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Application of recombinant Herpes Simplex Virus-1 (HSV-1) for the treatment of malignancies outside the central nervous system

Review Article

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Summary

Attenuated HSV-1 mutants are promising novel vectors for human gene therapy of cancer. In addition to their efficacy in treatment of experimental CNS tumors, HSV mutants have shown promise in treatment of extra-CNS tumors including mesothelioma, melanoma, breast cancer, epithelial ovarian carcinoma, colon carcinoma and non small cell lung carcinoma in various animal models. HSV mutants which have been partially attenuated can function as direct oncolytic agents capable of proliferating within three-dimensional tumors and causing tumor cell death. A major advantage of these replication-restricted HSV mutants is that they can selectively replicate in tumor cells and thus, potentially express transgenes in a higher percentage of the tumor cells. Alternatively, super-attenuated HSV mutants and amplicons can function as efficient vectors for gene therapy and have the ability to host large and multiple transgenes. A multi-pronged strategy for HSV-based anti-tumor therapy is currently emerging, where multi-attenuated viruses or the oncolytic HSV mutants are used as gene therapy vectors for intratumoral delivery of immunomodulatory or chemotherapy sensitizing transgenes. HSV-based tumor therapy has been reported to induce an anti-tumor immune response in some animal models. These findings may be due to the combination of co-expression of immunomodulatory molecules, immunogenic properties of the virus, necrosis of the tumor tissue and subsequent tumor antigen presentation. Thus, HSV oncolytic agents and gene therapy vectors show great potential as anti-tumor therapies. Further studies are required to test the efficacy and safety of these agents in extra-CNS malignancies.

I. Introduction

Therapeutic strategies for the gene therapy of malignancies have been designed along three main pathways: corrective gene therapies entail the delivery of wild-type tumor suppressor genes to tumors which have been shown to display alterations in those genes. This approach can lead to restoration of normal tumor suppressor function and to tumor regression (Favrot *et al.,* 1998). Secondly, suicide gene therapies are designed to deliver specific suicide genes, such as herpes simplex virus thymidine kinase or cytosine deaminase, into tumor cells (Singhal and Kaiser, 1998; Vile, 1998) which are rendered sensitive to the administration of prodrugs. The suicide gene converts the prodrug into toxic metabolites which can induce lysis in rapidly dividing cells. A third strategy involves the expression of immunomodulatory genes which may stimulate an anti-tumor response by the host immune system. These genes

include various cytokine genes (e.g. granulocyte/macrophage-colony stimulating factor (GM-CSF), interleukin (IL)-12, co-stimulatory molecules (e.g. B7.1) and allogeneic transplantation antigens (e.g. HLA-B7) (Pardoll, 1992). Combinations of the aforementioned strategies are also being investigated (Roth and Cristiano 1997).

Gene delivery remains one of the most important limitations in cancer gene therapy. The first generations of replication-incompetent adenoviral vectors, widely used in clinical trials for cancer gene therapy, may have limited therapeutic efficacy in bulky tumors, most likely due to localized gene delivery in three-dimensional tumors (Sterman *et al.*, 1998). Replication-selective viral vectors may offer a suitable alternative. Among replication-selective vectors, recombinant Herpes Simplex Virus Type-1 (HSV-1) mutants represent potentially powerful tools for the treatment of cancer. HSV has a large genome of 152 Kb (Fink and Glorioso, 1998). It may be able to accommodate more than 30 Kb of transgene inserts, making it a suitable vector for large and/or multiple transgenes (Fink and Glorioso, 1998). Although HSV-1 is a common pathogen in humans, it very rarely induces serious complications. Attenuation of HSV will most likely augment its safety profile. Recombinant viruses have been engineered to lack specific genes necessary for neurovirulence or viral replication in quiescent cells, resulting in replication-restricted viral mutants that selectively or preferentially replicate in and lyse tumor cells. Thus, depending on the degree of attenuation, HSV-1 mutants can be used not only as vectors for gene therapy but also as direct oncolytic agents.

