Follow-up MRI studies in patients who have received cranial irradiation will typically show cerebral atrophy. In one study that examined patients treated for germ cell tumors with a mean follow-up of 99 months, only 13% of patients maintained a performance status of 100%.[20]

Though not included as an assessment item for performance status, sterility caused by cisplatin therapy, which is an indispensable chemotherapeutic agent for germ cell tumors, should be seen as a major problem for long-term survivors. In addition, etoposide, which is one of the major drugs used in the treatment for germ cell tumors, is known to induce secondary neoplasms, albeit at a low rate.

Neurologic Deficits

After neurosurgical treatment for tumor excision or insertion of a CSF shunt, hydrocephalus is usually well controlled. For tumors originating around the optic pathway, there may be a high frequency of visual impairments affecting acuity and fields. Some patients will demonstrate limitations in upward gaze palsy as a result of involvement of tumor with the quadrigeminal plate. High-tone hearing loss has been observed after cisplatin therapy, which can also cause peripheral neuropathy. 4

Hormonal Disorder

For patients with suprasellar lesions, hormone replacement therapy is a common requirement after treatment, affecting almost 80% of patients. Diabetes insipidus or anterior pituitary dysfunction is common. Treatment of the former with DDAVP is usually long term. Saki et al emphasized that pituitary dysfunction present before treatment persisted or even worsened after patients went into remission, except for patients with small and localized masses on admission.[17] 5

Cerebral Injury after Radiation Therapy

Cerebral injury from radiation therapy is usually seen following whole-brain irradiation of 30 Gy or more. On MRI, generalized brain atrophy, multiple cerebral lacunae infarction, and high signal intensity areas in the white matter representing demyelination are generally observed. Associated with cerebral atrophy are mental and physical deterioration, hypothalamic or endocrinologic dysfunction, and impaired quality of life. A feared complication of radiation therapy is a radiation-induced secondary neoplasms. Sawamura et al also described 4 patients from a total of 84 intracranial germ cell tumor patients (4.7%) who developed radiation-induced neoplasms, including two glioblastomas and two meningiomas.[20] Moreover, there is another report describing a 12% occurrence of secondary neoplasms over a 20-year period.[13]

As an illustrative case, we describe a 14-year-old boy with a solid mass in the suprasellar area who underwent biopsy and proved to have an immature teratoma. Chemoradiation therapy was given, and a total radiation dose of 60 Gy was administered. After these treatments, MRI showed complete remission of tumor. The patient did well in school until 6 years after the initial treatment when he complained of headache and depression. A follow-up MRI study revealed a low-intensity lesion in the right basal ganglia with mild brain atrophy. After 6 months, a repeat MRI scan showed progressive brain atrophy (Figure 96-1). This case is typical for delayed brain injury induced by radiotherapy.

SUBCLASSIFICATION OF INTRACRANIAL GERM CELL TUMORS

According to the World Health Organization (WHO) classification of intracranial germ cell tumors, germinoma, embryonal carcinoma, yolk sac tumor (endodermal sinus tumor), choriocarcinoma, mature teratoma, immature teratoma, teratoma with malignant transformation, and mixed germ cell tumors are the main types.[11]

Germinomas

Pure Germinoma

Pure germinoma may be cured with a better than 90% 5-year survival rate using radiotherapy alone. In the report of Japan's Brain Tumor Registry in 2000, a remarkable improvement in cumulative survival for germinoma by radiation therapy was shown.[4] Several issues concerning quality of life, however, are known to be induced by radiotherapy. Therefore combined chemoradiotherapy has been used to reduce the total radiation dose to the brain. Nowadays, it is generally accepted that patients with germinoma can be cured by preirradiation chemotherapy followed by reduced doses of irradiation.

According to the analyses by the Brain Tumor Registry in 2000, relative survival rates for germinoma at 1-year, 2-year, and 5-year intervals were 96.6%, 94.3%, and 91.2%, respectively.[4] Brandes et al showed a 5-year survival rate of 96%.[3] Recently, Sano also showed 5- and 10-year survival rates of 96% and 93%, respectively.[19]

Germinoma with Syncytiotrophoblastic Giant Cells

Approximately 13% of germinomas contain syncytiotrophoblastic giant cells (STGC) positive for HCG-β.[21,22] This type of tumor has been shown to have a different response to chemoradiotherapy. Although the response rate is high, the tumor mass tends to regress much more slowly, and a complete response rate is generally lower than for pure germinoma. Also, germinomas with elevated HCG-β levels in serum or CSF are considered to have a higher risk of recurrence.[9] One report of patients with germinoma with elevated CSF HCG-β levels showed a 40% recurrence rate even after complete conventional radiotherapy.

To illustrate this point, a 15-year-old male who complained of headache received a MRI scan with gadolinium that showed an enhanced solid mass in the pineal lesion. CSF and serum HCG-β levels were elevated. After treatment with chemotherapy and focal radiotherapy, the MRI revealed complete remission. Four years after treatment, this patient had cerebellar ataxia. Repeat MRI showed complete remission of the original pineal lesion but diffuse dissemination of disease in the brain and spinal cord (Figure 96-2).

Teratoma

Mature Teratoma

It is generally accepted that mature teratoma should be treated by surgical resection without additional therapy. The completeness of tumor resection has been established as the most powerful prognostic parameter for this disease.[7] According to the analyses of the Brain Tumor Registry of Japan in 2000, it was reported that the relative survival rate of teratoma at 5 years was 81.2%.[4] Brandes et al showed a 5-year survival rate of 100%.[3]

Immature Teratoma

In this tumor, one finds mature teratoma along with primitive, malignant elements. Even after total-gross total resection, most cases show recurrence, and adjuvant therapy is essential to prolong survival. If serum HCG or AFP levels are elevated, prognosis is generally less favorable. However, Yoshida et al have described immature teratomas in which HCG and AFP tumor makers are negative, and the tumors are resistant to combination chemotherapy of.[25] Matsutani et al analyzed the long-term outcome of patients with immature teratoma and showed a 10-year survival rate of 70.7%.[12] Brandes et al reported a 5-year survival rate of 67%.[3]

6

As a case illustration, a 3-year-old boy sought treatment for vomiting. MRI with gadolinium showed an enhanced solid and cystic mass in the right occipital region. At surgery, a gross-total excision was performed, and histologic examination revealed an immature teratoma. Extensive chemoradiation therapy was performed. One year after treatment, follow-up MRI revealed a local recurrence with dissemination in the contralateral ventricle. After several months, regrowth of the original tumor with diffuse CSF dissemination were seen (Figure 96-3).

Teratoma with Malignant Transformation

Teratoma with malignant transformation includes the mature teratoma with a malignant component such as carcinoma or sarcoma inside the tumor mass. This tumor subtype is associated with a poor prognosis and a less than 50% chance of 5-year survival. The elements that may demonstrate malignant transformation include adenocarcinoma, squamous cell carcinoma, sarcoma, or mesenchymal carcinoma. That is why aggressive chemoradiotherapy has been performed. Despite heavy treatment strategies, Dearnaley et al reported a 5-year survival rate of only 18.2%.[5]

Other Malignant Germ Cell Tumors

Other malignant germ cell tumors include yolk sac tumors, choriocarcinomas, and embryonal carcinomas. Despite extensive surgical resection and aggressive postoperative treatment, these tumors show a poor response rate, early tumor recurrence, and frequent CSF dissemination into the spine. The prognosis for these patients remains poor at 20% to 40% chance of a 5-year survival.[12]

For these highly malignant germ cell tumors, extensive resection may be associated with improved survival, and neoadjuvant chemotherapy and high-dose craniospinal radiotherapy must be given.[8,23,24]

Yolk Sac Tumors (Endodermal Sinus Tumor)

Yolk sac tumors are rare, comprising less than 0.1% of all intracranial tumors. This tumor usually shows a remarkable elevation of AFP level in serum or CSF. This tumor has a tendency to disseminate within the CSF pathways. According to the analyses of the Brain Tumor Registry in Japan in 2000, it was reported that the 1-year, 2-year, and 5-year survival rates for patients with yolk sac tumors were 50.0%, 39.7%, and 27.2%, respectively.[4] One report suggests that extensive tumor resection followed by repeated intensive chemotherapy (PVB + PE) may improve prognosis.[16]

Choriocarcinoma

The rare tumor choriocarcinoma is mainly located in the pineal region and has a male predominance. Choriocarcinoma usually exists as a part of a mixed germ cell tumor.

