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ARTICLE

Effect of Preexisting Anti-Herpes Immunity on the Efficacy of Herpes Simplex Viral Therapy in a Murine Intraperitoneal Tumor Model

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HSV-1716, a replicating nonneurovirulent herpes simplex virus type 1, has shown efficacy in treating multiple types of human tumors in immunodeficient mice. Since the majority of the human population has been previously exposed to herpes simplex virus, the efficacy of HSV-based oncolytic therapy was investigated in an immunocompetent animal tumor model. EJ-6-2-Bam-6a, a tumor cell line derived from h-ras-transformed murine fibroblast, exhibit a diffuse growth pattern in the peritoneal cavity of BALB/c mice and replicate HSV-1716 to titers observed in human tumors. An established intraperitoneal (ip) tumor model of EJ-6-2-Bam-6a in naive and HSV-immunized mice was used to evaluate the efficacy of single or multiple ip administrations of HSV-1716 (4×10^6 pfu/treatment) or of carrier cells, which are irradiated, *ex vivo* virally infected EJ-6-2-Bam-6a cells that can amplify the viral load *in situ.* All treated groups significantly prolonged survival versus media control with an approximately 40% long-term survival rate (cure) in the multiply treated, HSV-naive animals. Prior immunization of the mice with HSV did not significantly decrease the median survival of the single or multiply treated HSV-1716 or the carrier cell-treated groups. These studies support the development of replication-selective herpes virus mutants for use in localized intraperitoneal malignancies.

Key Words: **HSV; cancer gene therapy; carrier cells; ovarian cancer; mesothelioma; oncolysis.**

INTRODUCTION

Replication-selective herpes simplex viruses that have deletions in the *RL1* gene (HSV-1716 and HSV-R3616), the ribonuclease reductase (ICP6) gene (hrR3), or both genes (G207, MGH-1) were first shown to exhibit efficacy against various brain tumors (1–3). HSV-1716, R3616, hrR3, and G207 have also shown efficacy in animal models against a wide variety of other human tumors, including melanoma (4, 5), malignant mesothelioma (6), colon cancer (7–9), breast cancer (10), ovarian cancer (11, 12), head and neck tumors (13), prostate cancers (14), and lung cancer (15, 16). Viruses with mutations in ICP6, the *RL1* region, or both show 10^3, 10^5, or greater reduced neurovirulence, respectively (17–19) and do not produce encephalitis when inoculated peripherally. Additionally, these mutants replicate as well as their wild-type parental strain in a variety of dividing cells lines but replicate poorly in cells not undergoing mitosis (12, 20).

Our group has focused on the use of replication-selective HSV-1 viruses in the treatment of localized peripheral tumors including malignant mesothelioma (6) and ovarian carcinoma (11, 12). Intraperitoneal injection of HSV-1716 or irradiated "carrier" cells infected with HSV-1716 into immunodeficient animals bearing established human malignant mesothelioma and ovarian carcinoma cells resulted in significant survival increases in treated animals with minimal systemic toxicity.

Although herpes viral gene therapy for malignant disease has shown promise in immunodeficient animal models, a major question is the influence of an intact immune system. Preexisting anti-herpes immunity is known to decrease lesion size with subsequent HSV-1 infection in both humans and mice (21–23). The effect of previously established immunity on the efficacy of herpes-mediated oncolytic therapy and the ability to administer repeated doses of virus remains an unresolved issue.

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Herrlinger *et al.* reported that preexisting HSV immunity markedly decreased the initial HSV-hrR3 uptake in an experimental brain tumor model (24), but Chahlavi *et al.* observed that anti-herpes immunity did not seriously impair efficacy in subcutaneous or brain tumor nodules in their mouse tumor model (25). The role of anti-herpes immunity has been a difficult question to study because oncolytic herpes viruses such as G207, hrR3, and HSV-1716 do not replicate as well in murine tissue and cells lines as human cell lines.