HSV-1 mutants have been shown to be efficacious in the treatment of experimental malignancies localized within the central nervous system (CNS) (Andreansky *et al.*, 1997; Chambers*et al.*, 1995; Jia *et al.*, 1994; Kesari *et al.*, 1995; Kramm *et al.*, 1997; Mineta *et al.*, 1995; Pyles *et al.*, 1997; Yazaki *et al.*, 1995). Two main lines of investigation have been followed. In the first discussed strategy, multi-attenuated viral vectors were engineered by deletion of multiple genes to be able to undergo at most one or two rounds of replication within cancer cells (Glorioso *et al.*, 1997). Alternatively, HSV amplicons, which have additional deletions of essential HSV genes and require helper virus or complementation of many HSV functions to replicate in any cells, can be used to express various transgenes (Fraefel *et al.*, 1996; Geller, 1993; Geller and Breakefield, 1991; Ho, 1994). Multi-attenuated viral vectors and amplicons were originally engineered for gene therapy of CNS hereditary conditions, such as neurodegenerative and neuromuscular diseases, based on their ability to express the transgene(s) but not HSV proteins in quiescent cells (Fink and Glorioso, 1998; Geller, 1993; Geller and Breakefield, 1991; Glorioso *et al.*, 1997; Ho, 1994; Huard *et al.*, 1997). These vectors may also be suitable for cancer gene therapy for transduction of suicide genes. In addition, these vectors can deliver cytokine genes, or costimulatory molecules to enhance tumor recognition and killing by the immune system.

In the second line of investigation, oncolytic HSV mutants have been engineered by deletion of one or more genes to replicate poorly or not at all in normal host epidermal and neuronal tissues but to be able to replicate 30-100 fold more efficiently in tumor cells. For example, viruses were initially attenuated by deletion of thymidine kinase or ribonucleotide reductase and were used as oncolytic agents of CNS malignancies. Since deletion of the thymidine kinase gene made the vector insensitive to the current anti-herpetic drugs, acyclovir and ganciclovir, which is an important safety mechanism in case of inappropriate HSV spread, other strategies are being pursued. Ribonucleotide reductase deletion mutants have been efficacious in the treatment of malignant gliomas in immunocompromised and immunocompetent mice (Boviatsis *et al.*, 1994). A third generation of viruses lacking both copies of ICP34.5 demonstrated efficacy in the treatment of several CNS tumors (Andreansky *et al*., 1997; Chambers *et al.*, 1995; Kesari *et al.*, 1995; Kramm *et al*., 1997; Yazaki *et al*., 1995). The HSV ICP34.5 mutants selectively replicated in tumor cells (McKie *et al.*, 1996; Randazzo *et al.*, 1997) and exhibited 10^5-10^6 fold attenuation in neurovirulence (Chou *et al.*, 1990; MacLean *et al.*, 1991; Valyi-Nagy *et al.*, 1994). Several strategies have been pursued to further augment the efficacy of these mutants. The efficacy for treatment of experimental human glioma by R3616, an ICP 34.5 mutant, was augmented by radiation therapy in an immunodeficient model (Advani *et al.*, 1998) and by co-expression of IL-4 (Andreansky *et al.*, 1998). Concomitant deletions of the ICP34.5 genes and ribonucleotide reductase (Mineta *et al.*, 1994; Mineta *et al*., 1995) or the uracil DNA glycosylase gene (Pyles *et al.*, 1997) led to further attenuation but preserved oncolytic efficacy in the treatment of various CNS tumors.

Recent evidence suggests that attenuated Herpes Simplex Virus-1 mutants can be also utilized for peripheral malignancies. The present review will offer a brief summary of HSV-1 mechanism of action, will provide the overall rationale for the utilization of mutant HSV-1 for treatment of malignancies in extra-CNS locations and summarize the evidence accumulated to date.