Clinically, intratumoral bleeding may occur as the initial symptom. The serum HCG levels correlate well with tumor progression, and it is a reliable tumor marker. According to the analyses of the Brain Tumor Registry in Japan in 2000, the 1-year, 2-year, and 5-year survival rates of choriocarcinomas are 55.9%, 44.8%, and 44.9%, respectively.[4] Jennings et al showed that none of 10 choriocarcinoma patients survived longer than 1 year.[10] To attain longer survival rates, radical surgery followed by intensive radiation therapy (total dose 52.2 Gy) and chemotherapy using ifosfamide, carboplatin, and etoposide (ICE therapy), has been proposed.[18]

7

Embryonal Carcinoma

Intracranial embryonal carcinoma usually exists as a part of mixed gem cell tumor with immature teratoma or choriocarcinoma. HCG-β or AFP may be positive in serum or CSF. This tumor is mainly located in the pineal region but sometimes within the suprasellar region. CSF dissemination is common. The 1-year, 2-year, and 5-year survival rates for embryonal carcinomas are 80.4%, 56.4%, and 50.6%, respectively.[4] Sawamura et al treated nine patients with embryonal carcinoma before 1990. No patients survived longer than 2 years after diagnosis. Packer et al treated six patients with embryonal carcinoma using radiation therapy, either alone or with adjuvant chemotherapy. All patients initially responded to therapy, but only one survived longer than 1 year.[14]

Mixed Germ Cell Tumors

Mixed germ cell tumors are composed of various combinations of two or more types of germ cell tumor elements. More than half of the described mixed germ cell tumors show combinations of germinoma and teratoma with or without immature components. The prognosis of mixed germ cell tumors relates to the most malignant element present. According to a report by Sano, germinoma components are found in 79%, teratoma components in 63%, yolk sac tumor components in 33.3%, and embryonal carcinoma components in 15.8% of mixed germ cell tumors.[19] Matsutani et al analyzed the long-term outcome of patients with mixed tumors whose predominant characteristics were germinoma and teratoma combined with minor elements of pure malignant tumor. The analysis revealed a 3-year survival rate of 70%.[12] Brandes et al reported a 5-year survival rate

of 69% for immature teratoma mixed with germinomas.[3] At least one report of a mixed germ cell tumor treated by extensive tumor resection followed by repeated intensive chemotherapy led to a survival of 4.5 years.[16]

The following is a case of a patient with a choriocarcinoma and teratoma located in the left basal ganglia who had right hemiparesis. The patient received extensive chemoradiation therapy and after treatment saw a marked reduction in tumor size. Six years after initial treatment, this patient complained of headache with progressive left hemiparesis and follow-up MRI revealed local recurrence with an irregularly enhanced huge mass in the same area. After repeat gross-total excision of tumor (Figure 96-4), the histologic examination revealed a mature teratoma. This patient has now been followed for more than 4 years and shows no recurrence.

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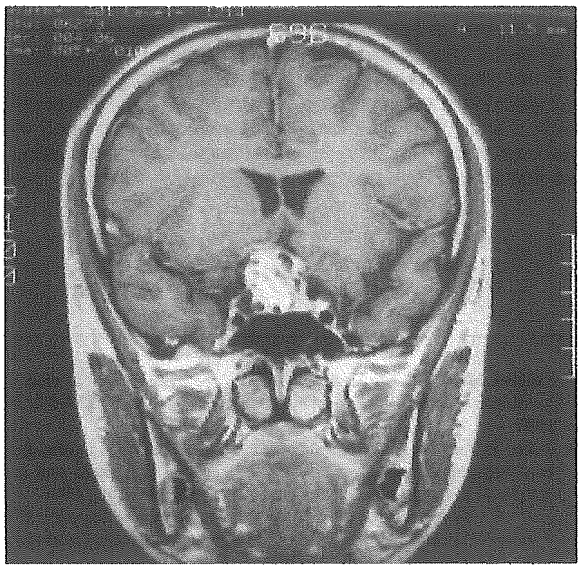

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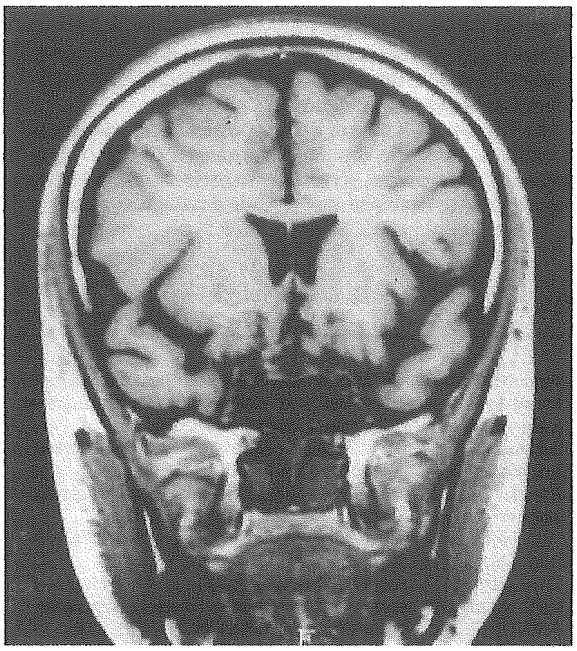