With our interest in mesothelioma and ovarian carcinoma, the first aim of this study was to develop an immunocompetent animal model of localized intraperitoneal malignancy in which we could study the potential therapeutic effects of a replication-selective HSV-1 vector. This was accomplished using an h-*ras*-transformed fibroblast that grows in a diffuse pattern on the various surfaces of the peritoneal cavity in BALB/c mice. The second aim was to evaluate the efficacy of administration of the replication-selective HSV-1 mutant, HSV-1716, in this immunocompetent model to determine the effects of treating a localized intraperitoneal murine tumor. The ability of single or repeated viral treatments to prolong survival was compared in naive and HSV-immunized animals. Our final aim was to evaluate the effect of anti-herpes immunity on the efficacy of treatment of tumors using HSV-1716-infected "carrier cells"—that is, cells that were irradiated, infected with the mutant herpes virus, and injected into the peritoneal cavity (11). We were interested in this delivery system because administering these carrier cells could theoretically protect the viral vector from host neutralizing antibodies.

MATERIALS AND METHODS

Herpes simplex vectors. HSV-1716, which was originally isolated in the laboratory of S. M. Brown (Glasgow, Scotland) (26), and KOS1.1 (27) were passaged for use as previously described (16). HSV-1716 contains a 759-bp deletion in each copy of the *RL1* region of the HSV I strain 17^+ genome (26). The *RL1* region encodes the ICP 34.5 and thus no ICP 34.5 protein expression occurs in HSV-1716-infected cells. KOS1.1 was used to induce anti-HSV immunity in mice, as previously described (28).

Cell lines and culture. EJ-6-2-Bam-6a is a VIH3T3 mouse fibroblast cell line transformed with the h-*ras* oncogene that was derived from a human bladder carcinoma (29) (American Type Culture Collection, Manassas, VA). It was maintained in DMEM with 10% fetal calf serum (FCS, Atlanta Biologicals, Norcross, GA), 100 U/ml penicillin G, 100 μg/ml streptomycin, and 2 mM glutamine (Mediatch, Washington, DC). Cells were harvested for tumor experiments during exponential growth of the cell culture.

In vitro cell viability assays. EJ-6-2-Bam-6a cells were infected with HSV-1716 (diluted in serum-free DMEM) at multiplicity of infection (m.o.i.) values of 0.01. 0.1, 1, and 10 for 4 h. After 4 h, medium containing 10% heat-inactivated FCS was added and plates were incubated at 37°C for 1 h. The cells were trypsinized and transferred to 96-well plates at a density of 2000 cells/well (n = 6 per condition). Viable cell number was assessed by colorimetric assay (CellTiter 96 aqueous nonradioactive MTS cell proliferation assay; Promega, Madison, WI) which measures viable cell dehydrogenase activity by absorbency at 490 nm. Error bars represent standard errors of the mean.

One-step viral growth curves. EJ-6-2-Bam-6a cells (10^6) were plated in T25 flasks plates and infected 24 h later with HSV-1716 at a m.o.i. of 0.01. A sample was harvested at time points of 0, 6, 19, 24, and 48 h by cell scraping and collection of the cell lysate, as previously described (16). The samples were frozen and stored at –80°C. The cell lysates were then thawed and titered by plaque assay on baby hamster kidney cell monolayers, as previously described (16). Burst size was defined by the calculation of fold increase in titer from t = 0 to t = 24 h.

Carrier cells. EJ-6-2-Bam-6a cells were plated in T75 flasks and grown until the cells reached 80% confluency (approximately 1×10^7 cells/flask). After cells were irradiated (3000 rads) in the flask, they were infected with HSV-1716 at a m.o.i. of 2 for 1 h (5 ml serum-free DMEM with rocking), and the medium was replaced with DMEM with 10% FCS. After a 3-h incubation, the cells were harvested with trypsin, counted, and injected intraperitoneally as described below.

Neutralizing antibody assay. Mouse serum was heat inactivated at 56°C for 1 h and then serially diluted (1:25–1:5000) in serum-free DMEM. The diluted serum was added in equal volumes to HSV-1716 (400 pfu/ml) and incubated at 4°C for 20 min. The mixture (100 μl) was then applied to 5×10^4 BHK cells (at 80% confluency) growing in 96-well plates. Viral plaques were measured as described (16) and the neutralizing antibody titer was defined as the highest dilution that yielded a 50% reduction in plaques.