II. HSV-1 replication

The replication cycle and epidemiology of HSV have recently been reviewed (Roizman and Sears, 1996; Whitley, 1996). HSV-1 is a DNA virus with a large genome of 152 Kb. To date, 80 HSV genes have been identified, but approximately 30 are non-essential for its replication *in vitro* in permissive Vero cells (Fink and Glorioso, 1998;

McGeoch *et al.*, 1988). In the immunocompetent human host, wild-type (wt) HSV-1 infects predominantly tissues of epidermal and neuronal origin (Whitley, 1996). Wt HSV infection of epidermal tissues results in a lytic infection and usually is accompanied by the induction of latency in peripheral neurons and the ganglia. Encephalitis, a lytic infection of the central nervous system, occurs only rarely. Briefly, the replication cycle begins with viral attachment to the cells, which is mediated by recognition of specific envelope glycoproteins, such as glycoprotein (g)B and gC to heparan sulfate (Laquerre *et al.*, 1998; Spear *et al.*, 1992). A cellular protein, EXT, can enhance the expression of heparan sulfate and has been shown to confer susceptibility of some cells to HSV infection (McCormick *et al.*, 1998). In addition, gD can specifically bind to cells via the Herpes virus entry mediator (HVEM) protein (Montgomery *et al.*, 1996) and by two additional, recently identified receptors (Geraghty *et al.*, 1998; Whitbeck *et al.*, 1997). Binding is followed by fusion of the viral envelope with the cell membrane of the infected host, partially mediated by viral gB, gD, and gH. The capsid is transported to the nucleus, where the viral DNA is released. During this process, VP16, a protein associated with the tegument, interacts with cellular transcription factors to activate transcription and expression of immediate early (α) genes ICP0, ICP4, ICP22, ICP27 and ICP47 (DeLuca and Schaffer, 1985; Fink and Glorioso, 1998; Honess and Roizman, 1974; Roizman and Sears, 1996). The viral early genes (β1 and β2 genes), which are mainly involved in nucleotide synthesis and viral DNA replication in quiescent cells, are then transcribed and translated. The late genes (γ1 and γ2) are subsequently expressed, resulting in the synthesis of the protein components of the capsid, tegument and viral envelope (Roizman and Sears, 1996; Subak-Sharpe and Dargan, 1998). There are some genes which are transcribed late as well as early and have been termed γ1 genes (Roizman and Sears, 1996 and ref. therein). Finally, the viral DNA is cleaved and packaged into capsids, and the DNA containing capsids appear to be enveloped at the nuclear membrane. The enveloped capsids transit through the cytoplasm in a multi-step process still under investigation and get released from the cell. Along this process the infected cell dies (Roizman and Sears, 1996 and ref. therein).

The mechanism by which HSV infected cells die is still a matter of investigation. Galvan and Roizman (Galvan and Roizman, 1998) recently indicated that some HSV-infected cells undergo apoptosis, while other cells die of non-apoptotic death. The type of cell death was found to be cell-type dependent (Galvan and Roizman, 1998). Normal proliferating cells, such as activated peripheral and cord blood derived T-lymphocytes, succumb to apoptosis when infected by wt HSV-1 (Ito *et al.*, 1997a; Ito *et al.*, 1997b) and this process is independent of the Fas/Fas ligand system (Ito *et al.*, 1997a). Although Ito *et al.* observed no change in frequency of apoptosis in non-activated cultures of T lymphocytes infected with HSV-1 vs. non-infected cells (Ito *et al.*, 1997b), wt HSV-1 has been reported to induce apoptosis in non-activated human peripheral blood mononuclear cells (Tropea *et al.*, 1995) as well as in other tissues (Irie *et al.*, 1998). The HSV genes which induce apoptosis in the infected cell are being investigated. Since HSV-1 can induce apoptosis at several checkpoints (Galvan and Roizman, 1998), it is likely that HSV-1 encodes several genes which can induce apoptosis. HSV encodes early genes that destabilize cellular RNA, disrupt cellular transcription and degrade cellular DNA (Johnson *et al.*, 1992; Kwong *et al.*, 1988; Roizman and Sears, 1996) and are likely candidates. Additional genes, including the genes which are non-essential for its replication *in vitro* (McGeoch *et al.*, 1988) may also be involved in induction of apoptosis in the infected host. Apoptosis of the HSV-infected cells can also occur in the absence of *de novo* protein synthesis, suggesting that proteins present in the virion may directly trigger some apoptotic pathways (Galvan and Roizman, 1998; Koyama and Adachi, 1997). Finally, oncolytic replication-restricted HSV-1 mutants lacking ICP34.5 may induce apoptosis (Chou *et al.*, 1994; Chou and Roizman, 1992) due to the loss of the protective effect that ICP34.5 exerts on the premature shut-off of total protein synthesis in the infected host (Cassady *et al.*, 1998a; Cassady *et al.*, 1998b).

