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Use of a "Replication-Restricted" Herpes Virus to Treat Experimental Human Malignant Mesothelioma

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ABSTRACT

Modified, nonneurovirulent herpes simplex viruses (HSVs) have shown promise in the treatment of brain tumors. However, HSV-1 can infect and lyse a wide range of cell types. In this report, we show that HSV-1716, a mutant lacking both copies of the gene coding ICP-34.5, can effectively treat a localized i.p. malignancy. Human malignant mesothelioma cells supported the growth of HSV-1716 and were efficiently lysed *in vitro*. i.p. injection of HSV-1716 into animals with established tumor nodules reduced tumor burden and significantly prolonged survival in an animal model of non-central nervous system-localized human malignancy without dissemination or persistence after i.p. injection into SCID mice bearing human tumors. These findings suggest that this virus may be efficacious and safe for use in localized human malignancies of nonneuronal origin such as malignant mesothelioma.

INTRODUCTION

The recent emergence of viral-based "gene therapy" as an approach to treat cancer has generated a great deal of enthusiasm and interest (1). For example, our group and others have explored the use of retrovirus-producing cells or adenoviruses to deliver the HSV*tk*[3] gene into localized malignancies, such as brain tumors and malignant mesothelioma (2–6). Although a "bystander" effect amplifies the efficacy of the HSV*tk*/ganciclovir system (7), it is likely that a major obstacle in treating actual patients with localized malignancies will be the ability to transduce a sufficient number of cells within a large tumor mass. One strategy that holds promise is the use of replicating viral vectors (8).

The idea of using replicating viruses to treat tumors was suggested as early as 1904 by George Dock (9); in fact, a number of clinical trials were conducted in the 1950s and 1960s with some success (10, 11), although interest in the use of viral-based cancer therapy waned thereafter. Advances in virology and molecular biology now allow the "engineering" of viruses with specific properties, suggesting that the idea of viral-based cancer therapy should be revisited. One promising virus in this regard is HSV-1. A number of HSV-1 mutants have recently been identified that appear to replicate preferentially in rapidly dividing transformed cells (12, 13). Because of the natural tropism of wild-type herpes virus for neuronal tissue, the published uses of modified, replicating HSV to treat cancer have centered on tumors of CNS origin (14–16). One of these mutants is HSV-1716, a virus that contains a 759-bp deletion in the genes coding for ICP 34.5 (12). This mutation, through mechanisms that are still unclear, severely attenuates the ability of HSV-1716 to replicate in normal tissues (12, 17, 18), but does not appear to affect the virus' ability to replicate in rapidly dividing malignant cells. We and others (18, 19) have shown that HSV-1716 is extremely nonneurovirulent in animal models and can be used to treat intracranial tumors in normal and immunodeficient mouse models (15, 16, 20).

Neurovirulent, wild-type HSV-1 is able to infect and lyse a wide variety of cell types. Therefore, we reasoned that a mutant HSV virus, such as HSV-1716, might be efficacious in the treatment of localized, non-CNS malignancies. In addition, the use of this virus in a location distant from the CNS might offer significant safety advantages. Accordingly, in this paper we report on the successful *in vitro* and *in vivo* use of HSV-1716 to treat a localized, non-CNS malignancy. We have chosen human malignant mesothelioma as a model tumor system for HSV-1716 because of the localized nature of this malignancy (allowing for direct virus administration) and because of the current lack of effective treatment (21).

MATERIALS AND METHODS

HSV-1716. HSV-1716 was originally isolated in the laboratory of S. M. Brown (Glasgow, Scotland) and passaged for use in this study by N. W. Fraser (Philadelphia, PA). The genome of this virus contains a 759-bp deletion located within each copy of the *Bam*HI fragment of the long repeat region of the genome (12). These deletions remove most of the gene encoding ICP 34.5, and the mutant fails to make the protein (12, 22)

***In Vitro* Studies of HSV-1716 on a Human Malignant Mesothelioma Cell Line.** A human malignant mesothelioma cell line call REN was isolated, characterized, and passaged as described previously by our laboratory (23). Human lung cancer cell lines A549, H322, and H358 were obtained from the American Type Culture Collection (Rockville, MD). The human osteosarcoma cell line SAOS was obtained from T. Halazonetis (The Wistar Institute). The human melanoma cell line WM451 Lu was obtained from M. Herlyn (The Wistar Institute) and was isolated, characterized, and passaged as described previously (24). To construct single-step viral growth curves, cells were plated on six-well plates at a density of 5×10^5 cells/well and infected 24 h later with HSV-1716 at a MOI of 0.01 (5×10^3 PFU/well). One well was harvested at 0, 6, 12, and 24 h by cell scraping and collection of the media. The samples were freeze/thawed and titered by plaque assay on baby hamster kidney cell monolayers. A cell viability assay was performed by plating cells in 96-well plates at a density of 5×10^3 cells per well. Twenty-four h later, the cells were infected with HSV-1716 at MOIs of 0, 0.001, 0.01, and 0.1. Six wells were infected at each MOI. A sufficient number of plates were used to allow for viability assay at 24, 48, 72, and 96 h after infection. Viable cell number was assessed by a colorimetric assay (CellTiter 96 Aqueous Nonradioactive MTT Cell Proliferation Assay; Promega, Madison WI) that measures viable cell dehydrogenase activity by absorbance. The percentage of control growth is defined as the ratio of the mean absorbance of six treatment wells at 490 nm to the mean absorbance of six untreated matched controls.