Animals and immunization. Female BALB/c mice (6 weeks old) were obtained from Taconic Laboratory (Germantown, NY) and maintained in the animal facility at the Wistar Institute (Philadelphia, PA). Mice were at least 8 weeks of age before use. In the indicated groups, mice were immunized with a herpes simplex virus type 1, KOS (5×10^5 pfu injected intraperitoneally), approximately 6 weeks prior to tumor injection. 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 No. 85-23, revised 1985) approved all animal protocols.

Intraperitoneal tumor model. Intraperitoneal tumor was established in BALB/c mice by intraperitoneal (ip) injection of 1×10^5 EJ-6-2-Bam-6a cells in 0.5 ml of serum-free DMEM. At 1 week, when macroscopic tumor was identified on the bowel mesentery in four of four sacrificed mice, the mice were randomly divided into groups (n = 10/group) and injected ip with one of the following treatments: medium (500 μl) alone once or three times (every third day), HSV-1716 (4×10^6 pfu/500 μl medium) once or HSV-1716 three times (every third day), HSV-1716-infected carrier cells (4×10^6 cells/500 μl DMEM) once or three times (every third day), or uninfected, irradiated EJ-6-2-Bam-6a cells (4×10^6 cells/500 μl DMEM) given three times (every third day). For the experiments in immunized mice, the presence of neutralizing antibodies (1:2000 ± 200) was confirmed, and mice were then injected with tumor. The presence of tumor was subsequently confirmed as described above and the mice were then randomized for treatment. The naive groups were age-matched. Survival curves are plotted using Kaplan–Meier analysis.

Statistical analysis. All *in vitro* cell viability data are expressed as means ± standard error of the mean. Survival studies were analyzed using the Mantel–Cox log-rank test.

RESULTS

HSV-1716 Replicates in EJ-6-2-Bam-6a Cells

To study the effect of an intact immune system and anti-herpes immunity on the efficacy of HSV-1716, a murine tumor cell line capable of growth within the peritoneal cavity and with the ability to support viral replication to the same extent as in human cell lines was sought. Human cell lines such as the mesothelioma cell line, REN, supports HSV-1716 viral replication with a burst size of 6600 (6). Some murine cell lines such as

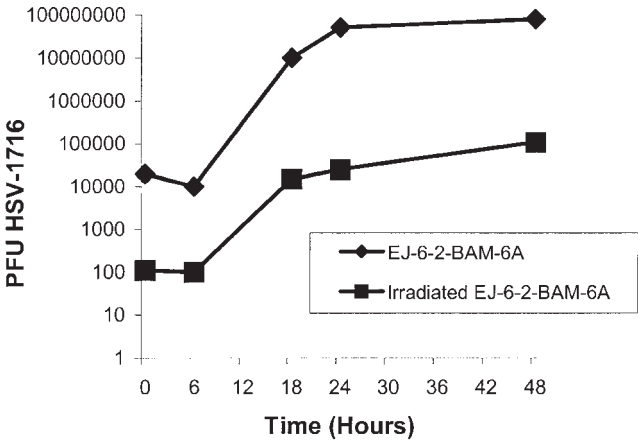

FIG. 1. One-step viral growth curves of HSV-1716 on EJ-6-2-Bam-6a Cells. EJ-6-2-Bam-6a cells and irradiated EJ-6-2-Bam-6a cells (3000 rads) were infected with HSV-1716 at a m.o.i. of 0.01. A sample was harvested at time points of 0, 6, 19, 24, and 48 h by cell scraping and collection of the cell lysate. The cell lysates were titered by plaque assay on baby hamster kidney cell monolayers. Burst size was defined by the calculation of fold-increase in titer from $t = 0$ to $t = 24$ h.