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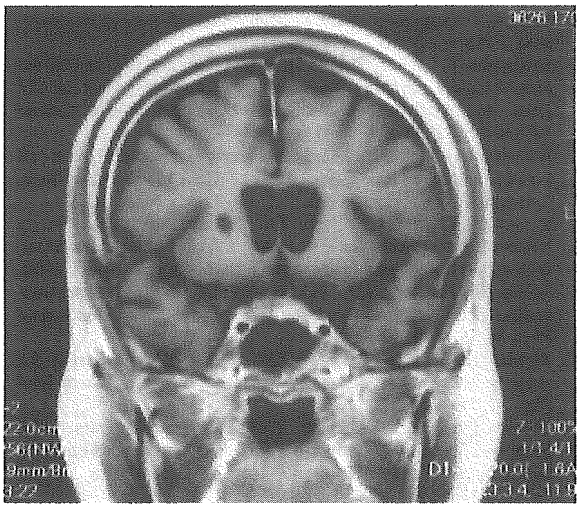
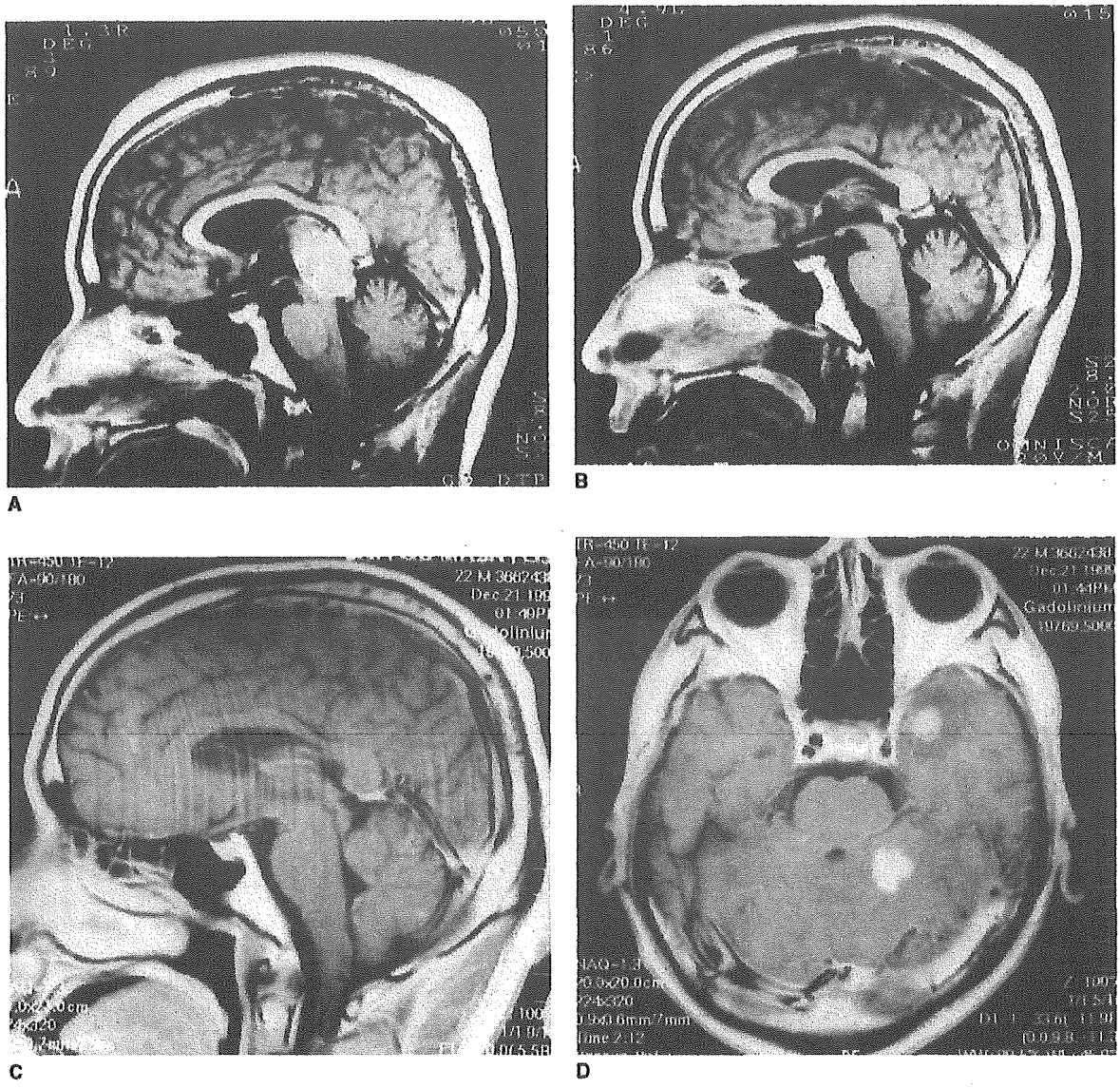
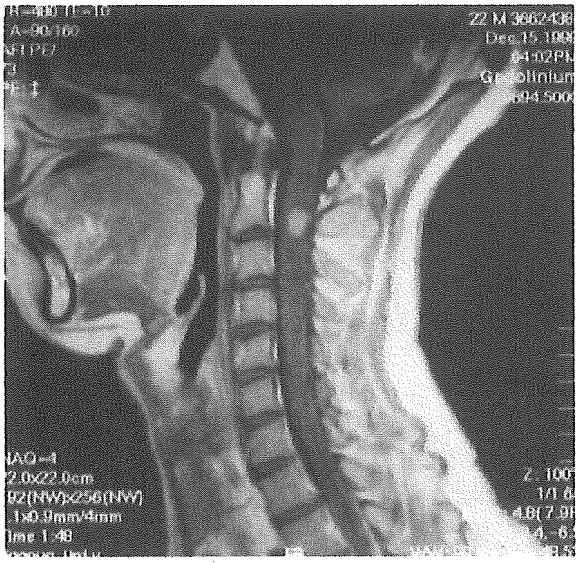


C

FIGURE 96-1 *A,* A 14-year-old boy complained of visual disturbance. Magnetic resonance imaging (MRI) with gadolinium enhancement revealed an enhanced solid mass in the suprasellar (neurohypophysis) region. Histologic examination revealed a immature teratoma. Extensive chemoradiation therapy was performed, and total radiation dose was 60 Gy (whole-brain 40 Gy + boost 20 Gy). *B,* After treatment, MRI revealed complete remission of tumor. Patient's condition was excellent and, after graduating from high school, he worked in computer programming. *C,* Six years after initial treatment, the patient complained of headache and slight depression. Follow-up MRI revealed a low-intensity lesion in the right basal ganglia with mild brain atrophy. After 6 months, his mental status gradually become worse. He is suspected to have brain damage induced by radiotherapy.



10 FIGURE 96-2 *A,* A 15-year-old patient complained of headache. Magnetic resonance imaging (MRI) with gadolinium showed an enhanced solid mass in the pineal region. The level of β-human chorionic gonadotropin was elevated in both CSF and serum. *B,* After treatment with chemoradiation, MRI revealed complete remission. *C,* Four years after treatment, the patient complained of a floating sensation and showed cerebellar ataxia. Repeated MRI still showed complete remission in the original pineal lesion. Multiple intramedullary dissemination is not only in the brain *(D)* but also in the spinal cord *(E).*



E

FIGURE 96-2 cont'd.

