Interleukin-6 Modulated Conditionally Replicative Adenovirus as an Antitumor/Cytotoxic Agent for Cancer Therapy

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Abstract
In this study, we report that an interleukin-6 (IL-6)-inducible E1A-substituting activity can be exploited for the production of infectious adenoviral particles during infection with the E1A-deleted adenovirus (Ad) Ad5dl312. The basal level of complementation can be increased by 1.5 log by induction of the HepG2 cells with recombinant human IL-6. Additionally, the IL-6-inducible E1A-substituting activity can complement E1A deletion in other cancer cell lines to render them Ad producer cells on induction with recombinant human IL-6, although the efficiency of complementation varies between cell lines. Ad5dl312 can replicate in, produce cytotoxic effect, and kill human tumor cells without addition of exogenous IL-6 in the context of tumor cells possessing an IL-6 autocrine arc, such as ovarian tumor cells. In contrast, normal human mesothelial cells isolated from normal human peritoneum lining do not support replication of Ad5dl312, even in the presence of exogenous IL-6. These results suggest that Ad5dl312 could be used as a cytotoxic agent to selectively kill tumor cells responsive to or possessing an IL-6 autocrine arc.

Introduction
The delineation of the genetic etiology of neoplastic diseases has made gene therapy a rational interventional strategy for the treatment of cancer (1–4). Many cancer gene therapy strategies have been used in experimental animal models, and promising results have been translated to Phase I clinical trials. To this end, many gene delivery systems have been developed for the different cancer gene therapy approaches. The choice of a gene delivery system is dictated by the nature of the interventional strategy and the context of the target disease (i.e., local/ regional disease versus metastatic tumors). For most in vivo strategies, vectors have been administered intratumorally, intraperitoneally, or into an anatomical compartment containing the tumor mass. The logic of these delivery modes is based on the achievement of high local vector concentrations that favor tumor cell transduction and limits vector dissemination. Nevertheless, underlying all of the cancer gene therapy approaches is the recognition that quantitative tumor cell transduction cannot be achieved with the vector systems presently available.

One way to overcome this limitation would be to design a vector that would amplify posttransductionally. This amplification effect could be achieved by methods such as replication of the delivered viral vector at the site of delivery. A further augmentation in this approach would be the development of a conditionally replicative system; a replication-competent virus would be used to selectively replicate within the transduced tumor cells and not in normal cells. In this schema, virus progeny made from the transduced tumor cells would then permit infection of the neighboring tumor cells. This would result in an augmented viral concentration that could be of therapeutic utility in two ways. First, tumor-specific replication of a viral vector encoding an antitumor gene would increase viral concentration at the tumor interface with spreading of the virions into the tumor mass. This should increase efficiency of tumor cell transduction and therapeutic efficacy of the antitumor gene. Second, the use of lytic viruses would allow viral-mediated oncolysis, which would occur regardless of the delivered antitumor gene. In both cases, amplification of the antitumor effect would be achieved.

In this regard, virus-mediated oncolysis has been previously used for experimental tumor therapy. Early studies involved the use of mumps virus (5, 6), vaccinia virus (7), myxovirus (8–11), West Nile virus (12, 13), and Newcastle Disease Virus (14) for different neoplastic diseases because of their apparent oncolytic properties. In the last few years, studies with the H-1 parovirus, Newcastle Disease Virus, and Herpes Simplex Virus type 1 have demonstrated that killing of tumor cells and tumor regression can be achieved by viral-mediated oncolysis (15–22). In addition, the Herpes Simplex Virus type 1-derived multimutant G207 has been shown to be effective in decreasing tumor growth and prolonging survival of mice bearing intracranial gliomas and meningiomas (23, 24). However, these schemas have generally demonstrated limited therapeutic utility, likely related to vector stability in vivo and host immunological response.
As an alternative, we considered Ad3 as a candidate for a conditionally replicative vector system. Ads are suitable candidate vectors for cancer therapy because their biology is well characterized and they possess a high level of in vivo stability. Recombinant Ads have shown a high level of utility for the in vivo transduction of tumors after in situ or vascular delivery (25–27). They have a broad host range and are not cell cycle-dependent. Thus, they can infect dividing cells as well as quiescent and terminally differentiated cells like neurons and hepatocytes (26, 27). In addition, Ads possess a lytic life cycle, and this oncolytic property could be exploited by engineering the virus to replicate specifically within tumor cells. Thus, the use of the adenovirus vector in the context of conditional replication is logical.

