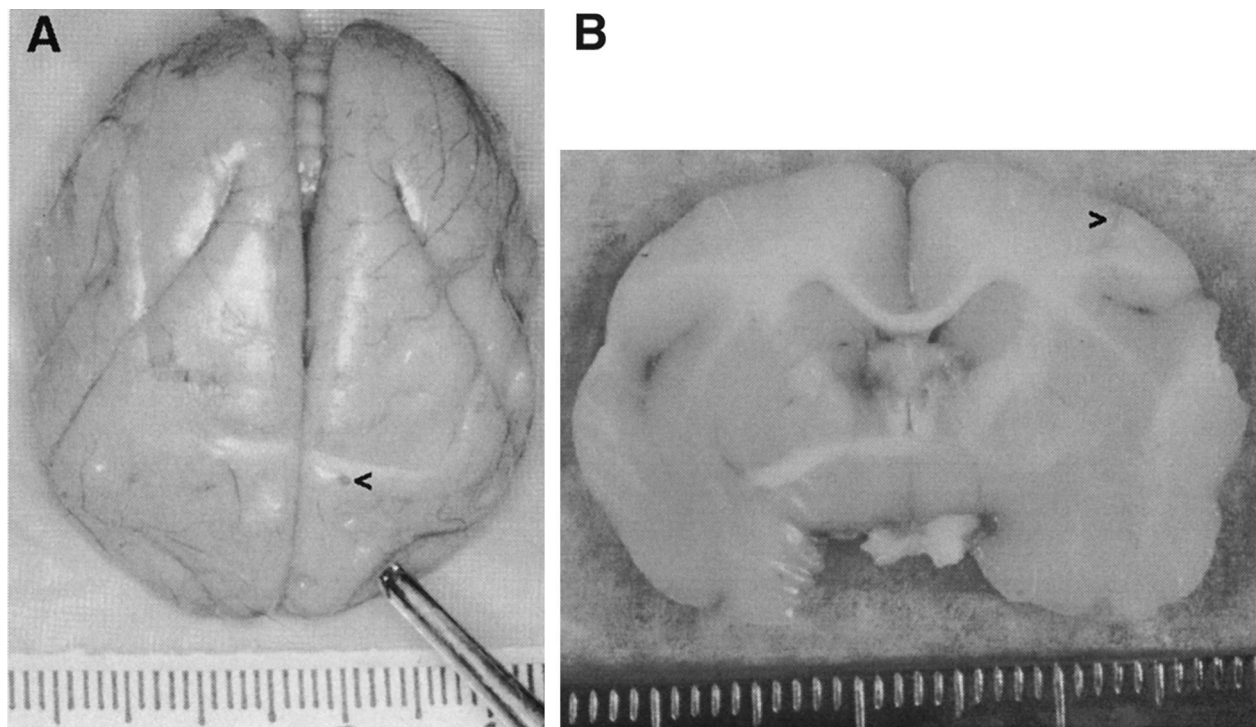


FIG. 1. Brains from G207-injected animals. (A) Brain from T9205 at time of necropsy, 6 months after injection of 10^7 PFU of G207, with frontal lobes at the bottom and brain stem at the top. (B) Coronal cut through the brain from T910805 at the injection site 4 months after injection of 10^7 PFU of G207. In both panels, the left side of the brain is on the right side of the figure, and the injection site is marked (>). Hatch marks on rulers are in millimeters. Photographic slides were scanned with a Polaroid SprintScan 35, and Adobe Photoshop was used to adjust the contrast and brightness of the images.

dodiamide (1 ml/kg; Omniscan; Nycomed Inc., Princeton, N.J.). The time frame for MRI imaging is indicated in Table 1.