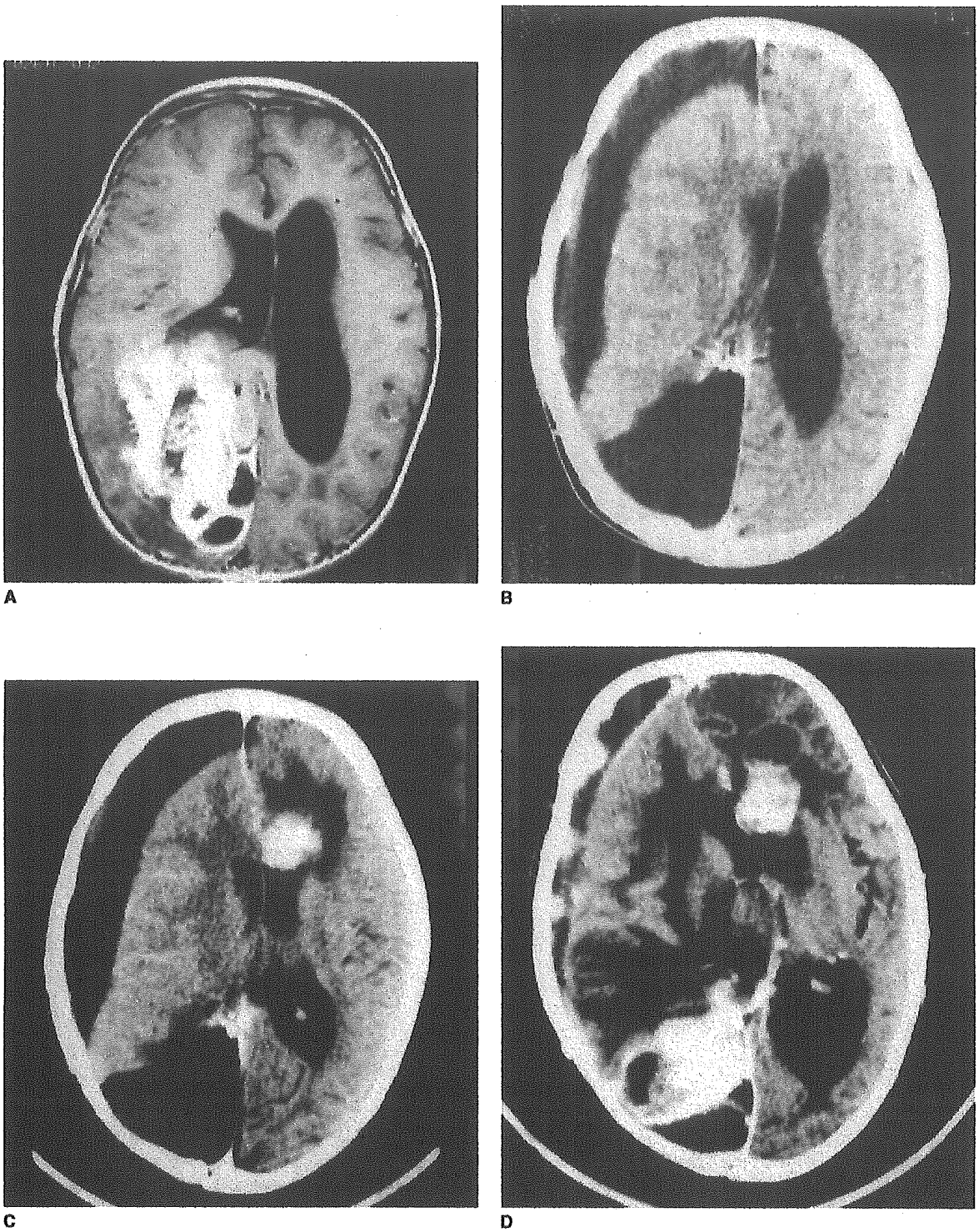


FIGURE 96-3 *A,* A 3-year-old boy experienced frequent vomiting. Magnetic resonance imaging (MRI) with gadolinium showed an enhanced solid and partly cystic mass in the right occipital lesion. *B,* After gross-total removal of the tumor, histologic examination revealed an immature teratoma. Extensive chemoradiation therapy was performed. *C,* One year after treatment, the 4-year-old patient had no complaints, but follow-up MRI revealed local recurrence with dissemination at the contralateral lateral ventricle. *D,* After several months, MRI showed local recurrence of tumor with diffuse dissemination to CSF.

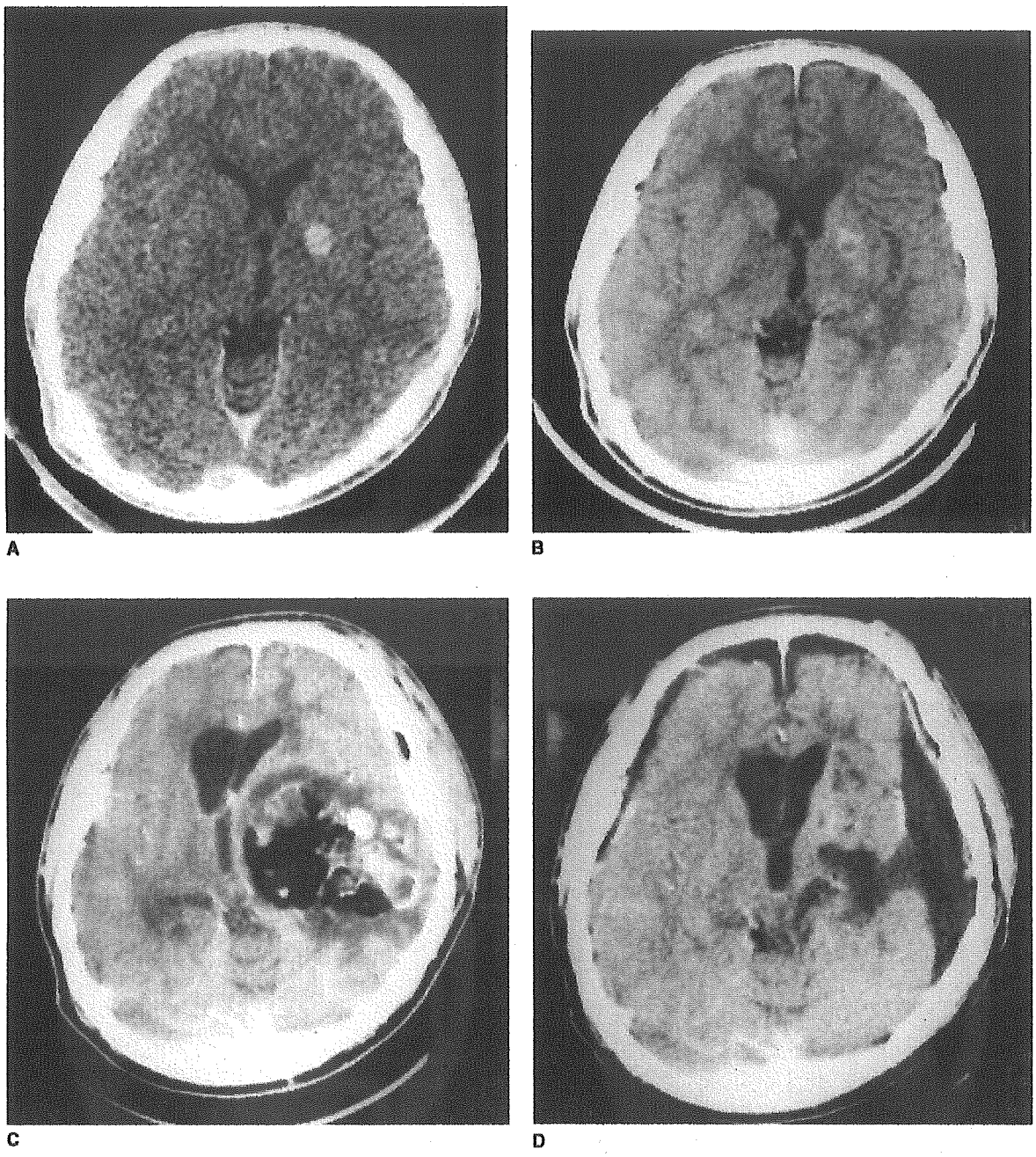


FIGURE 96-4 *A,* An 8-year-old boy showed mild right hemiparesis. Computed tomography (CT) revealed an enhanced solid mass in the left basal ganglia. We obtained a specimen by stereotactic biopsy, and histologic examination revealed a choriocarcinoma. Extensive chemoradiation therapy was performed and the patient's condition was quite good. *B,* After treatment, CT scan revealed reduced mass effect and a less enhanced tumor. *C,* Six years after initial treatment, the patient complained of headache with progressive left hemiparesis, and follow-up magnetic resonance imaging revealed local recurrence, with an irregularly enhanced huge mass. *D,* After gross-total removal of tumor, subsequent CT showed no tumor, but histologic examination revealed a teratoma. The patient received no additional treatment and at 4-year follow-up showed no recurrence.

13

Development of Oncolytic Replication-Competent Herpes Simplex Virus Vectors

The G207 Paradigm

Tomoki Todo and Samuel D. Rabkin

1. INTRODUCTION

Oncolytic virus therapy is a promising new strategy for treating cancer that involves replication-competent virus vectors that can replicate *in situ* in tumor cells, exhibit oncolytic activity by direct cytocidal effects, and then spread throughout the tumor. In addition, replication-competent virus vectors are capable of transferring and expressing foreign genes in host cells. These virus vectors are either genetically engineered (e.g., herpes simplex virus type 1 [HSV-1], adenovirus, vaccinia virus), naturally attenuated (e.g., Newcastle disease virus), or nonpathogenic in humans (e.g., reovirus), so they replicate selectively in tumor cells, but do not harm normal tissues *(1)*.