***In Vivo* Studies.** A previously described model of human malignant mesothelioma growing in the peritoneal cavity of SCID mice was used for all *in vivo* experimentation (23). Animal protocols were approved by the Animal Use committees of the Wistar Institute and the University of Pennsylvania in compliance with the Guide for the Care and Use of Laboratory Animals (NIH

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[3] The abbreviations used are: HSV*tk*, herpes simplex thymidine kinase; HSV-1, herpes simplex-1 virus; CNS, central nervous system; ICP, infected cell protein; MOI, multiplicity of infection; PFU, plaque-forming unit; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide.

No. 85-23, revised 1985). Briefly, SCID mice were obtained and housed at the animal facilities of the Wistar Institute. On day 0, animals were injected i.p. with 3×10^7 REN cells in 1 cc of cell culture media. For the tumor burden study, treatment animals were given 5×10^6 PFU of HSV-1716 in culture media by i.p. injection on day 14. Control animals received an equivalent volume of culture media. The animals were examined daily and sacrificed by cervical dislocation on day 28. The amount of tumor burden was assessed using a four-point semiquantitative scale, which accounts for both gross and microscopic disease. Briefly, animals were assessed for tumor in the following four areas: stomach/pancreas, portal region, retroperitoneum/diaphragm, and small bowel mesentery. On gross examination, animals received either a score of 0 if no tumor was present or a score of 1 in each of the four designated areas where gross tumor was seen. If no gross tumor was visible, H&E-stained paraffin-embedded sections of each organ from the designated area were examined in a blinded fashion by an anatomic pathologist. The sections were scored as either 0 for no microscopic tumor or 0.5 if microscopic tumor was present. Thus, the tumor scores ranged from 0 to 4.0. Organs including brain, heart, lungs, liver, stomach, pancreas, kidney, adrenals, spleen, gonads, small bowel, and diaphragm were obtained from each animal. Each organ was divided into thirds with equal samples designated for frozen section, formalin fixation, and DNA extraction.

For the initial survival study, animals were injected i.p. with 3×10^7 REN cells in 1 ml of cell culture media (day 0). On day 7, one animal was sacrificed for gross tumor confirmation, and the remaining animals were randomized to the treatment group and the control group. Treatment animals received 5×10^6 PFU of HSV-1716 by i.p. injection; control animals received an equal volume of culture media. The animals were checked daily and followed for survival. The same protocol was followed for the dose-response study, except the animals were randomized into a control group, a high dose group (5×10^6 PFU HSV-1716), a middle dose group (5×10^5 PFU HSV-1716), and a low dose group (5×10^4 PFU HSV-1716). A similar protocol was followed to evaluate the early deaths observed in the survival studies, except that the tumor-bearing animals were sacrificed 3 days after viral administration. Blood samples were obtained, pooled, and evaluated for serum creatinine and serum uric acid level. Serum chemistry analysis was performed in the Pepper Clinical Laboratory at the University of Pennsylvania Medical Center (Philadelphia, PA).

Histology and Immunohistochemistry. As reported previously, the REN cell line was isolated from a patient with the appropriate clinical and pathological features of malignant mesothelioma, including morphological and immunohistochemical staining pattern (CEA, LeuM1, and mucicarmine antibody negative; Ref. 23). To further assure the nonneuronal origin of these cells for the purposes of this study, REN-derived tumors were stained immunohistochemically with an antibody called TA-51 (a gift of J. Q. Trojanowski, University of Pennsylvania, Philadelphia, PA), which recognizes neurofilament H, a specific marker for neuronal lineage (25). REN cells were TA-51 negative (data not shown). Immunohistochemical staining for HSV antigens was performed on paraffin sections of tissues fixed in 10% phosphate-buffered formalin, using a commercially available rabbit anti-HSV polyclonal antibody (DAKO, Carpinteria, CA).