Necropsy and histopathology. Animals were sacrificed by intravenous administration of sodium pentobarbital (40 to 60 mg/kg) followed by intracardiac perfusion with 4% paraformaldehyde. Following all deaths, due to euthanasia or otherwise, a complete necropsy was performed. Histologic samples were obtained from the peripheral organs: adrenal gland, heart, intestines, kidney, liver, lymph node, lung, muscle, pancreas, skin, spleen, stomach, and testes or ovaries. Occasionally, the adrenal glands were not found at the time of necropsy. In disseminated HSV disease, usually in neonates and immunocompromised individuals, numerous organs can be involved, including the adrenal glands, pancreas, intestine, spleen, stomach, kidneys, and muscle (9). From the CNS, samples were taken from the frontal, parietal, temporal, and occipital lobes; cerebellum; upper brain stem; medulla; and spinal cord. The injection site in the left frontal lobe was identified at the time of necropsy by the visualization of local scar tissue on the scalp and the burr hole in the skull, and tissue was isolated from the scalp, skull, and left frontal lobe. Specimens were embedded in paraffin, and 10- μ m sections were obtained by using a microtome and then dehydrated, stained with hematoxylin and eosin, and coverslipped (29). Additional sections were immunohistochemically stained for HSV-1 antigens with rabbit anti-HSV-1 antibody (Dako) (4), followed by Vectastain indirect immunoperoxidase detection and then counterstained with hematoxylin. Histologic sections were evaluated by a board-certified neuropathologist (H.J.M.).

RESULTS

Intracerebral inoculation of G207. To test the safety of G207 and the extent of neurotoxicity associated with it, we performed stereotactic intracerebral inoculations in the left frontal lobe in three groups of animals: a single inoculation of 10^7 PFU of G207 in nine animals (group 1), a single inoculation of 10^9 PFU of G207 in two animals (group 2), and a second inoculation of 10^7 PFU of G207 1 year after an initial inoculation of 10^7 PFU in two animals (group 3) (Table 1). In the first group, one animal each was sacrificed at 3, 6, and 12 months for histopathological examination, three animals were alive and healthy more than 2.5 years after inoculation, and

three animals died from causes unrelated to HSV. Of the latter deaths, one resulted from extended fasting in preparation for an MRI study (T894), one resulted from an anesthetic complication during surgery to remove an inguinal hematoma (T432), and one resulted from acute pancreatitis (T910805) attributed to natural disease because there was no histologic or immunohistochemical evidence of HSV infection in any of the organs examined, including the pancreas. At the time of necropsy, the injection site was notable in some animals while the brains looked otherwise normal (Fig. 1). Among the three sacrificed animals, neuropathologic examination revealed no abnormalities of the CNS, with the exceptions of sparsely distributed interstitial and/or perivascular inflammatory cells and gliosis at the injection site (Fig. 2). For many animals the injection site was not apparent in histologic sections. The peripheral organs also revealed no evidence of toxicity attributable to G207, and no HSV-immunoreactive cells were seen in any of the examined sections, including those from the injection site. The hearts and kidneys of several monkeys contained mild fibrosis and interstitial nephritis, respectively, a common finding in *Aotus* (62).

A large inoculation volume (180 μ l) was required for group 2, the animals receiving a high dose of G207 (10^9 PFU). One of these animals (T413) had a tonic/clonic seizure 5 days postinjection and developed mild contralateral weakness, which resolved over 3 days. This animal remained healthy until it died 7 months after inoculation due to a ruptured aortic aneurysm. No histologic evidence of neural, systemic, or vascular HSV infection was found. The other animal (T558) experienced no adverse effects due to the surgery and remained healthy until sacrificed 20 months after injection. T558 had one of the lowest normal body temperatures (98.4 to 100.5°F).

