Systemic Cancer Therapy with a Tumor-selective Vaccinia Virus Mutant Lacking Thymidine Kinase and Vaccinia Growth Factor Genes


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ABSTRACT

We have demonstrated previously the oncolytic effects of a systemically delivered, replicating vaccinia virus. To enhance the tumor specificity of this vector, we have developed a combined thymidine kinase-deleted (TK−) and vaccinia growth factor-deleted (VGF−) vaccinia virus and investigated its properties in vitro and in vivo. The gene for enhanced green fluorescent protein (EGFP) was inserted into the TK locus of a VGF− vaccinia virus by homologous recombination creating a double-deleted mutant vaccinia virus (vRDD-GFP). Infection of resting and dividing NIH3T3 cells with vRDD-GFP yielded reduced viral recovery compared with wild-type (WT), TK−, or VGF− viruses from resting cultures but equivalent virus recovery from dividing cultures. Eight days after nude mice were injected i.p. with 107 plaque-forming units (pfu) of WT, TK−, VGF−, or vRDD-GFP vaccinia virus, tissues and tumor were harvested for viral titer determination. No virus was recovered from the brains of mice injected with vRDD-GFP compared with the other viruses, which ranged from 130 to 28,000 pfu/mg protein; however, equivalent amounts were recovered from tumor. There was no toxicity from vRDD-GFP because nude mice receiving 106 pfu of IP vRDD-GFP lived >100 days, whereas mice receiving WT, VGF−, or TK− virus had median survivals of only 6, 17, and 29 days, respectively. Similar results were seen when 105 pfu of vRDD-GFP were given. Nude mice bearing s.c. murine colon adenocarcinoma (MC38) had significant tumor regression after treatment with 105 pfu of systemic (i.p.) vRDD-GFP compared with control (mean tumor size, 180.71 ± 35.26 mm3 versus 2796.79 ± 573.20 mm3 12 days after injection of virus). Our data demonstrate that a TK− and VGF− mutant vaccinia virus is significantly attenuated in resting cells in vitro and demonstrates tumor-specific replication in vivo. It is a promising vector for use in tumor-directed gene therapy, given its enhanced safety profile, tumor selectivity, and the oncolytic effects after systemic delivery.

INTRODUCTION

Successful tumor-directed gene therapy is dependent upon a high percentage of tumor cells expressing a large quantity of gene product after systemic injection of a vector. Transduction efficiencies and levels of gene expression of currently available vectors remain low and limit their potential therapeutic effects, despite modification of these vectors to allow tumor targeting and tumor-specific gene expression (1–4).

Recently, replicating viruses have been explored and offer several advantages. Levels of gene expression are higher, transduction efficiency is improved through replication and infection of surrounding cells, and antitumor effects are seen attributable to virus-mediated cell death (5–7). Currently, viruses such as adenovirus, herpes virus, Newcastle disease virus, and vaccinia virus are being used as replicating vectors (6, 8–12). Vector-associated toxicity is a concern, and various modifications have been explored in an effort to improve their tumor specificity and safety. Previously, herpes virus vectors have been modified for gene therapy in a similar fashion using mutant vectors with inactivation of the thymidine kinase or ribonucleotide reductase genes (10, 13–15). This is the first report of a mutant vaccinia virus with multiple selective mutations to enhance tumor specificity.

Vaccinia virus has been used as a live vaccine in the smallpox eradication program and recently as a vaccine against cancer (16). It has not been widely accepted as a potential tumor-directed gene therapy vector because of concerns regarding the safety of a systematically administered replicating virus. Vaccinia has been modified previously to carry various antigens, cytokines, and immunostimulatory molecules including carcinoembryonic antigen, gp100, MART-1, granulocyte/macrophage-colony stimulating factor, B7-1, IL-1β, IL-2, and IL-12 (17–26). These studies have provided insights into the potential toxicities of systemically delivered replicating vaccinia virus. Although it is generally a safe vector, case reports of generalized vaccinia and vaccinia-associated encephalitis have been described, usually in the immunosuppressed population (27–31). Strategies toward improving the safety of this vector have been described (16, 24, 25, 32–34).