***In Vivo* Dissemination and Restriction Studies.** To look for dissemination of HSV-1716, we performed PCR looking for the HSV*tk* gene in the collected tissues. Genomic DNA was obtained by standard phenol/chloroform extraction and amplified by PCR. The PCR primers (sense ATGGCTTTTCGTACCCCTGCCAT and antisense GGTATCGCGCGGGGGGGTA) were designed to span a region of the HSV*tk* gene generating a 536-bp fragment. The genomic DNA extracted from each tissue sample was subjected to 35 cycles of PCR using the *tk* primers. The *tk* plasmid and DNA extracted from brain tissues of an animals infected with wild-type HSV-17+ were used as positive controls for the PCR reaction. PCR products were run on ethidium bromide containing 1.5% agarose gels, and the positives produced the expected 536-bp fragment. The gels were then overnight blotted onto Zeta-Probe GT blotting membranes (Bio-Rad Laboratories, Hercules, CA). The membrane was UV cross-linked and probed using a ^{32}P-labeled portion of the HSV*tk* plasmid.

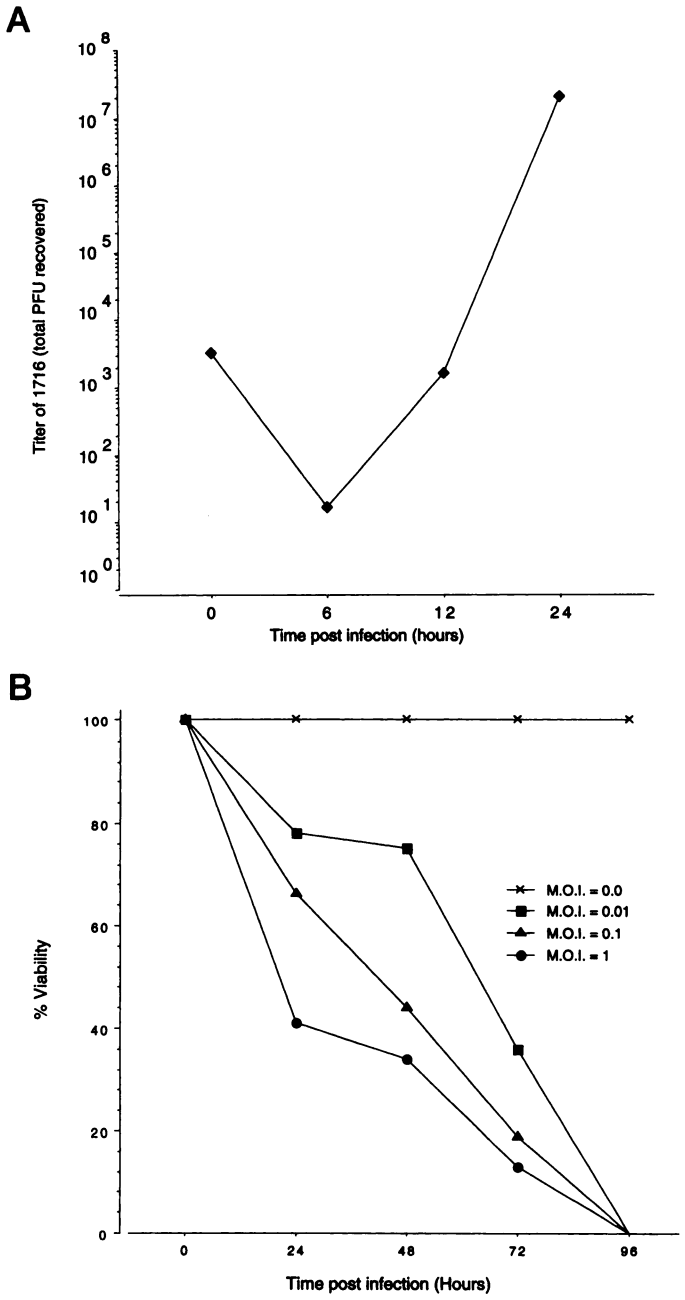

Fig. 1. *A*, HSV-1716 single-step viral growth curve on human malignant mesothelioma cells. Inoculum at time 0 was 5000 PFU of virus (MOI = 0.01). Note that at 24 h the amount of virus present had increased by four logs over the initial input inoculum. *B*, MTT assay for human malignant mesothelioma cell viability as a function of time and varying MOI. The percentage of control survival is the ratio of mean MTT activity in infected cells ($n = 6$ wells at each time point) to the mean activity in matched uninfected cells ($n = 6$ wells at each time point).