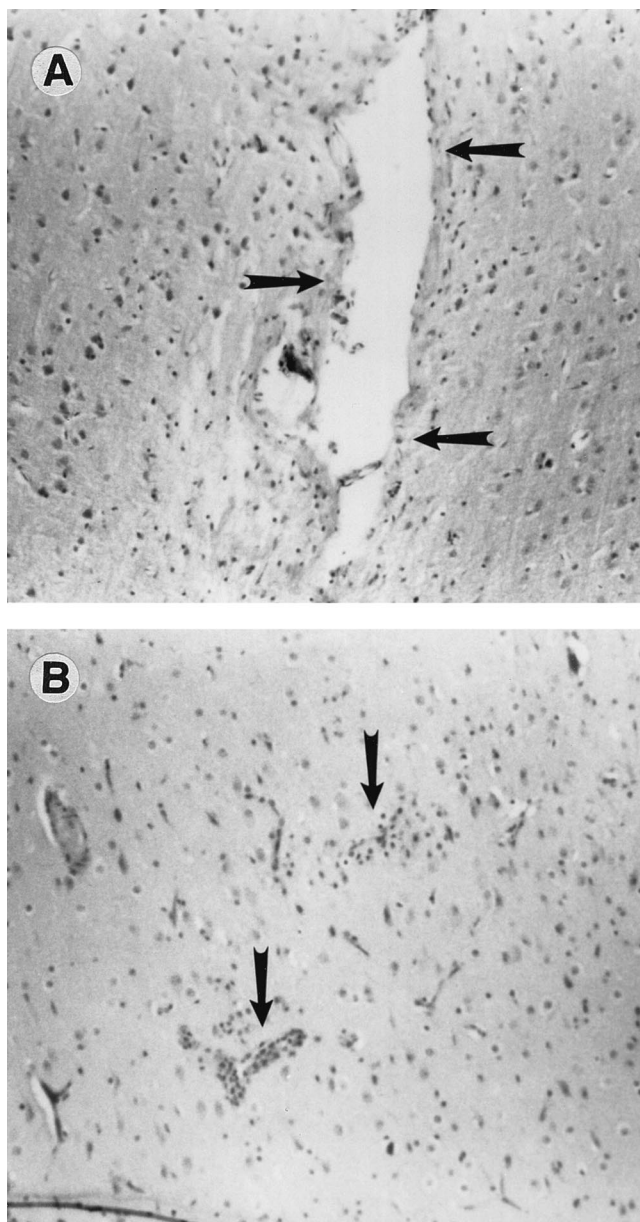


FIG. 2. Histology of brain from G207 injection site. Brain tissue was obtained at necropsy from animals T1122 (A) and T894 (B) 3 months and 25 days, respectively, after inoculation of 10^7 PFU of G207. Ten-micrometer paraffin-embedded sections were stained with hematoxylin and eosin. Mild perivascular inflammation adjacent to the injection site is visible (arrows).

In order to determine whether prior HSV infection might result in an enhanced inflammatory response or allergic encephalomyelitis, animals T442 and T348 were reinoculated with G207 1 year after the first inoculation at the same stereotactic coordinates. There was no evidence of any clinical disease or MRI-detectable changes in these animals and they were alive and healthy more than 2 years after the second inoculation.

Control intracerebral inoculations. To control for complications due to surgery and to provide a baseline for tissue histopathology, we stereotactically inoculated virus buffer into two animals, T421 and T357 (Table 1). T357 was euthanized 30 months after mock inoculation after becoming moribund due

to acute cardiopulmonary failure, and T421 was alive and healthy more than 3 years after surgery.

To confirm the exquisite sensitivity of *A. nancymae* to HSV and to determine the symptoms of HSV encephalitis in this species, one animal (T1057) was inoculated with 10^3 PFU of strain F, a dose of the wild-type parental strain of G207 4 to 6 log units lower than that of G207. This animal exhibited signs of encephalitis, with a rising fever (103°F on day 3 and 105°F on day 4) and lethargy. On day 5, the animal exhibited hypothermia (97°F), right hemiparesis, and left pupillary enlargement and was euthanized. Histologic signs of HSV encephalitis included meningitis, with thickening of the meninges, subarachnoid hemorrhage, and inflammatory cell infiltrate in the underlying brain parenchyma (Fig. 3A); hemorrhage within the basal ganglia (Fig. 3B) and ventricles; and acute cerebritis, necrotic neurons, and Cowdry A nuclear inclusions (Fig. 3C). Immunohistochemical staining for HSV-1 revealed numerous HSV-immunoreactive neurons in both frontal lobes (Fig. 3D), ipsilateral amygdala, and arachnoid cells. Hemosiderin and erythrophagocytosis were seen in the spleen and in the liver and lymph nodes, respectively, consistent with this animal's involvement in a previous malaria study. The remaining organs were normal.

Cerebral MRI studies. MRI has been shown to be a sensitive noninvasive test for identifying the tissue injury occurring in HSV encephalitis (12, 28, 53, 59). MRIs were obtained 10 days (Fig. 4A) and 1 (Fig. 4B), 6, 10 (Fig. 4C), 11, and 12 months after an initial inoculation of 10^7 PFU of G207; 3 and 13 months after a second inoculation of 10^7 PFU of G207; and 6 months (Fig. 4D) after an initial inoculation of 10^9 PFU of G207 (Table 1). Only subtle radiographic changes at the injection site and in the overlying calvarium, due to burr hole placement, could be identified (Fig. 4). These changes were attributed to the surgery itself since similar subtle findings are often observed after intracranial procedures in humans. No abnormal signal characteristics or contrast enhancements attributable to HSV toxicity were observed in dura or brain parenchyma at the injection site or throughout the rest of the brain.