For this application, we are taking advantage of the IL-6-inducible E1A-substituting activity described in the hepatocarcinoma HepG2 cell line by Spergel and Chen-Kiang (28) that allows viral DNA replication and early gene expression during infection with an E1A-deleted adenovirus, Ad5dl312. This IL-6-inducible E1A-substituting activity has been identified as NF-IL-6, a member of the C/EBP family (29). In a transient expression system, NF-IL-6 cDNA alone was shown to be sufficient to regulate E1A-responsive promoters, E2a and E1B, in the absence of E1A.

In this study, we report that the IL-6-inducible E1A-substituting activity can be exploited for the production of infectious adenoviral particles during infection of HepG2 cells with the E1A-deleted Ad Ad5dl312. The basal level of complementation can be increased by 1.5 log by induction of the HepG2 cells with rhIL-6. Additionally, the IL-6-inducible E1A-substituting activity can complement E1A deletion in other cancer cell lines to render them Ad producer cells on induction with rhIL-6, although the efficiency of complementation varies between cell lines. Ad5dl312 can replicate, produce cytopathic effects, and kill these tumor cells without the addition of exogenous IL-6 in the context of tumor cells possessing an IL-6 autocrine arc, such as ovarian tumor cells. In contrast, normal human mesothelial cells isolated from normal peritoneum lining do not support replication of Ad5dl312, even in the presence of exogenous IL-6. These results suggest that Ad5dl312 could be used as a cytopathic agent to selectively kill tumor cells responsive to or possessing an IL-6 autocrine arc.

Materials and Methods

Cell Lines and Culture Conditions. The human hepatocarcinoma HepG2, Hep3B, human cervical carcinoma HeLa, transformed human embryonal kidney 293 (30), human colon carcinomas LS174T and WiDr, human ovarian teratocarcinoma PA-1, and human ovarian carcinoma OVCAR-3 cell lines were obtained from the American Type Culture Collection (Manassas, VA). The human bladder carcinoma EJ, the human fibroblast W162 (31), the human ovarian carcinoma SKOV3ip1 (32), and the OV-4 cell lines were generous gifts from Kevin Scanlon (City of Hope, Duarte, CA), Gary Keiter (Johns Hopkins School of Hygiene and Public Health, Baltimore, MD), Janet Price (M. D. Anderson Cancer Center, Houston, TX), and Timothy J. Eberlein (Brigham and Women’s Hospital, Harvard Medical School, Boston, MA), respectively. Cells were maintained at 37°C in a 5% CO2 incubator. All media contained 10% heat-inactivated FBS (HyClone Laboratories Inc., Logan, UT), 2 mM l-glutamine (Mediatech, Herdon, VA), 100 units/ml penicillin, and 100 μg/ml streptomycin (Mediatech). HepG2 and Hep3B cell lines were grown in Earle’s MEM (Mediatech) supplemented with 10 mM MEM nonessential amino acids (Sigma Chemical Co., St. Louis, MO) and 1 mM sodium pyruvate (Mediatech). The LS174T and WiDr cell lines were grown in Earle’s MEM supplemented with 10 mM MEM nonessential amino acids and DMEM/Ham’s F12 (Mediatech), respectively. HeLa, 293, W162, SKOV3ip1, PA-1, and OV-4 cell lines were maintained in DMEM/Ham’s F12 medium. The OVCAR-3 cell line was maintained in RPMI (Mediatech) supplemented with 20% FBS and 10 μg/ml insulin (Life Technologies, Inc., Gaithersburg, MD). The EJ cell line was grown in RPMI 1640. Human primary ovarian cancer cells were isolated from ascites, as previously described (33), and maintained in RPMI 1640 supplemented with 2% FBS. Normal human mesothelial cells were isolated from fresh peritoneum lining tissue by mechanical disruption and collagenase D treatment (Boehringer Mannheim, Indianapolis, IN) and maintained in DMEM/Ham’s F12 medium. To propagate and maintain these cells in a proliferative state, 10 ng/ml EGF (Life Technologies, Inc.), and 0.4 μg/ml hydrocortisone (Sigma Chemical Co.) were added to the medium (34).