CT-26 have a burst size of 2–4; others such as Lewis lung exhibit a burst size of 20 (15). Although the NIH3T3 fibroblast line was susceptible to HSV, but not tumorigenic, the EJ-6-2-Bam-6a cell line, an h-*ras*-transformed fibroblast line of the NIH3T3 line, is indeed tumorigenic (29). A one-step growth curve was performed to determine the extent of HSV-1716 replication in both nonirradiated and irradiated EJ-6-2-Bam-6a cells (Fig. 1), since carrier cells must be rendered incapable of tumor formation (e.g., by radiation) for use in the clinical setting. It should be noted that inoculation of EJ-6-2-bam-6a cells (10^5) that had received 3000 rads did not induce tumors in five of five mice after intraperitoneal injection nor in five of five mice after subcutaneous bilateral flank injection ($n = 10$ flanks). The mice were euthanized 8 weeks after the initial tumor cell inoculum and necropsy revealed no evidence of tumors. The burst sizes for EJ-6-2-Bam-6a and for irradiated EJ-6-2-Bam-6a cells were 2210 ± 120 and 350 ± 20. Thus, these data indicate that EJ-6-2-Bam-6a can support HSV-1716 replication to a similar extent as many human cell lines.

HSV-1716 Is Oncolytic in the EJ-6-2-Bam-6a Cell Line

The *in vitro* oncolytic effect of HSV-1716 on EJ-6-2-Bam-6a was assessed at various m.o.i. values ranging from 0.01 to 10. Cell viability was determined after 6 days using a cell proliferation assay. As demonstrated in Fig. 2, EJ-6-2-Bam-6a cells were quite sensitive to the lytic effects of the virus. Cells exposed to m.o.i. values as low as 0.01 showed a greater than 75% cell death versus control. Cells infected at m.o.i. values of 1 or higher had a greater than 90% cell death versus control.

Efficacy of HSV-1716 Therapy in Mice with Established Intraperitoneal Tumor

The antitumor effects of HSV-1716 therapy were evaluated in survival studies in an immunocompetent, intraperitoneal tumor model. Injection of 1×10^5 EJ-6-2-Bam-6a cells into mice led to reproducible, diffuse intraperitoneal tumor nodules located on the small bowel mesentery, hepatic hilum, and surface of the diaphragm. Lesions less than 1 mm in size were identified 7 days post tumor inoculation. As the tumor burden increases, the abdomen becomes distended in the absence of ascites. Necropsy reveals multiple (>100) nodules in the abdomen measuring from 1 to 5 mm in size. These nodules are observed throughout the abdomen growing diffusely on the small bowel mesentery, omentum, and hepatic hilum with the greatest tumor burden in the small bowel mesentery (data not shown). Death of the animals was often due to intestinal obstruction secondary to tumor burden. Median survival was 4 weeks with virtually all animals dying by 50 days (Fig. 3). Since multiple administrations of HSV-based oncolytic viruses have improved efficacy in several models (14; Blank *et al.* and Abbas *et al.*, unpublished data), the efficacy of three treatments was also assessed.

After macroscopic tumor nodules were established and observed in four of four animals, groups of animals ($n = 10$) were injected ip with either vehicle or one dose of virus (4×10^6 pfu) or three doses of virus (4×10^6 pfu) at 3-day intervals. As shown in Fig. 3A, a single HSV-1716 administration significantly improved survival versus control ($P = 0.0002$, log-rank), although no long-term survival was observed. Three doses of HSV-1716 significantly prolonged survival over control ($P < 0.0001$, log-rank) and over a single injection ($P = 0.0179$, log-rank). Importantly, there was a 50% long-term survival rate (cure) in the multiply treated animals.

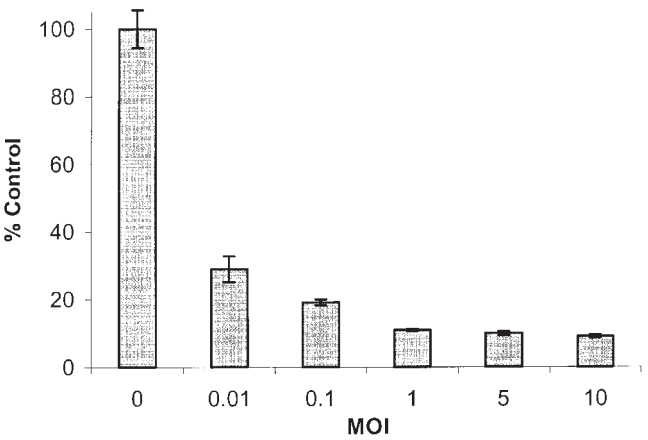

FIG. 2. *In vitro* oncolytic effect of HSV-1716 on EJ-62-Bam-6a cells. Subconfluent monolayers of cells were infected at varying m.o.i. values from 0.01 to 10 on day 0. Cell survival on day 6 was accessed via cell viability assay (see Materials and Methods). Error bars represent standard errors of the mean of three independent experiments.