Humoral response to G207 injection. Prior to inoculation, animals T9307, T348, T442, T558, T413, and T421 were found to be seronegative for anti-HSV-1 antibodies by ELISA. A single intracerebral injection of G207 (10^7 PFU) resulted in low serum anti-HSV titers that decreased over the course of a year, but T317 had a significantly elevated titer after 3 years (Fig. 5). A large increase in antibody titers occurred within 2 months of the second intracerebral injection of G207 in group 3 (1 year after the initial injection), which was still elevated 2 years later (T348 and T442) (Fig. 5). Even with this "booster" effect, no clinical, MRI, or pathological abnormalities were observed.

DISCUSSION

Replication-competent viral vectors that are cytotoxic have a number of attractive features for tumor therapy, such as the abilities to generate new infectious virus in situ while killing tumor cells and to spread within the tumor (49, 51). However, as a result of these properties, they are potentially more pathogenic than replication-defective viral vectors. When clinical trials with such viruses are being considered, it is imperative that adequate safeguards be engineered into the vector and that rigorous safety testing be performed. As an example of such a viral vector, we developed an attenuated, replication-competent HSV-1 vector, G207, as a therapeutic agent for brain tumors. G207 is derived from HSV-1 strain F, one of the

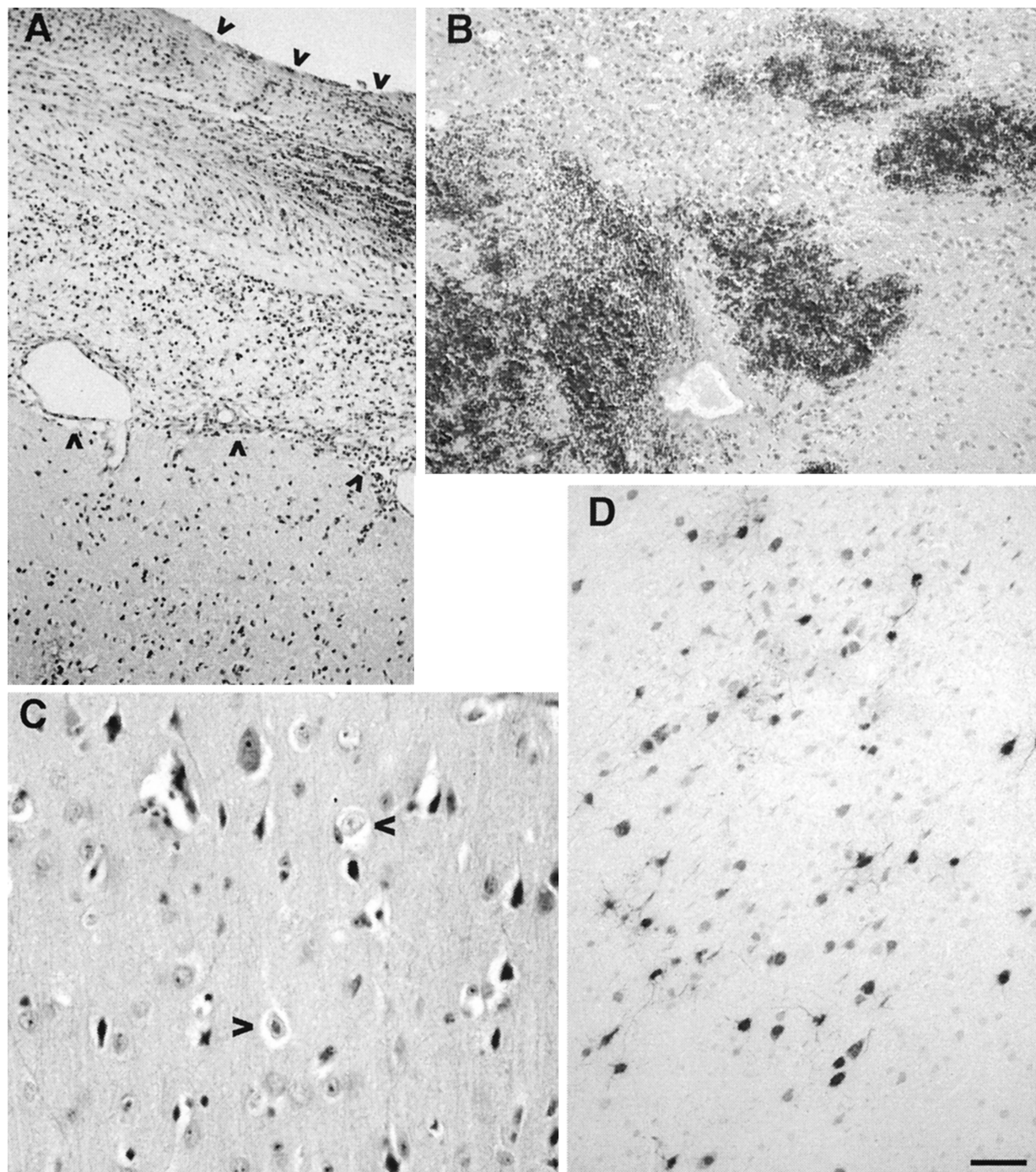


FIG. 3. Histology and immunohistochemistry of brain tissue from HSV-1 strain F injection. Brain tissue was obtained at necropsy from animal T1057 5 days after inoculation of 10^3 PFU of F. Ten-micrometer paraffin-embedded sections were stained with hematoxylin and eosin (A, B, and C) or were immunohistochemically stained with anti-HSV antibody (D). Changes consistent with HSV encephalitis are evident. (A) Meningitis, with thickened meninges, and inflammatory cell infiltrate (between arrows) as opposed to the normal condition of single-cell thickness. (B) Hemorrhage in the basal ganglia. (C) Cowdry A eosinophilic intranuclear inclusions (10) in the ipsilateral (arrowheads) and contralateral hemispheres. (D) HSV-immunoreactive cells (black) are seen throughout the cortex, in both ipsilateral and contralateral hemispheres. Photographic slides were scanned with a Polaroid SprintScan 35, and Adobe Photoshop was used to adjust the contrast and brightness of the images. Bar, 0.08 mm (A and D), 0.07 mm (B), and 0.03 mm (C).