RESULTS

HSV-1716 Efficiently Replicates in a Human Malignant Mesothelioma Cell Line and Lyses the Cells *in Vitro*. To determine the ability of HSV-1716 to replicate within a non-CNS human tumor cell line *in vitro*, we performed a single-step viral growth curve in REN cells. As shown in Fig. 1*A*, REN cells supported efficient growth of 1716. Immediately after infection (time 0), 70% of the input viral inoculum was recovered. By 6 h, the number of recovered active viral particles fell by a factor of 200, as expected due to viral uptake and disassembly in preparation for viral replication. However, at 24 h, a

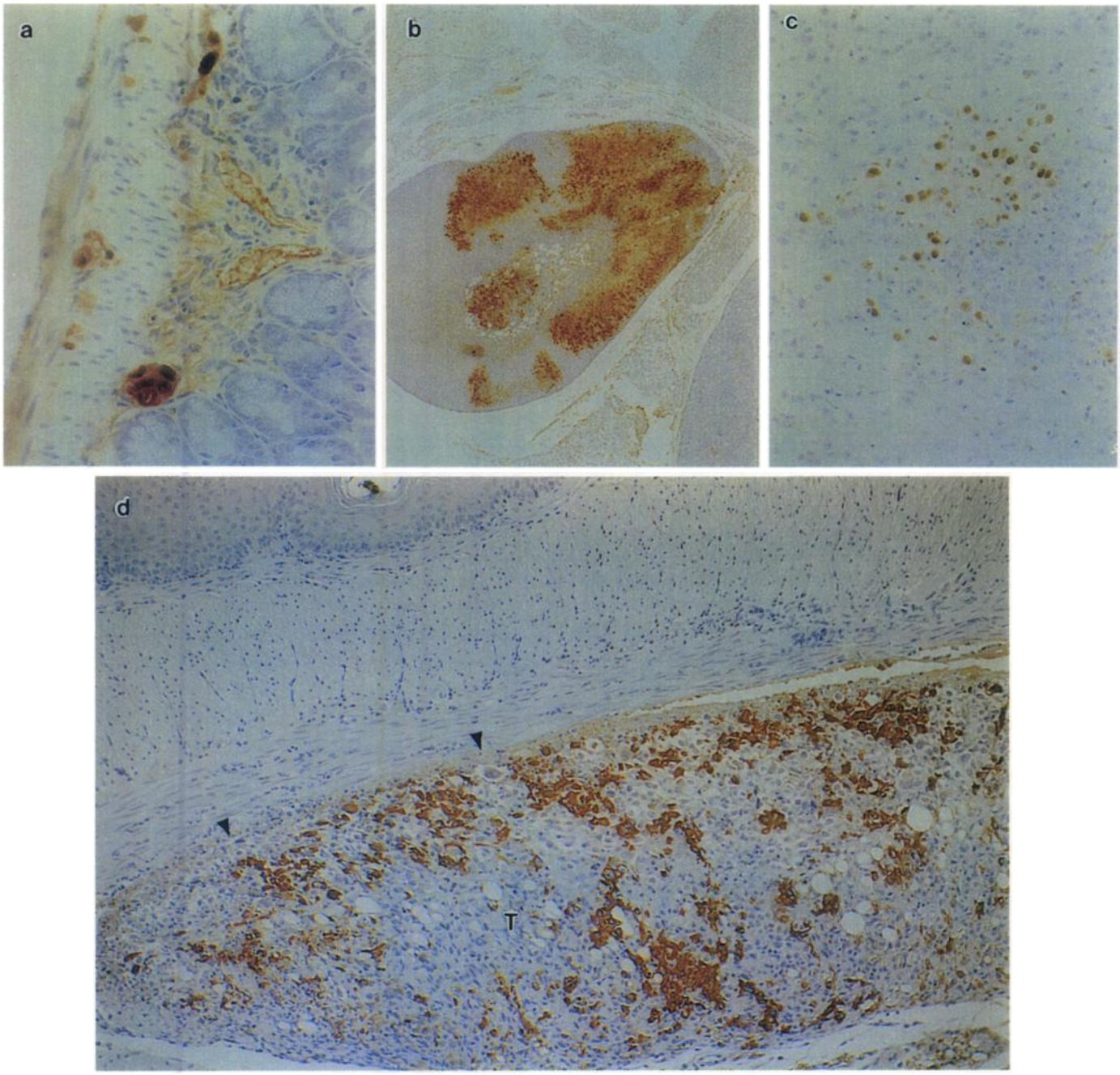

Fig. 2. Immunohistochemistry for HSV viral proteins in SCID mice. Micrographs 3–5 days after i.p. injection of wild-type virus HSV-17+. Positive immunostaining (*brown cells*) is seen in the normal tissues, including ganglia of the myenteric plexus (*a*), adrenal gland (*b*), and brain (*c*). In contrast, *d* shows a tumor-bearing animal 72 h after receiving 5×10^6 PFU of HSV-1716 by i.p. injection. Note the marked positivity of tumor cells with the lack of infection in the surrounding normal tissues (×200, *arrowheads* mark the interface between tumor (*T*) and normal tissues).