HSV-1 infection can also inhibit apoptosis such as that induced by cytotoxic T lymphocytes (Jerome *et al.*, 1998), hyperthermia (Galvan and Roizman, 1998; Leopardi and Roizman 1996), sorbitol treatment (Galvan and Roizman 1998; Koyama and Miwa 1997), anti-fas ligand (Galvan and Roizman, 1998), tumor necrosis factor alpha (TNFα) and C2 ceramide (Galvan and Roizman, 1998) in some cells. Wt HSV encodes at least two genes, ICP4 (Leopardi and Roizman, 1996) and Us3 (Leopardi *et al.*, 1997), which have been shown to protect some infected cells from undergoing apoptosis (Koyama and Miwa, 1997). In addition, as mentioned above, ICP34.5 exerts a protective effect on the premature shut-off of total protein synthesis in the infected host (Cassady *et al.*, 1998a; Cassady *et al.*, 1998b). Although bcl-2 expression does not play a major role in regulation of apoptosis in HSV-1 infected activated T lymphocytes in vitro (Ito *et al*., 1997b), it may play a role in some systems (Geiger *et al.*, 1997). The specific mechanisms by which apoptosis is regulated in the HSV-infected cells is the subject of current investigation.

III. HSV-1 mutants used as vectors for cancer gene therapy

HSV-1 vectors have been engineered following two different strategies. Recombinant viral vectors are derived

directly from wtHSV-1, and contain deletion or insertional mutations in various genes. Many investigators have taken the approach of producing HSV mutants with multiple gene deletions, as a means to increase the insertion capacity of the vector and thus be able to host multiple transgenes (Fink and Glorioso, 1998; Johnson *et al.*, 1994). For example, HSV mutants have been engineered with multiple mutations or deletions in genes which include ICP4, ICP27, ICP8, UL33, UL42 and gB and gH to attenuate viral replication (Breakefield and DeLuca, 1991; Glorioso *et al*., 1997). For example, HSV mutants with various combinations of deletions of ICP4, ICP22, ICP27 and ICP42 yield viral mutants with minimal cytotoxicity, due to their inability to replicate in normal cells (Huard *et al*., 1997; Johnson *et al*., 1992). Nevertheless, these vectors have been shown to achieve expression of transgenes in normal cells, in which the transgene is expressed with minimal expression of HSV genes. Recombinant multi-attenuated vectors have been utilized in experimental cancer gene therapy, and their use for suicide or immune gene therapy of extra-CNS malignancies is recently gaining interest (Glorioso *et al.*, 1995). A multi-attenuated HSV vector with alterations in ICP4, ICP22, ICP27 and ICP41 was utilized to transduce several ovarian cancer cell lines with the suicide gene HSV thymidine kinase, and was found to achieve high transduction efficiency (Wang *et al.*, 1998). Further studies are needed to determine whether sufficient cells can be transduced to yield a clinical benefit. Rees *et al.* (1998) constructed a mutated HSV vector that could undergo a single round of viral replication and express murine granulocyte colony stimulating factor (mG-CSF). This vector exhibited efficient transduction and achieved effective immunization in a murine syngeneic renal carcinoma model (Rees *et al.*, 1998).