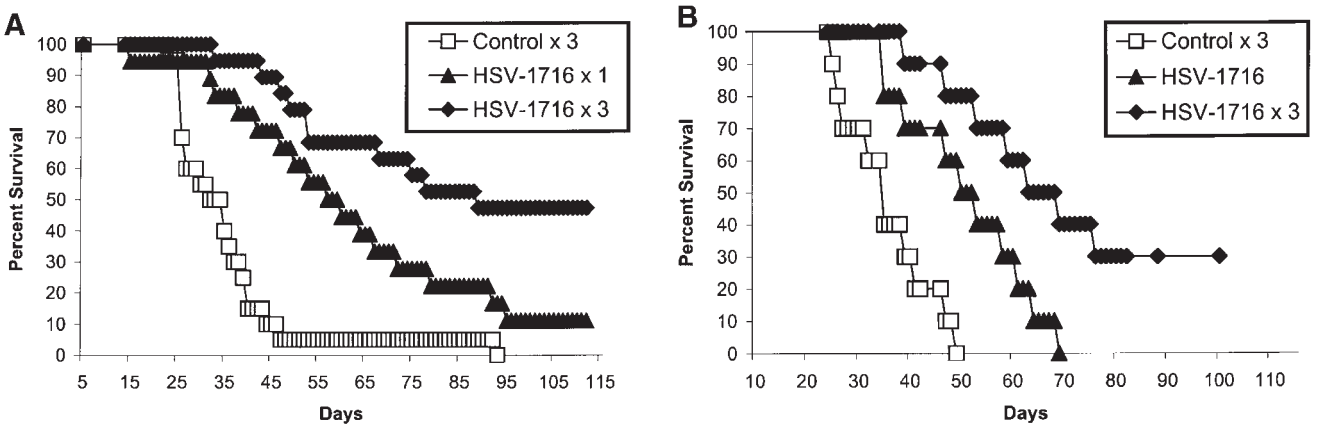

FIG. 3. Kaplan–Meier survival analysis of efficacy of HSV1716 treatment in an intraperitoneal tumor model in both naive (A) and immunized (B) BALB/c mice. Intraperitoneal tumor was established in an immunocompetent (BALB/c) mouse by intraperitoneal (ip) injection of 1×10^5 EJ-6-2-Bam-6a. Macroscopic tumor was identified in all mice observed ($n = 4$) and experimental mice were randomly divided into groups ($n = 10$/group) and injected intraperitoneally with medium alone once or medium alone three times (every third day) and HSV-1716 (4×10^6 pfu/500 μl medium) once or HSV-1716 three times (every third day). (B) In addition, a group of mice were immunized with a herpes simplex type 1 virus, KOS (5×10^5 pfu injected intraperitoneally), 6 weeks prior to tumor injection. After confirmation of the presence of neutralizing antibodies (1:2000 ± 200), mice were injected with tumor cells, tumor nodules were established, and then mice were randomized for treatment as in the naive experiments. Mice were followed daily for survival, and the data were analyzed using the Mantel–Cox log-rank test.

Impact of Preexisting HSV Immunity

Preexisting anti-herpes immunity is prevalent in the human population and may influence the efficiency of infection of our viral therapy (24). Thus, the efficacy of HSV-1716 therapy was also determined in mice that had been previously immunized with the wild-type herpes simplex virus 1 strain, KOS (5×10^5 pfu), intraperitoneally. Anti-HSV immunity was assessed by the induction of neutralizing antibody (NAb) as a function of time. As expected, the serum of control mice ($n = 4$) that had not been inoculated did not neutralize HSV-1716 at any dilution. Anti-HSV NAbs were detectable in all mice ($n = 5$) at a dilution of 1:2000 ± 200 by day 35 postimmunization. Intraperitoneal tumor was established in these immunized animals and age-matched naive mice. The mice were randomized and groups of animals ($n = 10$) were injected ip with vehicle (medium), one dose of HSV-1716 (4×10^6 pfu), or three doses of HSV-1716 (4×10^6 pfu) at 3-day intervals. The immunized, tumor-bearing mice treated with medium had virtually identical median survival times compared to the naive mice (Fig. 3B vs Fig. 3A). Median survival was 32 days with all control animals dead by 50 days.