HSV-1 in particular has many features that make it attractive for cancer therapy *(2)*: (1) HSV-1 infects most tumor cell types; (2) its life cycle is well studied *(3)*; (3) the HSV-1 genome has been sequenced; (4) the functions of the majority of genes have been identified *(4)*; (5) genes can be manipulated; and (6) the large size of the genome (153 kb) provides space for insertion of large amounts of deoxyribonucleic acid (DNA) *(4)*. Furthermore, HSV-1 has the following features that are well suited for clinical application: (1) total tumor cell killing in vitro can be achieved at a relatively low multiplicity of infection (MOI); (2) antiviral drugs are available that enable optional termination of the therapy *(5)*; (3) animal models are available for preclinical evaluation of safety and efficacy; (4) the viral genome does not integrate into the host cell genome; and (5) it can exist in a latent state without causing detectable damage to the infected cell *(6)*. HSV-1 is a neurotropic virus, and many of the genes necessary for neuropathogenicity are nonessential and can be mutated *(7)*. Therefore, the use of HSV-1 is especially advantageous for brain tumor therapy.

Research on oncolytic HSV-1 therapy has advanced rapidly from a basic concept to clinical studies. In the early days, replication-competent HSV-1 vectors were genetically engineered to have mutations in one nonessential gene associated with either virulence or viral DNA synthesis to restrict viral replication to transformed cells *(2,8)*. These so-called first-generation vectors demonstrated that HSV-1 vectors could in fact efficiently inhibit the growth of tumors without lethally harming the host animal. They also showed that oncolytic HSV-1 therapy could be applied not only to brain tumors, but also to a broad range of solid tumors *(9)*. There were concerns, however, regarding the use of these first-generation vectors in humans because their pathogenicity may not have been sufficiently attenuated, and a single mutation could potentially revert to wild type. To address these concerns, so-called second-generation vectors were developed that had genetically engineered mutations in two different genes.

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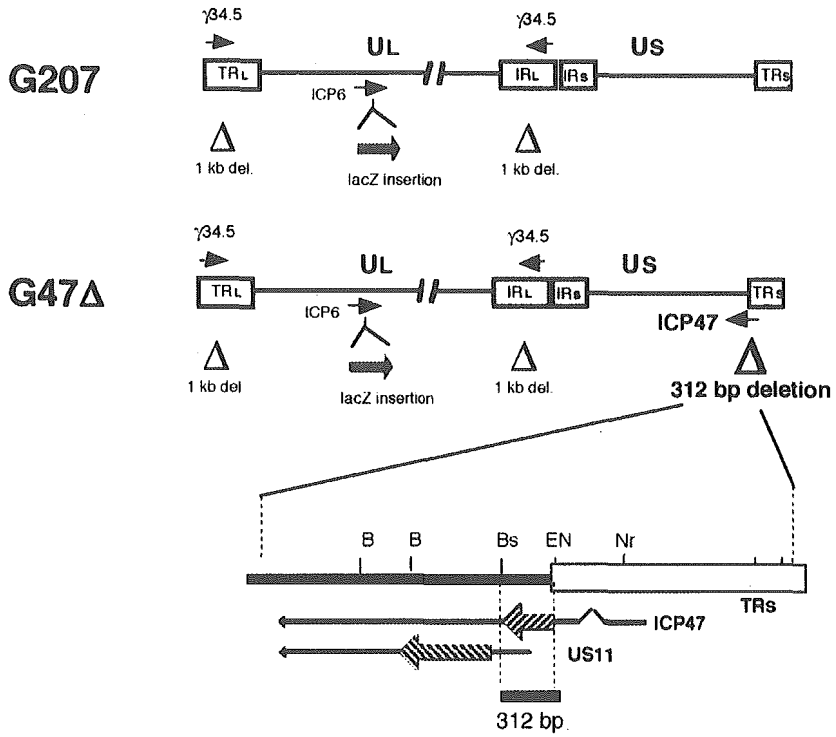

Fig. 1. Structures of G207 and G47Δ. The HSV-1 genome consists of long and short unique regions U_L and U_S, respectively, each bounded by terminal (T) and internal (I) repeat regions R_L and R_S, respectively. G207 was engineered from wild-type HSV-1 strain F by deleting 1 kb within both copies of the *γ34.5* gene and inserting the *E. coli lacZ* gene into the ICP6 coding region. G47Δ was derived from G207 by deleting 312 bp from the ICP47 locus. Because of the overlapping 3' coterminal transcripts of US11 and ICP47, the deletion also places the late gene US11 under control of the ICP47 immediate-early promoter. The ICP47 transcript contains an intron (indicated by Λ). Restriction site abbreviations: B, BamHI; Bs, BstEII; E, EcoRI, EN, EcoNI, Nr, NruI. (Modified from ref. *80*.)

2. G207

G207 was the first of the second-generation HSV-1 vectors *(10)*. It was originally designed for clinical application in patients with brain tumors, with an emphasis on employing ample safeguards. G207 has deletions in both copies of the γ34.5 gene (Fig. 1), the major determinant of HSV-1 neurovirulence *(11)*. The *γ34.5*-deficient HSV-1 vectors are considerably attenuated in normal cells, but retain their ability to replicate in neoplastic cells *(9)*.

In normal cells, HSV-1 infection induces activation of double-stranded RNA-dependent protein kinase R (PKR), which in turn leads to phosphorylation of the α-subunit of eukaryotic initiation factor 2α (eIF-2α) and a subsequent shutdown of host and viral protein synthesis *(12)*. The product of the *γ34.5* gene antagonizes this PKR activity. However, in tumor cells with an activated Ras signaling pathway, it has been suggested that PKR activity is already inhibited, thereby allowing *γ34.5*-deficient HSV-1 vectors to replicate *(13,14)*. Many of the oncolytic HSV-1 vectors currently used have deletions in the *γ34.5* gene *(8)*, including R3616 *(11)*, the parent of G207, and 1716 *(15)*.

G207 also has an insertion of the *Escherichia coli lacZ* gene in the infected-cell protein 6 (ICP6) coding region (UL39), inactivating ribonucleotide reductase, a key enzyme for viral DNA synthesis in

nondividing cells, but not in dividing cells *(16)*. This double mutation greatly minimizes the chances of G207 reverting to wild type or a pathogenic phenotype. It also confers favorable properties on the virus for treating human cancers: G207 replicates preferentially in tumor cells and is harmless in normal tissue because of attenuated virulence, G207 is about 10-fold more sensitive to ganciclovir/acyclovir than its parent virus R3616, and the reporter gene *lacZ* allows easy histochemical detection of G207-infected cells *(10)*. 3616UB is a similar, second-generation vector except uracil DNA glycosylase was inactivated instead of ICP6 *(17)*.

2.1. Antitumor Efficacy

G207 has been tested in more than 60 different cell lines, which revealed that the vast majority, although not all, of human tumor cell lines are susceptible to G207 infection and replication *(18)*. In human glioma and malignant meningioma cell lines, for example, G207 can achieve destruction of the entire cell population in culture within 2 to 6 days at an MOI of 0.1 *(10,19)*. In contrast, at the same MOI, G207 manifests no effect on primary cultures of rat cortical astrocytes or cerebellar neurons *(10)*.

This difference in G207 cytopathic effect observed in vitro between tumor cells and normal cells is directly reflected in the results of in vivo studies. In athymic mice harboring U87MG glioma or F5 malignant meningioma tumors intracranially or subcutaneously, a single intraneoplastic inoculation of G207 significantly inhibited tumor growth and prolonged animal survival *(10,19)*. Prominent *lacZ* expression from G207 replication within tumors could still be observed 24 days postinoculation *(19)*.