least virulent laboratory strains (13). It contains deletions of both copies of the $\gamma_{34.5}$ gene, the major viral determinant for neurovirulence (6, 8, 56, 58), and a *lacZ* insertion inactivating the ICP6 gene. ICP6 is required for efficient viral replication in nondividing cells (16, 45) and also is a determinant of neuro-

virulence (5, 21, 67). The presence of multiple mutations is important for safety, even with deletion mutants, because second-site suppressor mutations (41) or novel recombinants could arise through selective pressure during replication in the tumor or brain.



FIG. 4. Cerebral MRI studies obtained after G207 inoculation. Sagittal T1-weighted MRIs were obtained after the administration of contrast dye. Images were obtained from T894 (A), T9205 (B), and T348 (C) 10 days, 1 month, and 10 months, respectively, after inoculation of 10^7 PFU of G207 and from T558 (D) 6 months after inoculation of 10^9 PFU of G207. The left frontal region of the brain (*) and the site of G207 injection (arrow) are indicated in each panel. Normal postoperative incisional scarring and inflammation of the skin overlying the inoculation site are present in all panels but are best seen in panel C. There were no changes, such as edema, hemorrhage, or necrosis, that would indicate the presence of HSV encephalitis.

HSV has evolved elaborate mechanisms to survive in the nervous system, its natural reservoir in humans and the site where it causes the most serious disease. In humans, illness resulting from HSV can be manifested by a variety of syndromes, including disseminated virus; CNS infections; and exanthema localized to the eyes, skin, and mouth (9, 63). CNS infections, usually due to HSV-1, often cause a devastating and rapidly fatal encephalitis. Death occurs in approximately 70% of those who do not receive treatment (66). The signs and symptoms of HSV encephalitis include fever, mental status changes (altered consciousness and personality changes), headache, nausea, and focal neurological findings (i.e., seizures and hemiparesis) indicative of the infected brain area, usually the temporal lobes (9, 54, 66). In neonates, about half the infections involve encephalitis, alone or with disseminated disease, and death is often associated with brain stem involvement (55). Acyclovir is the treatment of choice. However, even when adequate antiviral treatment is rendered, approximately 20% of patients die and the majority of survivors suffer significant neurologic impairment (35, 54, 66). Since G207 is a derivative of HSV-1, it is important to demonstrate that intracerebral inoculation with G207 does not pose a potential health hazard.