Determination of IL-6 and Soluble IL-6-R Production. Human ovarian carcinoma cell lines OVCAR-3 and SW626 were seeded at 1 × 10⁶ cells/well in a 6-well plate. When confluent, culture medium was removed, cells were washed, and 1 ml of fresh medium was added. After 4, 8, and 24 h, the conditioned medium was removed and stored at −80°C until required. Levels of IL-6 were determined by an ELISA kit (R&D Systems, Minneapolis, MN) after the recommendations of the manufacturer. Levels of total sIL-6-R were determined using the following ELISA procedure. Microtiter 96-well plates were coated with 10 μg/ml of the antihuman IL-6-R monoclonal antibody 17.1 and blocked at 4°C in 0.5% BSA. Human sIL-6-R standards and samples of unknown concentration were added and incubated at RT for 2 h. To detect bound sIL-6-R, 50 ng/ml antihuman IL-6-R antibody 17.1 and blocked at 4°C in 0.5% BSA. Human sIL-6-R standards and samples of unknown concentration were added and incubated at RT for 2 h. To detect bound sIL-6-R, 50 ng/ml biotinylated antihuman IL-6-R (BAF-227) was added and incubated for 2 h at RT, followed by a 20-min incubation with streptavadin-horseradish peroxidase conjugate. Plates were washed with PBS containing 0.1% Tween 20 between each step. Horseradish peroxidase activity was determined using 3,3′,5,5′-tetramethylbenzidine dihydrochloride as a substrate. The reaction was stopped by the addition of 1.8 M H₂SO₄, and the absorbance was measured at 450 nm.

Virus Infection. Ad5dl312 has been characterized as carrying a 902-bp deletion in the E1A gene (35) and was kindly provided by Thomas Shenk (Princeton University, Princeton, NJ). WT adenovirus type 5 was obtained from the American Type Culture Collection. Ad5dl1014 contains deletions in the E4 region encompassing ORFs 1, 2, 3, and ORF6, and ORF6/7.
as well (36). This mutant Ad was a generous gift from Gary Ketner. Characterization of stocks revealed less than one WT particle/10^{12} of the defective virions. Before infection, cells were seeded at approximately 25% confluence (between 1 × 10^5 and 4 × 10^5 cells/well) in 6-well plates. The next morning, cells were infected in a total volume of 100 μl with WT, Ad5dl312, or Ad5dl1014 at multiplicities of infection of 10 in infection medium (DMEM/F12 50:50 containing 2% FBS, 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.8 mM L-arginine). The cells were incubated at 37°C with gentle rocking. After 1 h of infection, the cells were washed twice with PBS at RT to remove unbound virions. Subsequently, the infected cells were overlaid with 3 ml of infection medium containing none or 100 units/ml recombinant IL-6 protein (Genzyme Corporation, Framingham, MA) and incubated at 37°C. The next morning, infection medium was substituted for complete medium containing 0 or 100 units/ml recombinant IL-6 protein (Genzyme Corporation, Framingham, MA) and incubated at 37°C. Because DNA replication occurs ~6 h p.i. with WT virus and assembly between 24 and 48 h p.i., and because of the 4-day delay in DNA replication of Ad5dl312 using the IL-6-inducible E1A-substituting activity described by Spergel and Chen-Kiang could complement for production of infectious Ad particles, HepG2 cells were infected with WT or Ad5dl312 at MOIs of 10 in the absence or presence of 100 units/ml rhIL-6. Because DNA replication occurs ~6 h p.i. with WT virus and assembly between 24 and 48 h p.i., and because of the 4-day delay in DNA replication of Ad5dl312 using the IL-6-inducible E1A-substituting activity, we estimated that assembly of Ad5dl312 virions would be over by 5.5–6 days p.i. Therefore, 6 days after infection, HepG2 cells and medium were harvested together, submitted to four to five cycles of freeze-thaw to release the virus progeny, and supernatants were assessed for plaque formation on permissive 293 cells. Plaque assays were read 14 days p.i., and titers were compared (Fig. 1). Infectious virions were made from Ad5dl312 in the absence of rhIL-6 but, most importantly, IL-6 induction of HepG2 cells during infection resulted in an increased titer of 1.5 log. The titer obtained from Ad5dl312 infections in the presence of rhIL-6 was approximately 1.5 log lower than that of WT virus. Increasing the concentration of rhIL-6 during infection did not result in a significant augmentation of the titer (data not shown), and induction with IL-6 did not have any effect on the replication of WT virus. Analysis of the progeny Ad5dl312 revealed less than one particle of WT virus/10^{12} particles in the harvested cell lysates and media (data not shown). Thus, plaque assays reflected the actual production of progeny Ad5dl312. These results thus establish that the cellular IL6-inducible E1A-substituting activity can complement the E1A deletion during infection of HepG2 cells and, therefore, lead to production of infectious Ad particles.
Conditionally Replicative Adenovirus

...Ad5dl312 and Ad5dl1014 using an MOI of 10 without the addition of exogenous IL-6. A, six days p.i., cell lysates were collected and assessed for plaque formation on permissive 293 cells for WT and Ad5dl312 or on W162 cells for Ad5dl1014. Titers were read on day 14. B, seven days p.i., the viable cells were washed with PBS, fixed in fresh buffered formaldehyde, and stained with crystal violet.