Besides brain tumors, G207 has proven efficacious in a variety of other animal tumor models in which human, mouse, rat, or hamster tumors have been generated subcutaneously or in various organs, including the liver, peritoneum, sciatic nerve, urinary bladder, and cheek pouch *(18)*.

In addition to direct intratumoral inoculation, G207 has been successfully administered intravenously *(20–22)*, via portal vein *(23)*, intraarterially *(24)*, and intraperitoneally *(25,26)*.

2.2. Safety

Because HSV-1 is the most common viral cause of fatal encephalitis *(27)* and G207 was the first replication-competent HSV-1 vector, along with 1716 *(28)*, to be used in human brains, it was extensively evaluated for its toxicity in the brain. In BALB/c mice, the highest dose of G207 (10^7 plaque forming units [pfu]) caused no symptoms for over 20 weeks when inoculated intracerebrally or intraventricularly *(29)*. In A/J mice, one of the most susceptible mouse strains to HSV-1 infection *(30)*, intracerebral inoculation of clinical-grade G207 at 2×10^6 pfu caused only a temporary and slight hunching in 2/8 mice *(31)*. Furthermore, in BALB/c mice that survived an intracerebral inoculation of wild-type HSV-1 (strain KOS) at an LD_{50} dose (~10^3 pfu), a subsequent challenge with an intracerebral inoculation of G207 (10^7 pfu) at the same stereotactic coordinates did not result in reactivation of latent HSV-1 *(29)*.

Aotus nancymae (New World owl monkeys) are among the most sensitive nonhuman primates to HSV-1 infection *(32,33)*. A total of 22 *Aotus* primates have been used for safety evaluation of G207 (intracerebral and/or intraprostatic inoculation) *(34–36)*. In *Aotus*, a single intracerebral inoculation of G207, up to 10^9 pfu or repeat inoculations of 10^7 pfu, caused neither virus-related disease nor detectable changes in the brain as assessed by magnetic resonance imaging (MRI) and pathological studies *(34)*.

In contrast, an intracerebral inoculation of 10^3 pfu of wild-type HSV-1 (strain F) caused acute viral encephalitis, with the animal becoming moribund within 5 days of inoculation. Four *Aotus* were used to evaluate the shedding and biodistribution of G207 after intracerebral inoculation of clinical-grade, column-purified G207 (3×10^7 pfu) *(35)*. Using polymerase chain reaction analyses and viral culture, neither infectious virus nor viral DNA was detected from tear, saliva, vaginal secretion, blood, or urine samples at any time-point up to 1 month postinoculation. Analyses of tissues obtained at necropsy at 1 month showed G207 DNA distribution restricted to the brain, with no infectious

virus isolated. Histopathology revealed normal brain tissues, including the sites of inoculation *(35)*. All *Aotus* receiving an intracerebral G207 inoculation showed an increase in serum anti-HSV-1 antibody titers as early as 21 days postinoculation *(34,35)*.

2.3. Clinical Trial

A phase I clinical trial of G207 for recurrent malignant glioma was performed in 21 patients at two institutions in the United States *(37)*. This dose escalation study started at 10^6 pfu and increased to 3×10^9 pfu, with three patients at each dose. G207 was inoculated stereotactically into an enhancing region of the tumor, visualized by computerized tomographic scan with contrast enhancement. No acute, moderate-to-severe, adverse events attributable to G207 were observed *(37)*. Minor adverse events included seizure (2 cases) and brain edema (1 case). Among 7 biopsied or resected tumors analyzed, specimens from 2 patients were positive for G207 DNA by polymerase chain reaction analysis (56 and 157 days postinoculation). Of 19 patients, 5 were negative for serum anti-HSV-1 antibody prior to G207 treatment, and despite corticosteroid treatment of these patients, 1 patient seroconverted after G207 inoculation *(37)*.

The tools to evaluate efficacy included Karnofsky performance score and serial MRI *(37)*. An improvement in Karnofsky score was observed in 6 of 21 patients (29%) at some time after G207 inoculation. Of 20 patients that had serial MRI evaluations, 8 had a decrease in tumor volume (enhancing area) between 4 days and 1 month postinoculation. All patients, except 1 who died from cerebral infarction 10 months after G207 treatment, eventually showed tumor progression. Interestingly, this glioblastoma patient had no evidence of residual tumor at autopsy. Autopsy was performed in 5 cases, and histology of the brains showed no evidence of encephalitis, white matter degeneration, or inflammatory changes, and all were negative for HSV-1 immunoreactivity. In 3 cases, the tumor was localized to one region of the brain without significant tumor cell invasion into the surrounding brain tissue as usually observed with typical glioblastoma cases.

Overall, the phase I clinical trial confirmed the safety of G207 inoculated into the brain at doses up to 3×10^9 pfu. Currently, a phase Ib clinical trial for recurrent malignant glioma was performed [NIH 481 (2001-07)], and a phase II trial is planned. Similar results were obtained in phase I trials for glioma with 1716 in the United Kingdom *(28,38)*; 1716, which only contains deletions of *γ34.5 (15)*, was tested at a lower dose range (up to 10^5 pfu) *(28,38)*.

3. USE OF ONCOLYTIC HSV VECTORS FOR IMMUNE THERAPY

Although G207 proved safe in glioma patients and efficacious in animal tumor models, G207 is considerably attenuated, not only for pathogenicity, but also in its tumor cell-killing capability compared to wild-type HSV-1. One way to improve the efficacy of oncolytic HSV therapy would be to harness antitumor immune responses induced in the course of the oncolytic activity of HSV vectors.

3.1. Antitumor Immune Responses

A difficulty in investigating the immune effects of oncolytic HSV therapy has been the lack of suitable animal tumor models susceptible to HSV-1 infection. Many mouse strains and a majority of murine cell lines are relatively resistant to HSV-1 *(18,30)*. It was not recognized until development of immunocompetent mouse tumor models suitable for HSV-1 evaluation that the host immune response plays an important role in the antitumor activity of oncolytic HSV-1 vectors both in the brain and in the periphery *(39,40)*. Initially, murine N18 neuroblastoma cells, one of the more susceptible murine cell lines tested for G207 susceptibility, were used in syngeneic A/J mice. In A/J mice harboring established N18 tumors subcutaneously or in the brain, intraneoplastic inoculation with G207 caused a significant reduction in tumor growth or prolongation of survival *(39)*. Moreover, in A/J mice bearing bilateral subcutaneous N18 tumors, intraneoplastic G207 inoculation into one tumor alone caused growth reduction and/or regression of both the inoculated and the noninoculated contralateral

tumor, indicating induction of systemic antitumor immunity *(39)*. This inhibition of noninoculated tumor growth was also seen in animals bearing intracerebral brain tumors after subcutaneous tumor inoculation. Animals that were cured of their subcutaneous tumors by G207 were protected against tumor rechallenge, in either the periphery or the brain. Antitumor immunity was associated with cytotoxic T lymphocyte (CTL) activity that was specific to N18 tumor cells and persisted for at least 13 months.

G207-induced, systemic antitumor immunity was also observed in BALB/c mice bearing subcutaneous CT26 (colon carcinoma) tumors and DBA/2 mice bearing subcutaneous M3 (melanoma) tumors *(40)*. In the CT26 model, intraneoplastic inoculation of G207 induced CTL activity that recognized a dominant, tumor-specific, major histocompatibility complex (MHC) class I-restricted epitope (AH1) from CT26 cells. Similar systemic antitumor immunity induction by G207 was observed in Syrian hamsters bearing subcutaneous KIGB-5 (gallbladder carcinoma) tumors *(41)* and BALB/c mice bearing CT26 liver metastases *(42)*. Thus, in an immunocompetent condition, the oncolytic activity of G207 can be augmented by induction of specific and systemic antitumor immunity effective both in the periphery and in the brain.