Previously, deletion of either the TK gene or VGF genes was shown to significantly decrease pathogenicity compared with WT virus (32, 33). A TK− virus requires TTP for DNA synthesis from the nucleotide pool present in dividing cells. This leads to preferential viral replication in dividing cells and is the presumed explanation for the observed tumor specificity. We have shown previously that a systemically delivered TK− vaccinia virus expressing the firefly luciferase gene resulted in up to 3 logs higher gene expression in murine tumors compared with normal tissues (7, 35, 36). Further improvement in both tumor specificity and safety of vaccinia is required before its use as a systemic gene therapy vector in humans.

VGF is a secreted protein produced early in viral infection and acts as a mitogen to prime surrounding cells for vaccinia infection (37). Deletion of this growth factor causes decreased viral replication in resting cells and a 1000-fold increase in the LD50 of intracranial vaccinia (33). The combined effect of TK and VGF deletions on tumor specificity should be synergistic. In the absence of TK, viral replication will require TTP from DNA synthesis. Normal stimulation of dividing cells to divide will not occur in the absence of VGF; hence, replication will occur only in actively dividing cells. As well as decreasing pathogenicity, this is expected to maintain or enhance the tumor selectivity reported previously (7, 35, 36). Here we describe a unique tumor selective vaccinia gene therapy vector with deletions of both the TK gene and VGF genes. We examine in vitro replication, in vivo biodistribution, viral pathogenicity, and antitumor effects of this new vector in a mouse model.
Confluent wells of CV1 cells were infected for 2 h at 37 °C with vaccinia virus synthetic early/late promoter (39). It is flanked by portions of the pSEL-EGFP (Fig. 1). This placed the A/Sal sites of our shuttle plasmid pCB023-II (12), creating pSEL-EGFP (Fig. 1A). This placed the EGFP gene under the control of the vaccinia synthetic early/late promoter (39). It is flanked by portions of the vaccinia TK gene, which allows for homologous recombination into this locus. Confluent wells of CV1 cells were infected for 2 h at 37 °C with 10^8 pfu of VSC20 in 1.0 ml of MEM-2.5% FCS. Supernatants were removed, and a liposomal transfection (Superfect; Qiagen, Santa Clarita, CA) of pSEL-EGFP was performed using 0.7 ml of DMEM-10% FCS containing 2 μg of plasmid DNA and 10 μl of liposomes/well at 37 °C for 4 h. The transfection medium was removed and replaced with 3.0 ml of DMEM-10% FCS. After 3 days of incubation, cells were collected and sonicated in MEM-2.5% FCS. Serial dilutions (1.0 ml; 10^-2 to 10^-4 in MEM-2.5% FCS) were used to infect HUTK-143B cells at 37 °C. After 2 h, 2 ml of MEM-10% FCS containing bromodeoxyuridine (final concentration, 25 μg/ml; Sigma Chemical Co., St. Louis MO) were added for selection. After 24–48 h, cells were observed for green fluorescence and viral plaque formation. Six positive plaques were isolated, resuspended in MEM-2.5% FCS, and used to reinfect further HUTK-143B cells. After three to four cycles of selection, all plaques were positive for GFP.

DNA Extraction. Confluent CV1 cells were infected with unpurified recombinant vaccinia virus. After 2–3 days when complete viral cytopathic effect was seen, supernatant was removed, and cells were washed and harvested in 750 μl of PCR buffer [50 mM KCl, 10 mM Tris-Cl, 2.5 mM MgCl2, 0.1 mg/ml gelatin, and 0.45% NP40 (Sigma Chemical Co.), 0.45% Tween 20 (Bio-Rad, Richmond CA)], Proteinase K (Life Technologies, Inc., Rockville MD; 4.5 μl of 10 mg/ml) was added, and DNA was prepared by incubation for 1 h at 55 °C. Proteinase K was inactivated (10 min at 95 °C) prior to PCR, as described previously (40).