Fig. 3 In the context of tumor cells exhibiting an IL-6 autocrine arc-like human ovarian cancer cells, Ad5dl312 can selectively replicate in, produce new virus progeny, and kill the infected tumor cells by virus-mediated oncolysis without requiring the addition of exogenous IL-6. Human ovarian carcinoma OVCAR-3 cells were infected with WT, Ad5dl312, or Ad5dl1014 using an MOI of 10 without the addition of exogenous IL-6. A, six days p.i., cell lysates were collected and assessed for plaque formation on permissive 293 cells for WT and Ad5dl312 or on W162 cells for Ad5dl1014. Titers were read on day 14. B, seven days p.i., the viable cells were washed with PBS, fixed in fresh buffered formaldehyde, and stained with crystal violet.

Many cancer cells have been characterized as IL-6 responsive (37). We hypothesized that if cancer cells are responsive to IL-6, then the IL-6-inducible E1A-substituting activity might be expressed in these cells. To determine if the IL-6-inducible E1A-substituting activity was unique to HepG2 cells or shared by other cancer cells, cancer cell lines of various origins were screened for their capacity to support replication of Ad5dl312 using an MOI of 10 in the absence and presence of rhIL-6 (100 units/ml). Six days p.i., cells and medium were harvested, submitted to four to five cycles of freeze-thaw, and the supernatants assessed for plaque formation in 293 cells. The graph in Fig. 1 shows that almost all of the cell lines tested supported growth of Ad5dl312 in the presence of rhIL6, although to various degrees. In some cell lines (HeLa, SKOV3ip1, LS174T, OV-4, and Hep3B), there was almost no difference between titers obtained from infections in the absence and in the presence of rhIL-6. The most inductive cell lines in terms of Ad productive infection was the EJ bladder carcinoma cell line. On IL-6 induction of this cell line, the titer of Ad5dl312 increased by 3 logs, but never reached the titer of WT virus. Interestingly, in the PA-1 cell line, Ad5dl312 replication was as good as that of WT virus regardless of the status of exogenous IL-6. In contrast, the WiDr cell line poorly supported replication of Ad5dl312, even in the presence of exogenous IL-6. Taken together, these results demonstrate that Ad5dl312 virions can be produced in human cancer cells other than HepG2 in the presence of rhIL6 and that in some cell lines exogenous IL-6 does not seem to be required.

To demonstrate that replication of Ad5dl312 is due to an E1A-substituting cellular activity present in the IL-6-responsive tumor cells, the most inductive and Ad-productive cell line, EJ, was infected with Ad5dl312 or Ad5dl1014, an E4-deleted Ad mutant, in the absence and presence of rhIL-6. WT virus served as a control. In addition, preliminary studies documented the ability of Ad5dl1014 to replicate to WT levels on the permissive cell line W162 (data not shown). As in previous experiments, cell lysates were collected on day 6 p.i. and assessed for plaque formation on 293 cells for WT and Ad5dl312 samples or on W162 cells for Ad5dl1014 samples. Ad5dl1014, however, did not replicate in EJ cells, not even in the presence of exogenous IL-6 (data not shown). Thus, this result demonstrates that the IL-6-inducible cellular activity present in EJ cells is specific for complementation of E1A, therefore, suggesting a specificity to IL-6-responsive tumors for Ad5dl312 replication. Furthermore, the observed oncolytic effects were not based on contaminating WT virions.