4-log increase over the initial inoculum was observed demonstrating highly efficient replication of HSV-1716 on REN cells.

To demonstrate the ability of HSV-1716 to directly kill REN cells, we performed an *in vitro* cell viability assay at various times after viral infection. As shown in Fig. 1*B*, HSV-1716 efficiently killed target REN cells in a time- and dose-responsive fashion. At 96 h postinfection, there were no viable tumor cells remaining in cultures infected at MOIs ranging from 0.01 to 1.0. Similar results were obtained for several other nonneuronal cell lines tested in this viability assay, such as the human osteosarcoma line SAOS, the human lung cancer line H358, and the human melanoma cell line WM 451 Lu (data not shown). However, two other nonneuronal cell lines examined, the human lung cancer lines A549 and H322, were lysed poorly by both HSV-1716 and wild-type HSV-17+ (data not shown).

In Contrast to Wild-Type HSV-17+, HSV-1716 Infection and Replication Is Restricted to Tumor Cells in an *in Vivo* SCID Mouse Model of Human Mesothelioma. As expected, i.p. injection of SCID mice with 5×10^6 PFU of wild-type HSV-17+ led to rapid spread of the virus, signs of encephalitis (*i.e.*, ataxia and hind limb paralysis), and death of all animals by 7 days. To determine the extent of HSV infection, organs from animals sacrificed 3–5 days after wild-type HSV-HSV-17+ injection were analyzed immunohistochemically with a polyclonal antibody recognizing HSV antigens. Reactive cells were clearly seen in the myenteric ganglia of the small

intestine (Fig. 2*A*), adrenal glands (Fig. 2*B*), and brain (Fig. 2*C*). In contrast to wild-type HSV-HSV-17$^+$, SCID mice (n = 5) injected with 5 × 10^6 PFU of HSV-1716 remained alive at 100 days without signs of encephalitis. Immunohistochemistry for HSV antigens was negative at days 3, 8, and 14 in all tissues analyzed. Specifically, liver, kidney, adrenal, spleen, small bowel myenteric plexuses, and brain were negative for HSV antigen.

To assess the ability of HSV-1716 to infect and replicate within human tumors *in vivo*, SCID mice were injected i.p. with 30 million human REN cells. After 14 days, diffuse macroscopic 5–8-mm tumor nodules were present. At this time, 5 × 10^6 PFU of HSV-1716 were instilled into the peritoneal cavity, and the animals were sacrificed 72 h later. Microscopic examination revealed that virtually all tumor nodules showed necrosis, multinucleated cells, and nuclear inclusions consistent with active herpetic infection. Infiltration with mononuclear inflammatory cells was also present. In contrast, no viral cytopathic changes were seen in normal tissues. To directly detect HSV infection, tumor tissue and organs were stained with an anti-HSV polyclonal antibody. Fig. 2*D* shows a representative tumor nodule growing on the inferior aspect of a SCID mouse stomach. Three days after HSV-1716 administration, a large percentage of the tumor cells stained positively for HSV antigens, whereas surrounding normal tissues, as well as all other normal tissues examined, showed no positive staining. Specifically, liver, kidney, adrenal, spleen, small bowel myenteric plexuses, and brain were negative for HSV antigen. Similar results were obtained at days 5, 7, and 14 after infection; however, the number of virally infected tumor cells appeared to decrease at the later time points, possibly due to a decrease in available tumor substrate.

To more sensitively monitor the dissemination of HSV-1716 in tumor-bearing animals, we used the PCR followed by Southern blotting to detect the HSV*tk* gene. This assay can detect HSV DNA at a level of sensitivity equivalent to one genome copy per 100,000 cells.[4] Fig. 3*A* shows the results from a tumor-bearing mouse 3 days after i.p. installation of 5 × 10^6 PFU of HSV-1716. HSV DNA was detected in the tumor nodule (Fig. 3*A*, *Lane 6*) at this time; however, no viral DNA was detected in brain, spinal cord, adrenal gland, lung, or liver, confirming the immunohistochemistry results. In contrast, wild-type HSV led to detectable HSV*tk* sequences in the brain (Fig. 3*B*, *Lane 1*). Fig. 3*B* shows the results from two tumor-bearing animals 14 days after i.p. injection of HSV-1716. Again, no viral DNA was detected in any of the normal tissues assayed.