PCR. A standard PCR was performed using primers external to the site of recombination (P1, sense 5'-ATCGCATTTTCACTAAGTGTATGATG-3'; P2, antisense 5'-TACCTACGACACAAACATCATT-3'), within the newly recombined segment (P3, EGFP sense 5'-ATGGTGAGCAAGGGCGAGGAGC-3'; P4, antisense 5'-TACATGCTACCCCTGGGACAACC-3') and within the VGF gene (sense 5'-TGACGGGGTCACCCACACTGTGCCCATCTA-3' and antisense 5'-CTAGAAGCTTGGGACTGGGAG-3'). In brief, 25 μl of the digested vaccinia DNA, 1 μl of each primer, 1 μl deoxynucleotide triphosphates (10 mM; Life Technologies, Inc.), 2.5 units Taq polymerase (Promega Corp., Madison WI), and PCR buffer to 50 μl final volume were added. PCR parameters consisted of 15 s denaturing (94 °C), 30 s annealing (55 °C), and 3 min extension (72°C) for 35 cycles (GeneAmp PCR System 9700, Perkin-Elmer, Norwalk CT).

In Vivo Viral Yield. Dividing NIH3T3 cells were infected with 200 pfu/m (multiplicity of infection = 0.0005) of either F13L+, VJS6, VSC20, or vvDD-GFP in 1 ml of MEM-2.5% FCS for 2 h at 37 °C. MEM-10% FCS was added, and cells were incubated until harvesting at 24, 48, and 72 h after infection. To establish resting cultures, confluent NIH3T3 cells were washed with PBS and incubated for 5 days in DMEM with 5% FCS (33). These resting cultures were infected as above and harvested at the same time points after infection. After a single freeze-thaw cycle, virus was quantified by plaque titrating on CV1 cells as described previously (41).

Mice. Female athymic (C57BL/6) and C57BL/6 immunocompetent mice, 6 weeks of age, were obtained from the NIH small animal facility (Frederick, MD). They were housed in standard conditions and given food and water ad libitum. All animal studies were approved by the Animal Care and Use Subcommittee of the Animal Sciences Branch, National Cancer Institute.

In Vivo Viral Pathogenicity. Viral pathogenicity was assessed with tissue histology, in vivo viral replication, and mouse survival. Seven days after i.p. injection of 10^7 pfu of F13L+, VJS6, VSC20, or vvDD-GFP, whole sections of brain, liver, spleen, testes, bone marrow, ovary, and tumor were homogenized in HBSS (Biofluids) and kept at −70 °C until use. Five hundred μl of the homogenate were incubated on CV1 cells at 37 °C in 5% CO2, and titers were determined as described previously. Virus titers were standardized to total protein.

Survival studies were performed on 6-week-old nude or immunocompetent mice. Non-tumor-bearing mice were injected i.p. with 10^6 to 10^7 pfu of F13L+, VJS6, VSC20, or vvDD-GFP in 2 ml of HBSS and followed for survival.

Immunohistochemistry. Five days after i.p. injection of 10^7 pfu of F13L+, VJS6, VSC20, vvDD-GFP, and HBSS control into nude mice, whole sections of brain, liver, spleen, ovarian, and tumor were harvested, fixed in 10%
formalin (Fisher Scientific, Pittsburgh, PA), embedded in paraffin, and stained with H&E stain. Selected tissues were used for vaccinia virus antigen immunohistochemistry (Fig. 3). A rabbit polyclonal anti-vaccinia antibody was used at a dilution of 1:8,000–16,000 with the Vectastain Rabbit Elite kit (Vector Laboratories, Inc., Burlingame, CA). Diaminobenzidine was the chromogen. All results were interpreted by a pathologist (J. M. W.) blind to the virus treatment of the sections.

**Antitumor Effect.** MC38 tumor cells (10⁵) in 100 μl of DMEM were injected s.c. into the right flanks of 6-week-old female athymic (C57BL/6) mice and allowed to grow for 7–10 days. When the tumors reached 75–125 mm³ in volume, 10⁶ pfu of vvDD-GFP or HBSS control were injected i.p. (in 2 ml of HBSS/0.1% BSA; Calbiochem, La Jolla, CA). Tumors were measured by an investigator blind to the treatment of the animals. Tumor volume was calculated as \(\left(\text{width}^2 \times \text{length}\right) / 2\). (42).