A single HSV-1716 treatment of tumor-bearing, immunized mice significantly improved survival versus control ($P = 0.0021$, log-rank) with no long-term survival. There was no significant difference in the efficacy of a single HSV-1716 treatment of established ip tumor between naive and immunized mice ($P = 0.1$). Multiple HSV-1716 treatments significantly prolonged survival over control ($P < 0.0001$, log-rank) and over a single treatment ($P = 0.0228$, log-rank). Again, a significant number of animals (30%) in the multiply treated group were long-term survivors compared to none of the singly treated animals. The efficacy of three administrations of HSV-1716 was not significantly different in naive and immunized mice ($P = 0.2$).

These studies indicate that both single and multiple injections of HSV-1716 have significant antitumor activity in an immunocompetent mouse model of peritoneal tumor, even after immunization against HSV. Multiple injections of virus, even in immunized animals, showed significantly greater efficacy and led to long-term survival in 30–50% of animals.

Viral Delivery via Carrier Cells

HSV infection can spread via release of virus and subsequent uptake of virus in adjacent cells and it can also spread via cell-to-cell contact (30). We have previously shown that HSV-1716-infected PA-1 carrier cells can preferentially bind to epithelial ovarian cancer cells and significantly prolong survival in a xenogeneic EOC tumor model (11). It was likely that our EJ-6-2-Bam-6a carrier cells bound to tumor cells at least as well as to peritoneal mesothelial surfaces. Since anti-HSV neutralizing antibody does not inhibit cell-to-cell spread of virus (31), we hypothesized that anti-HSV NAb will not decrease the efficacy of our carrier cell delivery system. To investigate the utility of our carrier cell viral delivery system, survival studies were performed in naive and HSV-1-immunized mice bearing tumors. After macroscopic tumor nodules were identified 7 days post tumor cell inoculation, groups of animals ($n = 10$) were injected ip with one of the following treatments: (i) vehicle; (ii) infected, irradiated carrier cells (4×10^6 cells); (iii) three injections of carrier cells (4×10^6 cells/dose) at 3-day intervals; or (iv) three injections of non-HSV-1716-infected irradiated carrier control cells (4×10^6