HSV-1716 Reduces i.p. Tumor Burden and Increases Survival in a SCID Mouse Model of Human Mesothelioma. To determine the ability of HSV-1716 infection to eradicate established tumor, animals that had previously received 30 million i.p. REN tumor cells were given 5 × 10^6 PFU of HSV-1716 by i.p. injection. At the time of viral administration, animals had established i.p. tumors consisting of multiple 5–8-mm nodules with portal invasion and gall bladder distention. Two weeks later, animals were sacrificed, and the tumor burden was assessed using a previously developed semiquantitative scale that accounts for both gross and microscopic tumor (23). The tumor score ranges from 0 (no gross or microscopic tumor) to a maximum score of 4.0. There was a significant reduction in the mean tumor score at day 28 in tumor-bearing animals (n = 12) treated with HSV-1716 compared with the mean tumor score in control animals (n = 8). The mean tumor score in the treatment group was 1.4 ± 0.2 compared with a mean tumor score in the control group of 3.9 ± 0.1 (P < 0.001). All animals in the control group survived the study period. There was one death in the treatment group that occurred 5 days after viral administration from unknown cause.

To determine whether the decrease in tumor mass conferred a survival advantage to SCID mice bearing established i.p. REN tumors, a group of tumor-bearing animals were injected with 5 × 10^6 PFU of HSV-1716 2 weeks after i.p. injection of tumor cells. The animals were followed for survival. The median survival was increased from 47 days in the control group (n = 9) to 95 days in the treatment group (n = 10). All deaths in the control group were a result of bulky i.p. disease; no external tumor nodules were visible at the initial tumor injection site. It is interesting that deaths in the treatment group occurred at two distinct time points. Three animals died 2–5 days after HSV-1716 administration. There was no evidence of bulky disease at this time. The majority of the remaining treated animals died around day 100 due to bulky malignant disease that extended from large s.c. nodules arising on the anterior abdominal wall. At 102 days, the three remaining treatment animals were sacrificed and necropsied. These animals also had nodules corresponding to the site of the initial tumor injection, and tumor appeared to be growing inward from the anterior abdominal injection site with invasion into the peritoneal cavity.

A second survival study was performed to determine the viral dose response. Tumor-bearing animals were randomized to control (n = 11) and treatment groups (low dose: 5 × 10^4 PFU HSV-1716, n = 10; middle dose: 5 × 10^5 PFU HSV-1716, n = 10; high dose: 5 × 10^6 PFU HSV-1716, n = 10). As shown in Fig. 4, treatment with high dose HSV-1716 significantly improved survival when compared with control animals (P = 0.0011 by Mantel-Cox log-rank test). Animals in the high dose group had a mean survival of 102 days; again, surviving animals developed s.c. tumor nodules on the anterior abdominal wall corresponding to the initial tumor injection site by day 100. Six of these animals subsequently died from bulky tumor extending from the anterior abdominal wall injection site into the peritoneal cavity. The low and middle dose treatment animals also demonstrated a significant improvement in survival when compared with the control animals (P = 0.0003 for control *versus* middle dose and P = 0.0019 for control *versus* low dose by Mantel-Cox log-rank test). There was no difference in survival between the low and middle dose animals (P = 0.65).

In both of the survival studies, approximately 20% of tumor-bearing animals died suddenly 2–5 days after HSV-1716 administration. Because of autolysis, only limited autopsies could be performed, and the cause of these early deaths remains unknown. These animals did not display signs of encephalitis (*i.e.*, no ruffled fur, no paralysis, and no ataxia) before their deaths. Immunohistochemistry and PCR data (see above) ruled out widespread viral dissemination. To study the possibility that early deaths in treated tumor-bearing animals were due to acute renal failure after massive tumor lysis, serum creatinine and uric acid were also studied. The serum creatinine level in a pooled specimen from four tumor-bearing animals 3 days after HSV-1716 administration was 0.2 mg/dl, and the uric acid level was 4.0 mg/dl. The serum creatinine level in matched untreated tumor-bearing mice was 0.3 mg/dl, and the uric acid level was 3.9 mg/dl.

DISCUSSION

In this report, we demonstrate that the mutant "replication-restricted" HSV-1716 (an ICP 34.5 null mutant) can reduce tumor burden and significantly prolong survival in an animal model of localized non-CNS human malignancy. Furthermore, we show that after i.p. injection of HSV-1716 into SCID mice bearing human tumors, replication is restricted to the tumor and does not disseminate or replicate outside the tumor. Although HSV is normally a neurotropic virus, these findings highlight the tumor-killing potential of HSV-1716 for

[4] K. Amin, unpublished data.