**Statistics.** Statistical analysis was performed using the Mann-Whitney test for nonparametric data when appropriate. Tumor volumes between groups were assessed using the ANOVA for repeated measures. Survival analysis was performed using the method of Kaplan-Meier (43), and differences between curves were assessed using the log-rank test (44). All statistics were generated using StatView Software (Abacus Concepts, Inc., Berkeley, CA), and Ps <0.05 were considered significant.

**RESULTS**

**Creation of a TK and Vaccinia Growth Factor Deleted Vaccinia Virus.** A shuttle plasmid containing the gene for EGFP (Clontech, Palo Alto, CA) was created (Fig. 1A). The parental VGF-deleted virus (VSC20) was created previously by the insertion of the lacZ gene into the VGF sites (33). The shuttle plasmid was used to insert EGFP into the TK locus of the VGF-deleted vaccinia virus by homologous recombination as described above, creating the double-deleted virus, vvDD-GFP. PCR using primers designed to amplify the TK gene, VGF gene, and spanning the sites of recombination confirmed the deletion of these genes (Fig. 1B). Staining of vvDD-GFP infected cells with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (ICN Biomedicals, Inc., Aurora, OH) demonstrated the presence of the lacZ gene and fluorescent microscopy confirmed the presence of the EGF gene (data not shown).

**In Vitro Replication of vvDD-GFP.** Synchronous infection of dividing (nonconfluent) NIH3T3 cells with WT, VGF−, TK−, or vvDD-GFP vaccinia yielded similar amounts of virus at various time points after infection (Fig. 2A). However, when resting (confluent) NIH3T3 cells were used, the yield of vvDD-GFP was significantly less than the other viruses at all time points (Fig. 2B). This supports the hypothesis that a vaccinia virus deleted of both TK and VGF would be attenuated in resting cells but show comparable growth to WT virus in dividing cells that have adequate sources of nucleotides for DNA synthesis.

**In Vivo Replication of vvDD-GFP.** Tumor-bearing (s.c. MC38) and non-tumor-bearing nude mice were injected with 10⁵ pfu i.p. of WT, VGF−, TK−, or vvDD-GFP vaccinia. Eight days after injection of virus, samples of brain, liver, spleen, testes, bone marrow, ovary, and tumor were harvested, titered on CV1 cells, and viral yield was calculated per milligram protein (Table 1). vvDD-GFP was recovered at significantly lower titers in brain compared with WT virus and markedly reduced titers in spleen and bone marrow. It was also recovered at lower titers compared with the TK-deleted or VGF-deleted viruses, particularly in the brain. Notably, all four viruses were equally infective in tumor and ovary. Previously, we have shown that although virus was recovered from the bone marrow, no myelosuppression was apparent (7). Patterns of infectivity were the same for the non-tumor-bearing group (data not shown). When this experiment was repeated in immunocompetent mice, similar patterns were seen; however, the peak infectivity occurred earlier, and most organs were negative for all four viruses by day 8 (data not shown).

Histology of these organs, 5 days after injection with virus, supported the viral recovery data (Table 2). Although all sections of brain were found to have a mild vasculitis on H&E staining, this did not necessarily correspond to the degree of viral staining on immunohistochemistry. Immunohistochemistry demonstrated more dramatic differences with less viral antigen in the brain and ovary after vvDD-GFP infection compared with the other viruses, whereas tumor staining was remarkably similar for all four viruses (Fig. 3). In the ovary, viral antigens were found in follicles and in adjacent ovarian stromal tissues. Oviducts were often immunoreactive. Primary tumors had strong immunoreactivity within viable tumor cells. Necrotic areas were less immunoreactive or nonimmunoreactive.