When high-dose dexamethasone was given to A/J mice bearing subcutaneous N18 tumors for an extensive period (16 days), G207 retained antitumor activity and caused a significant suppression of tumor growth when inoculated into the tumors *(43)*. However, all immunosuppressed (dexamethasone-treated) mice treated with G207 displayed tumor regrowth despite initial shrinkage, whereas 50% of the G207-treated mice not immunosuppressed were cured. Dexamethasone administration significantly reduced neutralizing serum antibodies against G207 after intraneoplastic G207 inoculation, but this did not affect the amount of infectious G207 isolated from tumors. The most striking effect of dexamethasone administration was the abolition of G207-induced CTL activity against N18 cells *(43)*. These results further support the importance of tumor-specific CTL induction in the course of oncolytic HSV-1 antitumor activity.

The effect of circulating anti-HSV-1 antibodies on the efficacy of oncolytic HSV-1 therapy has been investigated because the majority of the population is HSV-1 seropositive *(44,45)*. A/J and BALB/c mice were immunized by repeated intraperitoneal inoculations of wild-type HSV-1 (strain KOS) and then the antitumor efficacy of G207 on established subcutaneous N18 and CT26 tumors was determined *(46)*. In both tumor models, the antitumor efficacy of G207 was the same whether the mice were immunized or not for HSV-1.

In a study using intraocular immunization, treatment of M3 melanoma tumors in DBA/2 mice with HSV-1 1716 was actually more effective than in nonimmunized mice *(47)*. Because HSV-1 predominantly spreads cell to cell, circulating antibodies known to neutralize free virus may have little effect on HSV-1 directly inoculated into tumors. When NV1020, at a low dose (10^6 pfu), was administered intravenously to immunized BALB/c mice with CT26 tumors in the liver, there was a detectable decrease in efficacy *(48)*. This efficacy attenuation with intravenous delivery was overcome by administering a higher dose (10^7 pfu) of NV1020.

3.2. *Third-Generation Oncolytic HSV-1 Vector*

The therapeutic benefits of oncolytic HSV-1 vectors depend on the extent of both intratumoral viral replication and induction of host antitumor immune responses. We are developing new generations of HSV-1 vectors by enhancing these properties and retaining the safety features of G207. G47Δ is one such vector created from G207 by introducing another genetic alteration, deletion of the *α47* gene and the overlapping *US11* promoter region *(31)* (Fig. 1). Because the *α47* gene product (ICP47) inhibits transporter associated with antigen presentation, which translocates peptides across the endoplasmic reticulum, the downregulation of MHC class I that normally occurs in human cells after infection with HSV-1 does not occur *(49)*. G47Δ-infected human cells in fact presented higher levels of MHC class I than cells infected with other HSV-1 vectors *(31)*. Further, human melanoma

cells infected with G47Δ were better at stimulating their matched tumor-infiltrating lymphocytes in vitro than those infected with G207. Unfortunately, the interaction of ICP47 with transporter associated with antigen presentation is species specific and is exceedingly inefficient in rodent cells *(50)*. Therefore, it is not possible to test the immune effects in vivo in mouse tumor models.

The deletion also places the late *US11* gene under control of the immediate-early *α47* promoter, which results in suppression of the reduced growth phenotype of *γ34.5*-deficient HSV-1 mutants *(51)*, including G207. In the majority of cell lines tested in vitro, G47Δ replicated better than G207, resulting in the generation of higher virus titers, and exhibited greater cytopathic effect *(31)*. In athymic mice bearing subcutaneous U87MG human glioma tumors and A/J mice bearing subcutaneous Neuro2a neuroblastoma tumors, G47Δ was significantly more efficacious than G207 at inhibiting tumor growth when inoculated intraneoplastically *(31)*.

Improved antitumor efficacy of G47Δ has also been shown in other immunocompetent mouse tumor models, including prostate and breast cancer *(65)*. Nevertheless, this deletion does not suppress the attenuated pathogenicity of *γ34.5* deletion mutants *(52)*, and the safety of G47Δ remained unchanged from G207 following injection into the brains of HSV-1-sensitive A/J mice *(31)*.

Thus, compared with the parental virus G207, G47Δ demonstrated (1) better induction of human antitumor immune cells; (2) better growth properties, leading to higher virus yields and increased cytopathic effect in vitro; (3) better antitumor efficacy in both immunocompetent and immunoincompetent animals; and (4) preserved safety. These features make G47Δ highly attractive for clinical application.

3.3. Combination With Immune Gene Therapy

Our experience using various HSV-1 vectors to treat tumors, including wild-type HSV-1, indicates that there is a limit to improving the antitumor efficacy of oncolytic vectors by simply bringing the replication capability closer to that of wild-type viruses, putting aside the difficulty of doing so without increasing pathogenicity. In developing new vectors, therefore, more emphasis is currently placed on enhancing the ability to induce antitumor immunity. The combination of oncolytic HSV-1 vectors with defective vectors expressing immunostimulatory molecules can improve therapeutic efficacy significantly (Fig. 2) *(53–55)*. In this approach, the oncolytic HSV-1 vector acts as a helper virus for the propagation of plasmid-based defective vectors *(56)*. An advantage of this approach is that different defective vectors can be generated with different oncolytic helper viruses for a multiplicity of combinations without creating new vectors.

We have developed an immune gene therapy strategy that would work for brain tumors as well as other cancers. The brain is considered an immune-privileged site, and patients with brain tumors are often under an immune-suppressed condition because of immunosuppressive factors secreted by the brain tumor and/or corticosteroid administration. On the other hand, a robust, nonspecific inflammatory response in the brain can cause undesirable brain edema.

To meet these requirements, we created a defective HSV vector (dvB7Ig) expressing a soluble form of B7-1, one of the most potent costimulatory molecules, and used it in combination with G207 *(54)*. Soluble B7-1 was designed as a fusion protein of the extracellular domain of B7-1 and the Fc portion of immunoglobulin G, so that it is secreted by tumor cells rather than expressed on the cell surface. Secreted soluble B7-1 should provide antigen-presenting cells increased T-cell stimulatory activity, activate T cells in an anergic state, and because it is in a dimeric form, provide a strong stimulation to T cells by crosslinking neighboring CD28.

The in vivo efficacy was tested in the poorly immunogenic murine neuroblastoma Neuro2a in A/J mice. Intraneoplastic inoculation of dvB7Ig/G207 at a low titer successfully inhibited the growth of established subcutaneous tumors, despite the expression of B7-1-immunoglobulin detected in only 1% or fewer tumor cells at the inoculation site, and prolonged the survival of mice bearing intracerebral tumors *(54)*. Inoculation of dvB7Ig/G207 induced a significant influx of $CD4^+$ and $CD8^+$ T cells in the tumor. In vivo depletion of immune cell subsets further revealed that the antitumor effect