A variety of malignant tumors and cell lines have been shown to contain or synthesize IL-6, and an autocrine growth stimulation has been suggested as a possible mechanism for the action of IL-6 (38 – 40). In the context of gynecological malignancies, an IL-6 autocrine loop has been demonstrated in cervical (41), chorio (42), and ovarian carcinomas (43). We hypothesized that tumor cells possessing an IL-6 autocrine growth stimulation would support replication of Ad5dl312 without requirement for addition of exogenous IL-6. To determine if this was the case, we used the human ovarian carcinoma cell lines OVCAR-3, known to exhibit an IL-6 autocrine arc, and SW626. Previous studies had demonstrated that OVCAR-3 cells possessed a functional IL-6 autocrine arc (43). Indeed, we could confirm both an exuberant secretion of IL-6 (Fig. 2A) and the presence of IL-6-R (Fig. 2B) in both cell lines. Cells were infected with WT, Ad5dl312, or Ad5dl1014 Ads in the absence of exogenous IL-6 using an MOI of 10. Six days p.i., the cell lysates were collected and subjected to plaque formation on permissive 293 cells. As shown in Fig. 3A, Ad5dl312 replicated in OVCAR-3 cells as efficiently as WT Ad and achieved an equivalent titer (2 × 10⁹ PFU/ml for WT versus 1 × 10⁹ PFU/ml for Ad5dl312). Of note, the addition of exogenous IL-6 during infection of these cells did not result in an increased titer (data not shown). Moreover, OVCAR-3 cells selectively supported replication of Ad5dl312 because the E4-deleted Ad Ad5dl1014 did not replicate in these cells (Fig. 3A). These results, therefore, suggest that cancer cells possessing an IL-6 autocrine loop can selectively support the replication of Ad5dl312 without requirement for addition of exogenous IL-6.
Because Ads possess an oncolytic life cycle, we sought to exploit this oncolytic property as an antitumor/cytotoxic agent and couple it to a tumor-specific characteristic, the IL-6 autocrine arc of human ovarian cancer cells. In this experiment, OVCAR-3 cells were infected with Ad5dl312 at an MOI of 10; WT and Ad5dl1014 viruses served as positive and negative controls, respectively. Seven days p.i., the cells were stained with crystal violet. As shown in Fig. 3, WT virus infection led to 100% killing of infected SW626 cells, and the two samples of primary ovarian tumor cells, OVCA-CR1 and OVCA-CR7. In addition, Ad5dl312 replicated in and efficiently killed the infected tumor cells by virus-mediated oncolysis (Fig. 4), as observed in OVCAR-3 cells (Fig. 3). Of note, the replication of Ad5dl312 in these cells occurred without the addition of exogenous IL-6. Similar results were also obtained with the human ovarian cancer cell line PA-1 and other samples of human primary ovarian tumor cells (data not shown). Taken together, these results demonstrate that Ad5dl312 can replicate in and kill ovarian tumor cells based on their IL-6 autocrine arc.

In anticipating toxicity problems given the possible ability of Ad5dl312 to replicate and disseminate beyond the tumor compartment (i.e., the peritoneal cavity in the context of ovarian carcinoma), we tested the capacity of Ad5dl312 to replicate in and kill normal human mesothelial cells isolated from peritoneal lining tissue. Cells were infected with Ad5dl312 and WT using an MOI of 10 in the absence and presence of rhIL-6. Twelve days p.i., the cells were stained with crystal violet (Fig. 5). WT virus replicated in and killed normal human mesothelial cells. However, Ad5dl312 did not replicate in these cells, even in the presence of exogenous IL-6. Equivalent results were obtained with two other samples from different patients of normal human mesothelial cells (data not shown). Thus, these results suggest that while not permitting replication of Ad5dl312, the human peritoneal lining would act as a natural barrier to prevent toxicity coming from replication of Ad5dl312 in nontumor cells in the context of a treatment for ovarian carcinoma.

Discussion

We report here the use of an E1A-deleted mutant adenovirus, Ad5dl312, as an antitumor/cytotoxic agent in tumor cells that are responsive to or exhibit an IL-6 autocrine growth stimulation by taking advantage of an IL-6-inducible E1A-substituting activity. We have shown that the IL-6-inducible E1A-substituting activity present in HepG2 cells can be exploited for the production of Ad5dl312 infectious particles. This cellular activity is present in other human cancer cell lines and allows replication of Ad5dl312 in the presence of rhIL-6, although to various degrees. In addition, we have shown that the IL-6-inducible cellular activity specifically complements the E1A deletion. More importantly, we have demonstrated that in the context of tumor cells exhibiting an IL-6 autocrine arc, Ad5dl312 can replicate in human ovarian cancer cell lines and human primary ovarian tumor cells, leading to death of the infected tumor cells by virus-mediated oncolysis. In addition, normal human mesothelial cells derived from peritoneal lining tissue did not support replication of Ad5dl312 even in the presence of endogenous IL-6. These results, therefore, suggest that this novel conditionally replicative Ad could be of utility for treatment of ovarian carcinoma and other malignancies for which an IL-6 autocrine arc exists.