**In Vivo Pathogenicity of vvDD-GFP.** Nude mice were injected with systemic (10⁶ pfu i.p.) WT, TK−, VGF−, or vvDD-GFP vaccinia virus and followed for survival (Fig. 4A). As expected, WT virus was extremely virulent, with nude mice surviving a median of 5 days. Both the VGF− and TK− virus were attenuated compared with the WT virus (median survival in nude mice, 17 and 29, days respectively; \(P < 0.0001\) compared with WT) as expected from previous reports (32, 33). vvDD-GFP was highly attenuated in nude mice, and
all lived >100 days (P < 0.0001). When this experiment was repeated using a 10-fold higher dose (10^9 pfu) of vvDD-GFP, similar results were seen with an insignificant left shift of the curve (Fig. 4B). 10^9 pfu were still significantly less toxic than 10^8 of the TK− virus (P < 0.0001). Interestingly, when this study was repeated in C57BL/6 immunocompetent mice, less toxicity was seen in all groups except the group receiving WT virus, which remained very toxic (Fig. 4C). In experiments using nude mice, the animals receiving 10^9 pfu of vvDD-GFP began to die from viral pathogenicity before 100 days, as shown. This was not seen in the immunocompetent mice. Likely the virus is never eliminated by the immune system in athymic mice.

**Antitumor Effect.** As demonstrated above, vvDD-GFP is able to selectively replicate in tumor tissues. Nude mice bearing s.c. MC38 tumors were injected systemically (i.p.) with 10^7 pfu of vvDD-GFP or HBSS control. A significant antitumor effect was seen in the mice treated with vvDD-GFP (P < 0.001), including one complete response (Fig. 5). Remarkably, this antitumor effect is attributable to the replication of virus alone because no therapeutic genes have been included.

**DISCUSSION**

The creation of a TK− and VGF-deleted vaccinia virus offers several advantages for use as a tumor-directed vector for cancer gene therapy. We have demonstrated decreased replication of this virus both in vitro and in vivo in nondividing cells. Decreased viral pathogenicity was demonstrated, including minimal recovery of vvDD-GFP from the brain tissue of nude mice. As well, a significant antitumor effect was seen after systemic injection because of selective replication of this virus in tumor cells. The inefficiency of currently available vectors underscores the need for a highly efficient and safe vector such as the one described.

**Table 2.** *Nude mouse pathology after viral injection*  

<table>
<thead>
<tr>
<th>Saline/Virus</th>
<th>Tumor necrosis</th>
<th>Ovarian necrosis</th>
<th>Brain lesions</th>
<th>Liver necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBSS</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>vvDD-GFP</td>
<td>++</td>
<td>+/−</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td>VGF−</td>
<td>++</td>
<td>+/−</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td>TK−</td>
<td>++</td>
<td>+/−</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td>WT</td>
<td>++</td>
<td>+/−</td>
<td>+</td>
<td>+/−</td>
</tr>
</tbody>
</table>

* Degree of the lesion: −, not present; +/−, minimal; +, mild; ++, moderate; ++++, severe.

**Table 1.** Median viral recovery from nude mouse tissues

Table 1 Median viral recovery from nude mouse tissues

<table>
<thead>
<tr>
<th></th>
<th>WT (10^3)</th>
<th>TK− (10^5)</th>
<th>VGF− (10^5)</th>
<th>vvDD-GFP (10^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>2.8 (2.4–4.9)×10^6</td>
<td>1.3 (21–20)×10^2</td>
<td>1.5 (76–43)×10^2</td>
<td>0 (0–8)*</td>
</tr>
<tr>
<td>Liver</td>
<td>3 (8–11)</td>
<td>7.6 (6–13)</td>
<td>1 (24–1)</td>
<td>0.1 (0–2)</td>
</tr>
<tr>
<td>Spleen</td>
<td>5.1 (59–21)×10^2</td>
<td>12 (6–16)</td>
<td>23 (16–308)</td>
<td>8 (0–16)</td>
</tr>
<tr>
<td>Tissues</td>
<td>54 (0.1–200)</td>
<td>12 (0.1–24)×10^2</td>
<td>6 (0–0.8)</td>
<td>6.8 (0.7–28)</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>1.0 (0.8–10)×10^3</td>
<td>3.0 (075–76)×10^6</td>
<td>1.1 (41–2100)×10^3</td>
<td>5.0 (0–12)×10^2</td>
</tr>
<tr>
<td>Ovary</td>
<td>7.1 (2.6–9.7)×10^9</td>
<td>9.3 (2.3–15)×10^9</td>
<td>2.1 (41–39)×10^9</td>
<td>8.6 (6.172)×10^9</td>
</tr>
<tr>
<td>Tumor</td>
<td>17.0 (1.2–14)×10^10</td>
<td>4.6 (3.6–6)×10^8</td>
<td>2.3 (0.5–2.6)×10^9</td>
<td>6.5 (4.6–5)×10^10</td>
</tr>
</tbody>
</table>