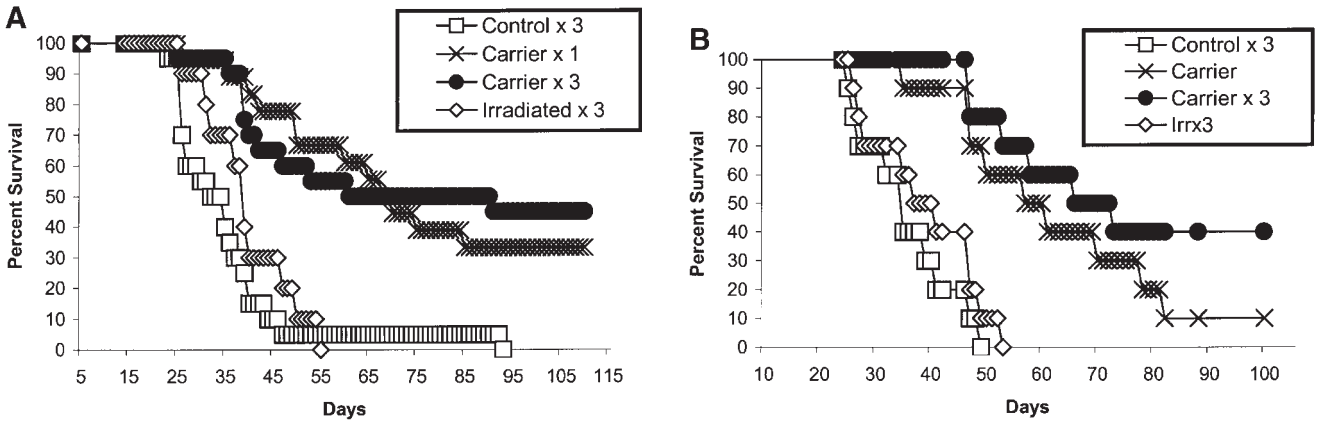

FIG. 4. Kaplan–Meier survival analysis of carrier cell therapy in this intraperitoneal tumor model in both naive (A) and immunized (B) BALB/c mice. As with the HSV-1716-treated mice, tumor was established by intraperitoneal (ip) injection of 1×10^5 EJ-6-2-Bam-6a cells. Macroscopic tumor was identified at 1 week, and remaining mice were randomly divided into groups (n = 10/group) and injected intraperitoneally with medium alone, three times (every third day), carrier cells (4×10^6 cells/500 μl medium) once, or carrier cells three times (every third day) or irradiated EJ-6-2-Bam-6a cells only given three times (every third day). In addition, a group of mice were immunized with KOS, a herpes simplex virus type 1 strain (5×10^5 pfu injected intraperitoneally), 6 weeks prior to tumor injection (10^5 EJ-6-2-Bam-6a). Tumor inoculation and treatment schedules were the same as described for the groups of naive mice. Mice were followed daily for survival that was analyzed using the Mantel–Cox log-rank test.

cells/dose). Treatment with noninfected, irradiated control cells did not significantly improve survival over the medium control, suggesting that the irradiated tumor cells were not very immunogenic. In naive animals (Fig. 4A), both single and repeat intraperitoneal injections of carrier cells improved median survival over control ($P < 0.0001$, log-rank) and both treatments led to 40–50% long-term survivors. Unlike the viral injections, repeat injections of carrier cells provided no further survival advantage versus a single treatment ($P = 0.7401$, log-rank). In naive animals, carrier cells provided a comparable survival advantage versus injection of virus alone after one injection ($P = 0.1544$, log-rank) or three injections ($P = 0.5998$, log-rank).

In immunized animals (Fig. 4B), a survival advantage was also seen in groups that received single ($P = 0.0013$, log-rank) or three treatments of HSV-1716-infected carrier cells ($P < 0.0001$, log-rank) versus control. Although repeat injections did not provide significant prolongation in median survival versus single administration ($P = 0.2005$, log-rank), there were more long-term survivors. In immunized animals, a single administration of HSV-infected carrier cells did not significantly improve survival versus a single injection of virus ($P = 0.0876$, log-rank). There was no significant difference in survival between naive or immunized groups receiving one dose of carrier cells ($P = 0.24$, log-rank) or three doses of carrier cells ($P = 0.84$, log-rank). Similarly, comparable survival was observed in immunized animals that received three treatments of virus or HSV-1716-infected carrier cells ($P = 0.7167$, log-rank).

Taken together, these experiments show that administration of HSV-1716 using carrier cells resulted in comparable antitumor efficacy in the established intraperitoneal tumor model.

DISCUSSION

Replication-selective herpes simplex viruses have demonstrated efficacy and safety in established human tumors in immunodeficient mice. Based on these data, and favorable toxicity profiles in mice (32) and *Aotus* monkeys (18), the HSV mutants G207 and HSV-1716 are being evaluated in clinical trials for the treatment of malignant glioma (33, 34). Patients in clinical trials will likely be HSV-1 seropositive, a potentially confounding factor in this viral-mediated cancer therapy. As in most viral-based cancer gene therapy, the impact of the immune system is an important issue, especially with the use of a replication-restricted virus. Will the oncolytic effect and spread of the vector be limited by preexisting immunity or will an immune-mediated antitumor effect be induced in immunized animals?