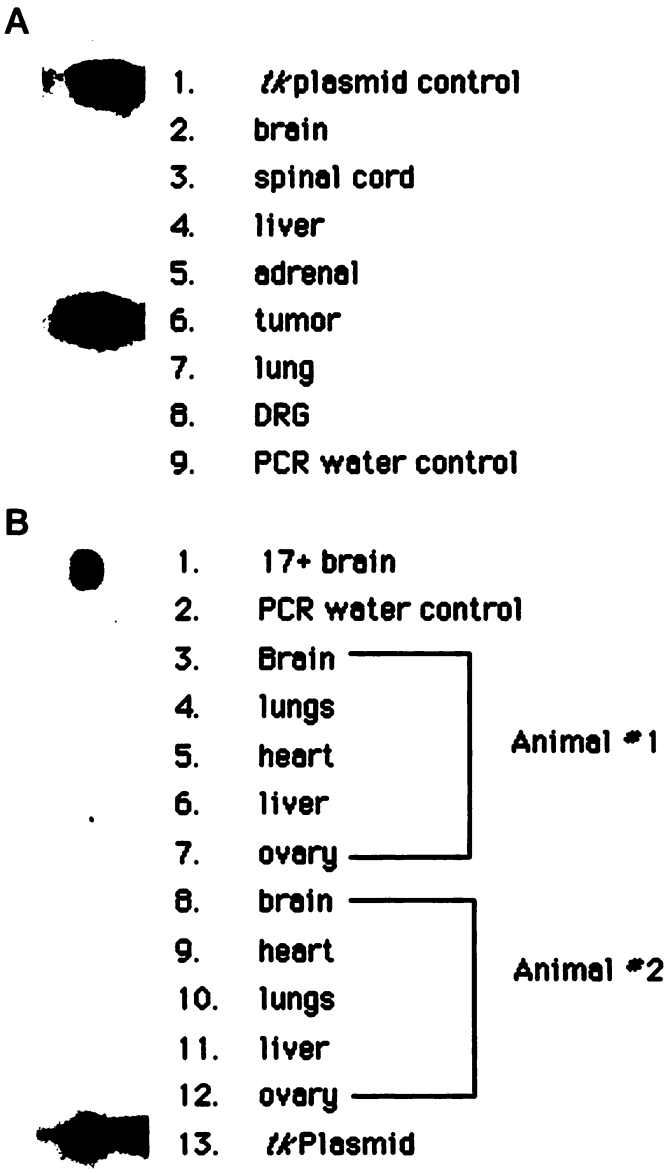

Fig. 3. *A*, PCR using primers for the HSV*tk* gene were performed on a control plasmid containing the HSV*tk* gene (*Lane 1*) and from DNA extracted from tissues in a tumor-bearing animal 3 days after i.p. HSV-1716 virus administration (*Lanes 2–8*). The Southern blot demonstrates HSV*tk* signal in the tumor nodule and lack of signal in normal tissues. DRG, dorsal root ganglion. *B*, PCR using primers for the HSV*tk* gene were performed from DNA extracted from the brain of an animal 5 days after i.p. injection of wild-type HSV-17 (*Lane 1*), from tissues of tumor-bearing animals 14 days after i.p. virus administration (*Lanes 3–12*), and on a control plasmid containing the HSV*tk* gene (*Lane 13*). The Southern blot demonstrates the presence of viral HSV*tk* in the brain of the animal infected with wild-type virus. In contrast, there is no HSV DNA detected in tissues from animals treated with HSV-1716.

localized malignancies of nonneuronal origin such as malignant mesothelioma. In addition, we found that a variety of nonneuronal tumor cell types are susceptible to HSV lysis *in vitro*, but that susceptibility is not universal. Recently, we have shown that some cell lines derived from tumors of the CNS can also be essentially resistant to lytic infection by wild-type HSV and/or ICP 34.5 null mutants (26). We are presently screening a large number of tumors of both neuronal and nonneuronal lineage with the goal of better defining the cellular factors that restrict lytic HSV infection.

The "replication-restriction" and marked attenuation of neurovirulence of HSV-1716 results from deletion of the gene coding for ICP 34.5. The precise mechanisms responsible for these properties are still unknown but are under active investigation in a number of laboratories. The *ICP 34.5* gene encodes a protein of 263 amino acids consisting of a large amino-terminal domain, a linker region of three amino acid repeats (Ala-Thr-Pro, ATP codons), and a carboxyl-terminal domain (27). The carboxyl-terminal domain is homologous to domains of MyD116 and growth arrest and DNA damage gene *34* (GADD 34; Refs. 28 and 29), cellular genes involved in cell cycle regulation and programmed cell death. Recent work suggests that the HSV *ICP 34.5* gene precludes cells from triggering total protein synthesis characteristic of programmed cell death, at least in a neuroblastoma system (30). However, this does not appear to be a universal response, and at this time all that can be said is, that in contrast to normal cells, malignant cells have abnormalities in cell cycle and/or protein synthesis pathways that appear to render them permissive for viruses lacking the *ICP 34.5* gene. We have recently demonstrated in a range of primary human glioblastoma cells, that permissivity for HSV-1716 replication correlates positively with the rapidly dividing nature of the cells (26).