*P = 0.011.

*Tests samples obtained in a separate experiment.

and RNA synthesis in the cytoplasm and is therefore less dependent on the cellular factors than other viruses. Remarkably, the native and synthetic vaccinia promoters in use are very strong and contribute to its efficiency (39, 47, 48).

The most important feature of this virus, however, is the ability to selectively replicate and express genes in tumor tissues compared with normal tissues. We have demonstrated previously a 3-log higher expression of luciferase in tumors compared with normal tissue in a s.c. tumor model after systemic administration of the virus (7). The complete explanation for this tumor selectivity has yet to be elucidated; however, contributing factors include the enhanced ability of macromolecules to extravasate through permeable tumor vasculature (49) and the replication selectivity shown by vaccinia within the preferred metabolically active environment of tumor cells. Evidence for the contribution of permeable vasculature comes from smallpox literature. Smallpox was known to replicate preferentially in areas of increased vascular permeability secondary to injury and histamine release (50). The biodistribution of vaccinia (7, 35, 36), predominantly to tumor and ovarian follicles (both sites of increased vascular permeability; Refs. 49, 51), is also suggestive. Recently, we have shown that hyperthermia, which increases vascular permeability, leads to increased uptake of vaccinia virus. Of interest, ovaries have been shown to have high levels of VEGF (which also increases vascular permeability) and may explain the propensity for the virus to localize there as well as in tumor (52). Tumor-selective replication has been demonstrated previously (7, 53) and is thought to be largely attributable to the rapid division of tumor cells that provide nucleotides, specifically TTP, to complement a TK-deleted vector. The hypothesis for the high level of tumor selectivity shown by the current vector is a combination of the two. vvDD-GFP travels intravascularly and escapes at sites of increased vascular permeability, such as the tumor and ovary. Because of its ultimate reliance on dividing cells for replication, it is only able to replicate efficiently within tumor cells or ovarian follicles. Other sites of cellular replication such as bone marrow and gastrointestinal mucosa do not demonstrate the same levels of vaccinia infection, possibly because of a lack of leaky vasculature.

In addition to the high levels of gene expression resulting from the tumor-specific replication, we have shown that vaccinia alone, in the absence of a therapeutic gene, is capable of causing an antitumor effect from viral replication and subsequent cell death. This was dramatic in the rapidly growing MC38 cell line described above and will likely be similar in other cancer cell lines. The fact that a single systemic injection resulted in a complete antitumor response is an illustration of the efficient replication of this vector in tumor tissue. Despite a small percentage of cells initially infected, within 12 days
the virus spreads from cell to cell until a complete response is achieved. The effect in slow-growing human tumors and in immunocompetent models is currently under investigation.

The potential for serious infection leading to disease or death is a major concern when using any replicating virus. Extensive human trials with vaccinia virus as a smallpox vaccine showed mainly local side effects in normal subjects. However, encephalitis in infants and spreading infection in immunodeficient individuals, specifically those with deficits in cellular immunity were recognized (28, 30, 54, 55). The latter corresponds with current thinking regarding the importance of cell-mediated immunity in recovery from poxvirus infections (54).