In humans, it has been observed that prior immunity is not sufficient to prevent recurrent HSV infections (35, 36). Additionally, Morrison and Knipe showed that viral replication occurs at the site of injection even in the presence of protective immunity (37). Reports on the effect of anti-herpes immunity in tumor-bearing animals treated with replication-selective viruses have been contradictory, partly complicated by the lower susceptibility of rodent cells to HSV-1 infection. Herrlinger *et al.* reported that preexisting herpes simplex immunity led to an 85% reduction in marker gene expression in an intracranial glioma model (D74 cells) in rats using the herpes mutant hrR3 that is defective in ribonuclease reductase (24). They also described treatment studies in which administration of the hrR3 virus to brain tumors in nonimmunized animals led to only a 2-day increase in median survival. These survival findings are consistent with a high degree of resistance of the rodent cells to the herpes

simplex virus. In contrast, Chahlavi *et al.* studied the effects of G207 on the growth of CT26 colon carcinoma cells and N18 neuroblastoma cells growing subcutaneously in the appropriate syngeneic host mice (25). Intratumoral treatment with G207 inhibited tumor growth to a similar extent regardless of whether or not the mice were immunized against herpes (25). Similar to our study, it was observed that multiple tumor injections were more effective in both naive and immunized hosts (25). Additionally, Yoon *et al.* successfully treated mouse colon carcinoma localized to the liver by intravascular hrR3 in both naive and immunized mice (9).

Our studies using the EJ-6-2-Bam-6a *h-ras* transformed murine fibroblast line in an intraperitoneal model are consistent with the findings of Chahlavi *et al.* (25) and Yoon *et al.* (9). In our model of intraperitoneal malignancy, we found that multiple doses of replication-competent HSV-1716 were superior to single doses and that prior herpes immunity did not significantly hinder therapeutic efficacy.

We also studied the efficacy of vector delivery via carrier cells. We originally postulated that carrier cells might be important to evade anti-herpes humoral immunity. However, immune responses did not significantly blunt therapeutic efficacy in our intraperitoneal tumor model. The efficacy of the carrier cells was not significantly different from the efficacy of the virus alone-treated group, in spite of the fact that HSV-1716-infected carrier cells had a burst size of 350. One possible explanation is that the infected carrier cells did not release that level of virus or an alternative possibility is that increased dosages of virus administered did not significantly enhance the efficacy. We believe this latter possibility is less likely since multiple administrations of virus did improve rates of long-term survival. In comparison, in our human xenogeneic model of epithelial ovarian cancer, administration of HSV-1716- and HSV-infected carrier cells significantly prolonged survival of EOC-bearing mice (11). Although modest, administration of carrier cells significantly augmented the survival of mice bearing EOC tumors to which the carrier cells could bind five- to eightfold preferentially. This raises the question as to whether the efficacy of carrier cells would be enhanced by engineering the carrier cells to more specifically target the tumor nodules in comparison to the normal mesothelium.

In addition, engineering of these carrier cells to augment the inflammatory milieu of the tumor and thus increase the potential to mount an antitumor immune response may provide a means to potentially improve efficacy. In support of the feasibility of this strategy, Todo *et al.* provided evidence that an intact immune system enhanced the efficacy of the replication-competent virus G207 treatment and led to antitumor immune responses (38). Furthermore, the importance of the antitumor immune response was elucidated by coadministration of corticosteroids that blunted the immune response to tumor and that decreased efficacy of G207 treatment (39). The antitumor responses could be augmented by coadministration of an IL-12-expressing HSV-1 amplicon (40) or by administration of a HSV-ICP34.5 deletion mutant that expressed IL-12 (41). In addition, Andreansky *et al.* demonstrated improved efficacy of an ICP34.5-deleted Herpes mutant, R3616, in an experimental tumor model of glioma by coexpression of IL-4 but not IL-10 (42). We postulate that carrier cells could be engineered to express one or multiple cytokines, chemokines, or other immunomodulators while still remaining permissive for viral replication, and these engineered carrier cells may augment the induction of an antitumor response and HSV-based oncolytic efficacy.

In summary, we have developed a murine model for intraperitoneal malignancy in an immunocompetent animal that resembles ovarian cancer and mesothelioma and have shown that the replication-competent herpes virus mutant HSV-1716 is highly effective in increasing survival after intraperitoneal injection in both naive and herpes-immunized animals. Multiple injections of virus significantly improved efficacy. In addition, delivery of virus using carrier cells was feasible and had similar efficacy as direct viral delivery. These studies support the development of replication-selective herpes virus mutants for use in intraperitoneal and other non-central nervous system malignancies. Although the effects of preexisting immunity will have to be assessed ultimately in humans, this paper suggests that patients in clinical trials using HSV-based oncolytic therapy should not be excluded because of their prior exposure to HSV.

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