The ultimate clinical utility of replication-restricted viruses such as HSV-1716 will be determined by the balance between efficacy and safety. The experiments presented here, and by others in brain tumor models, indicate that mutant herpes viruses can selectively replicate in human and murine malignant cells to induce the regression of established tumors. Two factors inherent in an HSV-based approach to mesothelioma therapy provide potentially valuable safety features. (*a*) The retention of the HSV*tk* gene in HSV-1716 will allow treatment with the antiviral drugs acyclovir or ganciclovir at any point to abort the infection. (*b*) Administration of virus to a peripheral location, such as the pleural or peritoneal cavity rather than directly into the brain, should increase the margin of safety.

One issue that will likely affect the efficacy of replication-restricted HSV mutants will be the immune response of the host to virus. A critical balance between optimal viral replication leading to tumor destruction and the immune-mediated elimination of viral infection must be achieved. The efficacy of replication-restricted HSV infection in immunocompetent animals with non-CNS malignancy, and even more importantly in immunocompetent HSV-primed animals, will need to be analyzed.

Safety issues, specifically the possibility of viral dissemination

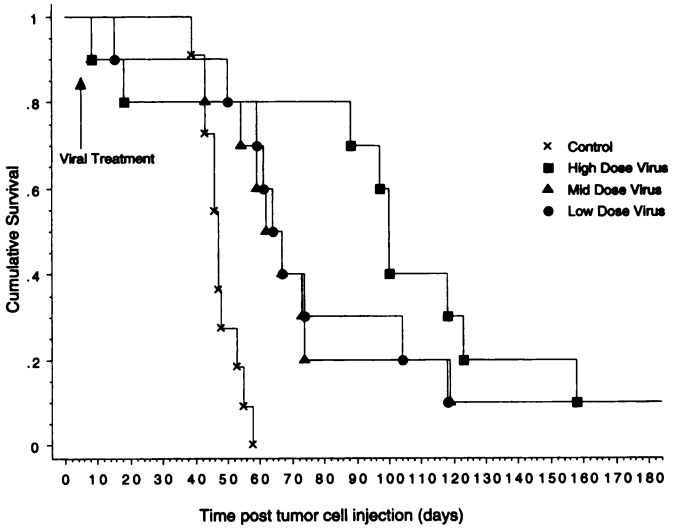

Fig. 4. HSV-1716 viral dose-response survival study. SCID mice received 3×10^7 human malignant mesothelioma cells on day 0. Seven days later, one animal was sacrificed to confirm tumor. The remaining animals were randomized into four groups: control (n = 11, culture media), low dose (n = 10, 5×10^4 PFU HSV-1716), middle dose (n = 10, 5×10^5 PFU HSV-1716), and high dose (n = 10, 5×10^6 PFU-1716).

resulting in CNS infection with or without neural latency, will also determine the ultimate clinical utility of replication-restricted viruses such as HSV-1716. Administration of HSV-1716 to the peritoneal cavity did not result in detectable viral dissemination as evaluated by immunohistochemistry or a sensitive PCR assay. Specifically, no viral proteins or DNA were identified in the spinal cord or brain at 3 or 14 days after treatment. Our inability to detect the presence of HSV-1716 in normal tissues of treated mice suggests that the replication of HSV *ICP 34.5* gene mutants outside the tumor is severely restricted. In work reported elsewhere, we have transplanted human skin onto SCID mice and infected the grafts with HSV-1716, wild-type HSV-17+, or a revertant (HSV-1716R in which the ICP 34.5 deletion has been repaired; Ref. 31). Wild-type HSV-17+ and revertant virus (HSV-1716R) replicated briskly within the human skin and led to ulceration and gross destruction of the grafts. In contrast, replication of HSV-1716 was severely restricted in this normal human tissue homologue.

Approximately 20% of HSV-1716-treated tumor-bearing mice died shortly after viral inoculation. We did not detect dissemination of HSV-1716 by immunohistochemistry or PCR in mice at these time points. Although these deaths are likely due to host-tumor-virus interactions, we were unable to establish their exact etiology. Tumor lysis appears to be an unlikely cause, because the creatinine and uric acid levels in the serum of treated mice did not differ from untreated tumor-bearing controls. Clearly, a more complete understanding of this phenomenon, as well as other potential side effects, will be important before the clinical use of this or other HSVs is attempted.

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