Currently, no human trials exist delivering replicating vaccinia systemically, and the development of a safe, tumor-selective vaccinia virus for this purpose is timely. Vaccinia has been used successfully in humans as an intratumoral vector with no reported toxicity (53, 56–58).

The major advantage of the currently described vector is the marked decrease in *in vivo* toxicity demonstrated. The use of a nude mouse model stringently tested the pathogenicity of this vector. Of interest, viral replication in tumor was demonstrated, and infection of the brain with vaccinia was significantly abrogated. This was demonstrated by immunohistochemistry for vaccinia in the organs of interest as well as viral recovery from these organs. The decrease in vaccinia recovered from the brain may explain the decreased pathogenicity because postvaccinal encephalitis has been a complication after smallpox vaccination (31). Decreased pathogenicity from all but the WT vaccinia was seen in immunocompetent mice as expected because there is an excellent immune response to vaccinia virus in mice. This is unlike the situation seen with adenoviral vectors, where the immune response seems to enhance toxicity.

Disadvantages to vaccinia and other viral vectors for cancer gene therapy still remain. The rapid immune response and clearance of viruses limit their utility in immunocompetent hosts, although the immune response to a tumor-specific virus may be an advantage if it leads to antitumor immunity as well. The initial injection results in the rapid formation of circulating, neutralizing antibodies, which limits the use of repeated injections; however, strategies for masking viruses from these neutralizing antibodies, such as coating them in liposomes, polyethylene glycol, or other biological agents, are under investigation (59, 60). As well, the previous immunization program for smallpox created a population that is generally immune to vaccinia. However, smallpox immunization was discontinued in 1978; therefore, future cancer patients will not have preformed immunoreactivity against this vector. Finally, the propensity of vaccinia to infect ovaries may lead to sterility if this treatment were to be considered for female
cancer patients; however, this risk is currently unknown, and the virus may target only the corpus luteum.

We have created a tumor-selective vaccinia virus by deletion of both its TK gene and VGF genes. It is capable of selective tumor replication and is significantly less pathogenic than other forms of the virus. The potential utility of this vector is broad in that it may be used as an antitumor agent on its own or by expressing suicide or cytokine genes. It may be useful in immunotherapy trials expressing tumor-associated antigens and costimulatory molecules. Perhaps most importantly, given its large capacity for foreign gene insertion, it can be used for all of the above. Its efficiency and selectivity compares favorably with other replicating viral vectors currently in clinical trials (61). This is a new vector for the tumor-directed gene therapy of cancer and worthy of consideration as a systemic vector in human cancer trials.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 5. Mean tumor volume after i.p. treatment of s.c. MC38 in nude mice; bars, SE. On day 0, 8 days after inoculation with tumor, mice were injected with 10^8 pfu of replicating vvDD-GFP ( ), n = 10) or HBSS control ( , n = 10). Control HBSS had no effect on tumors.

Fig. 4. A, survival of nude mice after treatment with 10^8 pfu of replicating WT (F13L+, solid line), TK− (VJS6, dotted line), VGF− (VSC20, medium dash), or vvDD-GFP (long dash) by i.p. injection (n = 10). Both the TK− and VGF− infected mice had significantly prolonged survival (P < 0.0001) compared with WT. The double-deleted vaccinia virus (vvDD-GFP) had no evidence of toxicity by day 100 (P > 0.0001 compared with WT). B, survival of nude mice after treatment with 10^8 pfu (solid line) or 10^9 pfu (dashed line) of vvDD-GFP compared with 108 pfu of the TK− (VJS6, dotted line). Both groups of mice infected with vvDD-GFP had significantly prolonged survival (P < 0.001) compared with the TK− infected mice. C, survival of C57BL/6 (immuno-competent) mice after treatment with 10^8 pfu of replicating WT (F13L+, solid line), TK− (VJS6, dotted line), VGF− (VSC20, medium dash), or vvDD-GFP (long dash) by i.p. injection (n = 10). All mice died after treatment with WT virus; however, less toxicity was seen in the groups receiving the TK− or VGF− virus compared with nude mice. No toxicity was seen in the mice receiving vvDD-GFP.
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