A. nancymae is an excellent animal model for characterizing the pathogenesis of HSV. These nonhuman primates are ex-

tremely sensitive to HSV infection and disease (24, 38), similar to human neonates and immunocompromised patients (37). They develop clinical symptoms of HSV infection similar to those of humans. *Aotus* has a somewhat elevated and variable body temperature compared to humans, which might affect the pathogenicity of G207. However, strain F has a temperature sensitivity similar to that of G207 (27), and the animal inoculated with 10^3 PFU of strain F rapidly developed an encephalitis so severe that euthanasia was required within 5 days of inoculation. An examination of the brain tissues from this animal revealed typical histologic findings of HSV infection, including meningitis, inflammatory infiltrates, neuronal necrosis, Cowdry A intranuclear inclusions, and hemorrhage (24, 38). In contrast, a single intracerebral inoculation with 10^7 or 10^9 PFU of G207 produced no neurologic symptoms, and some animals were still alive and healthy 41 months after injection. One animal had a brief seizure 5 days after its inoculation with 10^9 PFU of G207; this could have been due to cortical irritation along the needle track, the location of the inoculation, the volume inoculated, or the virus. However, the symptoms were self-limiting and required no treatment, and no viral pathology was detected in the brain when it was examined 7 months after inoculation.

Noninvasive diagnostic tests for HSV encephalitis include

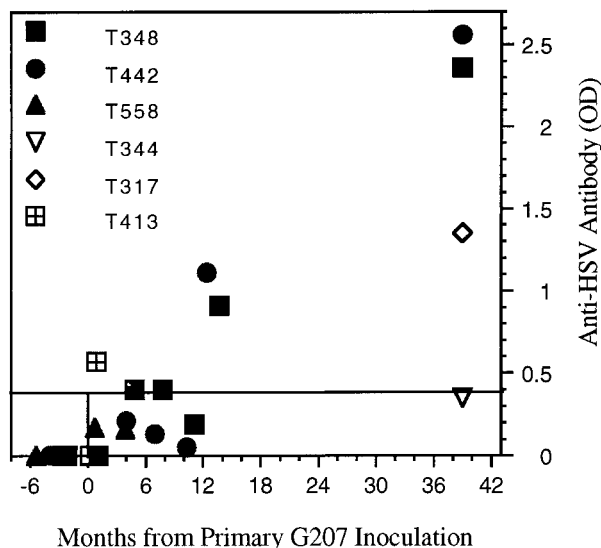


FIG. 5. Serum anti-HSV antibody levels. Anti-HSV antibody titers in serum were determined by ELISA (Table 1) at various times (months) pre- and post-G207 injection. Optical density (OD) values of ≥ 0.4 are positive, and values between 0.17 and 0.39 are equivocal. All preinjection sera had OD values of 0.

electroencephalography, computerized tomography, single-photon emission-computed tomography, and MRI (14, 19, 42, 52). MRI is highly effective at detecting the changes in brain water content that accompany brain tissue inflammation and is particularly useful for detecting the early changes associated with HSV encephalitis (28, 53, 59). We obtained MRI scans from 10 days to 1 year following G207 inoculation, and no radiographic evidence of encephalitis was found.

All animals tested developed a detectable but equivocal serum anti-HSV antibody response after intracerebral inoculation of G207. The two animals that received a second G207 inoculation 1 year after the first developed a rapid boost in antibody titer that lasted for at least two more years. In humans experiencing a primary genital infection (HSV-1 or -2), anti-HSV serum antibodies detected by complement fixation, radioimmunoprecipitation, or ELISA are generally not detected in the acute phase of infection (within 1 to 2 weeks) but are present in the early convalescent phase (2 to 8 weeks) after the onset of symptoms (2, 23, 69). In a 9-month-old child that developed HSV encephalitis, neutralizing serum antibody was not detected on day 5 after disease onset but was detected on day 9, increasing to a maximum at days 14 and 26 and then decreasing to about half this level in 14 months (32). While intracerebral inoculation is not a reasonable approach for vaccination, these studies suggest that G207 may be a useful live, attenuated strain for HSV vaccination in the periphery.

These studies demonstrate that G207 can be inoculated safely into the brain at doses of up to 10^9 PFU, at least 6 log units higher than a lethal dose of wild-type HSV-1. Even animals with humoral immunity due to prior exposure to G207 experienced no adverse consequences, and neither demyelination nor other neurologic sequelae occurred following a repeat intracerebral inoculation. This extensive neural toxicity testing suggests that G207 should be safe for intracerebral use in humans.

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