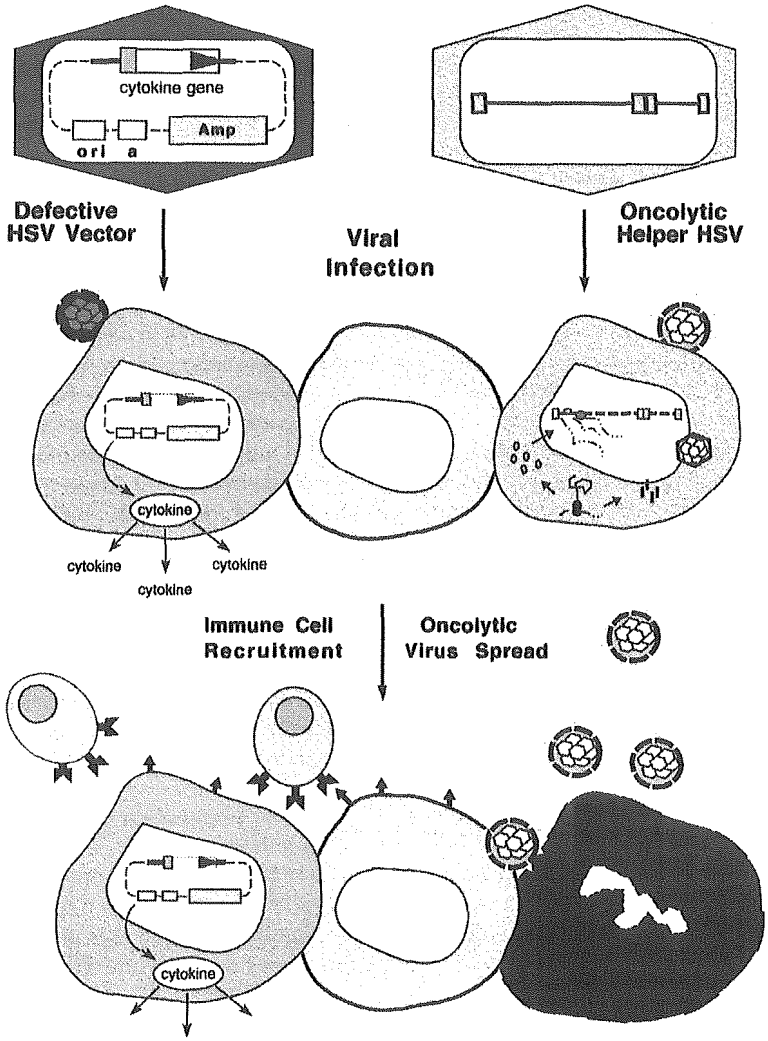

Fig. 2. Schematic diagram of immunomodulatory gene therapy using oncolytic HSV-1 vectors (like G207) as helper virus in combination with a defective HSV-1 vector expressing an immunomodulatory molecule. Defective HSV vector stocks are a mixture of defective particles (upper left) containing tandem repeats of an amplicon plasmid and HSV helper viruses (upper right) *(81)*. The amplicon plasmid consists of the cytokine/immunomodulatory gene, an HSV origin of replication (ori), and an HSV cleavage/packaging signal (a), but no viral coding sequences, and is packaged as a full viral genome length (~150 kb). Any conditional-lethal or replication-competent HSV mutant can be used as helper virus. When a mixture of helper and cytokine-expressing defective vector is inoculated into a tumor, the helper virus replicates, kills the infected cell, and spreads to other tumor cells (right side). On the other hand, tumor cells infected with the defective vector produce the cytokine and recruit immune cells (left side) that augment the antitumor immune response elicited by the oncolytic helper virus.

required $CD8^+$ T cells, but not $CD4^+$ T cells *(54)*. DvB7Ig/G207 treatment conferred tumor-specific protective immunity on cured animals. Thus, this approach proved to be a potent and clinically applicable means of treating brain tumors and other cancers.

A defective HSV vector expressing murine interleukin 12 (IL-12) in combination with G207 was very effective in treating subcutaneous CT26 tumors in BALB/c mice and inducing a tumor cell-specific $CD8^+$ CTL response *(53)*. An IL-2-expressing defective vector in combination with G207 had enhanced efficacy in murine squamous cell carcinoma and rat hepatocellular carcinoma models compared to G207 alone *(55,57)*. However, granulocyte-macrophage colony-stimulating factor (GM-CSF) expression from a defective vector did not have any increased efficacy over G207 alone in treating CT26 tumors (M. Toda and S. D. Rabkin, unpublished results, 1998). Whereas GM-CSF expression from replication-deficient HSV vectors did significantly enhance antitumor activity *(58, 59)*, as a tumor vaccine, GM-CSF-transduced cells have been found to be among the most effective *(60)*. This suggests that HSV infection may be inducing an immune response similar to that of GM-CSF expression, and that the spectrum of cytokines that will be effective in combination with oncolytic HSV vectors will be different from those used in tumor vaccines.

Replication-competent HSV-1 vectors that contain immunostimulatory molecule transgenes (i.e., IL-4, IL-10, IL-12, GM-CSF) have been created *(61–63)*. In particular, replication-competent HSV-1 vectors that express IL-12 have been shown in several animal tumor models to manifest direct oncolytic activity and express sufficient amounts of IL-12, which significantly augments antitumor activity without increasing toxicity, compared with the parental HSV-1 vectors *(62–65)*.

4. FUTURE DIRECTIONS

Now that it has been demonstrated in several clinical trials that oncolytic HSV-1 vectors can be administered safely in humans *(28,37,38,66)*, further development of oncolytic HSV-1 vectors will be directed toward improving antitumor efficacy. Doing so without compromising the safety of the vectors is the key to prevailing in this type of therapy. G47Δ is a good example of providing such an improvement in efficacy yet retaining safety features. A syncytial mutant (Fu-10) generated from G207, which forms tumor cell syncytium, was more efficacious in a lung metastases model than the parent, G207 *(22)*.

Expression of foreign transgenes, for example, "suicide" or immunostimulatory molecules, is another promising method to augment the activity of oncolytic HSV-1 vectors. A number of suicide genes, cytochrome P450 (CYP2B1) and cytosine deaminase (CD), have been incorporated into oncolytic HSV-1 vectors, and treatment with prodrugs significantly improved efficacy *(67,68)*. With the addition of foreign transgenes, it is important to be aware that they may increase the toxicity of the vector, decrease safety, and/or interfere with viral replication and decrease efficacy.

A practical method for improving the efficacy of oncolytic HSV-1 vectors is to combine them with conventional therapies. For example, a combination with cisplatin was shown to enhance the antitumor effect of G207 against human head and neck cancer *(69)*, and mitomycin C with 1716 was more effective than either treatment alone against human non-small cell lung cancer *(70)*.

Others have shown that ionizing radiation amplifies the replication of HSV-1 R3616 *(71)*, leading to improved survival of athymic mice bearing intracerebral U87MG tumors *(72)* and NV1020 (R7020) in some hepatoma tumor cell lines *(73)*. Although we did not observe such an enhancing effect of ionizing radiation with G207 in prostate cancer *(74)*, others have shown such an effect with G207 and cervical cancer *(75)*. Systemic delivery to brain tumors after intracarotid artery infusion can be enhanced by disruption of the blood–brain barrier using mannitol, bradykinin, or RMP-7 *(76–78)*. The replication and spread of oncolytic HSV-1 vector hrR3 in brain tumors after RMP-7 can be further enhanced by intraperitoneal administration of cyclophosphamide *(79)*. The combination of oncolytic HSV-1 vectors with established therapies should be rapidly translatable to the clinic.

5. CONCLUSION

Oncolytic virus therapy is an attractive treatment strategy because it is based on a new concept that the antitumor agent can amplify specifically at the tumor site after administration. This strategy

also has features that make it attractive for clinical application: (1) tumor cells are targeted irrespective of their genetic makeup; (2) it can be combined with conventional therapies such as surgery, radiation therapy, and chemotherapy; (3) combination with immunotherapy has potential synergistic effects; and (4) it can act as a vehicle for gene delivery in vivo. An increasing number of clinical trials using oncolytic viruses have been initiated or planned in recent years. We anticipate that oncolytic virus therapy will be established as an important modality of cancer treatment in the near future.

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