IL-6 is a cytokine with pleiotropic activities and has been shown to play a central role in immune host-defense mecha-
nisms, the acute phase reaction and hematopoiesis (44, 45). IL-6 has also been shown to play an important role in several types of malignancies. IL-6 can enhance or inhibit proliferation of carcinoma cells, whereby it can function as a paracrine and an autocrine growth factor (38, 40–43, 46–50). In some instances, IL-6 transits from a paracrine growth inhibitor to an autocrine stimulator, and this has been shown to correlate with progression of disease (51). Moreover, endogenous IL-6 can block programmed cell death induced by cytotoxic agents, transforming growth factor β1 and WT p53 (52–54). It has also been shown to act in cooperation with other cytokines/growth factors (55). In addition, IL-6 has been suggested to be involved in cell invasion and metastases through its effect on cell-cell association, attachment, and migration (48, 56). Thus, a role for IL-6 in several malignancy pathophysologies has clearly been established and makes IL-6 a rationale target for treatment of cancer.

The strategy presented here takes advantage of the IL-6 responsiveness of several human tumor cells to specifically complement and selectively support the growth of an E1A-deleted mutant Ad for the achievement of tumor cell killing by virus-mediated oncolysis. Our in vitro results obtained with human ovarian cancer cell lines and primary ovarian tumor cells have established the potential utility and clinical relevance of the mutant Ad Ad5dl312 for treatment of ovarian carcinoma. Ads have been used in the past for experimental tumor therapy. Smith et al. (64) have injected patients with advanced cervical carcinoma with WT Ad based on the luxuriant growth of Ads on HeLa cells. Sixty-five percent of the patients showed necrosis and cavity formation in the pelvic tumors. To achieve preferential and/or selective replication in target tumor cells, replication-competent restricted Ads have recently been developed (64, 65). Bischoff et al. (65) have reported an E1B-55 kDa-deleted Ad that specifically replicates in and kills p53-deficient human tumor cells in vitro and in vivo. Although this virus would be a good general antitumor agent because the loss or inactivation of the p53 gene is thought to contribute to the development of 50% of all human cancers, the mutant virus replication is not as efficient as WT virus infection in vitro (100-fold less than WT virus). The optimal replicative Ad should be able to replicate as effectively as WT virus for achieving a useful therapeutic end point. In contrast, our results have demonstrated that Ad5dl312 can replicate in vitro, as efficiently as WT virus, at least in cancer cells exhibiting an IL-6 autocrine arc as demonstrated in ovarian tumor cells. In the study of Rodrigues et al. (66), a different approach was used whereby the E1A gene was placed under the control of the PSA promoter to achieve PSA-specific killing of prostate cancer cells in vitro and in vivo. This PSA-specific engineered Ad replicated as efficiently as a WT virus in vitro, however, the comparison in vivo has not been shown. Prostate cancer cells are IL-6-responsive, and paracrine as well as autocrine growth stimulations have been demonstrated as the IL-6 mechanism of action in
these cells (50). Therefore, it is possible that Ad5dl312 could also be of clinical utility for treatment of prostate cancer, and this strategy would not be restricted only to PSA-positive or androgen-sensitive prostate tumor cells.

Our results and that of others suggest that replication-competent restricted Ads may be of utility for the treatment of cancer. However, some issues remain to be addressed. It is well recognized that adenoviral vectors used in gene therapy strategies produce a dose-dependent inflammatory response in rodents and primates and that vector-associated toxicity has also been observed in human clinical trials. These problems, therefore, threaten to prevent adenoviral vectors from realizing their full potential as vectors for human gene therapy (67). Such an immune response against the virus may affect virus spread and decrease efficacy of tumor cell killing. However, an immune response directed at the infected tumor cells might be desirable since an antitumor immune response might be elicited and would be additive to the virus-mediated oncolysis. Furthermore, since an antitumor immune response might be directed at the infected tumor cells might be desirable, an immune response against the virus may affect virus spread and may prevent vector dissemination beyond the i.p. cavity. Additional toxicity studies will define if the present specificity profile of replication translates into an acceptable therapeutic index.

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References

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