A second type of multi-attenuated vectors, the amplicon vectors, are engineered utilizing plasmids carrying the HSV DNA packaging signal, the HSV origin of DNA replication, expression cassettes regulating the transgenes of interest together with an *E-coli* origin of DNA replication and antibiotic resistance genes (Frenkel *et al.*, 1994; Geller, 1993; Geller and Breakefield, 1991; Ho, 1994). Although propagation of amplicon vectors initially required co-infection with HSV helper virus (Frenkel *et al.*, 1994; Geller, 1993; Geller and Breakefield, 1991; Ho, 1994), amplicons can now be propagated by complementation using plasmids (Fraefel *et al.*, 1996). Amplicon HSV vectors have been utilized to rapidly transduce hepatoma cells from cultured cells or tissue explants with IL-2 or GM-CSF genes (Karpoff *et al.*, 1997; Tung *et al.*, 1996). Administration of these transduced cells into rats or mice, respectively induced an immune response to the hepatomas. Toda *et al.* (1998a) showed that co-expression of IL-12 by an HSV amplicon in the presence of an oncolytic G207 helper virus augmented the anti-tumor effect. Preliminary data indicated that an HSV amplicon vector carrying IL-2 was found to achieve high therapeutic efficacy in treating intraperitoneal metastatic gastric carcinoma in nude mice and to increase the killing activity of splenocytes (Tsuburaya *et al.*, 1998). Furthermore, subcutaneous murine lymphoma nodules were eradicated in approximately 85% of tumor-bearing mice by co-administration of HSV amplicon vectors expressing the chemokine RANTES and the T cell costimulatory ligand B7.1 (Kutubuddin *et al.*, 1998).

IV. HSV-1 mutants used as direct oncolytic agents

Molecular alterations in certain genes of the HSV genome have led to the engineering of replication-restricted HSV mutants, which maintain the ability to infect and rapidly kill proliferating cancer cells but still maintain low (or undetectable) replication rates in normal diploid cells. Several genes have been the target of alterations including the thymidine kinase (UL23) (Jia *et al.*, 1994; Martuza *et al.*, 1991; Sanders *et al.*, 1982), the ICP6 gene (UL39) encoding the large subunit of HSV ribonucleotide reductase (RR) (Boviatsis *et al*., 1994; Idowu *et al.*, 1992; Kramm *et al*., 1997), the uracil DNA glycosylase (UNG) gene (Pyles *et al*., 1997) and the ICP34.5 (Chambers *et al.*, 1995; Kesari *et al*., 1995; Mineta *et al*., 1995). The thymidine kinase-negative HSV-1 mutant (Jia *et al*., 1994; Martuza *et al*., 1991) was shown to efficiently cause tumor growth inhibition after intraneoplastic inoculation of subcutaneously and subrenally implanted experimental human gliomas with minimal toxicity in immunodeficient mice. It may also be effective for treatment of other solid tumors localized in the periphery. Although HSV*tk*$^-$ mutants were sensitive to foscarnet and phosphonormal acid (Jia *et al.*, 1994), a potential disadvantage of these strains relates to their resistance to commonly used anti-herpetic drugs such as acyclovir or ganciclovir and has spurred the engineering of alternate attenuated HSV vectors. HSV mutants lacking the ribonucleotide reductase through a deletion or mutations of ICP6 gene were also shown to be replication-restricted and demonstrated efficacy in CNS malignancies. The HSV-1 ribonucleotide reductase deficient (RR$^-$) mutant hrR3, containing an *E-coli LacZ* gene insertion in the ICP6 gene, was recently tested in an experimental metastatic colon carcinoma with liver metastases in an immunodeficient mouse model (Carroll *et al.*, 1996). This mutant displayed selectivity only for the intrahepatic tumors *in vivo* and did not spread to the surrounding normal liver after intrasplenic injection, supporting the notion that it replicated only in dividing cells, which provided RR in complementation (Carroll *et al.*, 1996). HSV oncolytic agents have also been generated by mutations or deletions of the ICP34.5 genes, altering both copies in the HSV genome (Chambers *et al.*,

1995; MacLean *et al.*, 1991). Its protein product is implicated in the prevention of the protein synthesis premature shut-off in the infected host, through inhibition of the phosphorylation of the eukaryotic translation initiation factor eIF2α (Cassady *et al.*, 1998b), as well as in viral exit from the cell (Brown*et al.*, 1994). ICP34.5$^{-/-}$ mutants have proven efficient in treating several types of CNS malignancies in experimental rodent models (Andreansky *et al.*, 1997; Chambers *et al.*, 1995; Kesari *et al.*, 1995; Kramm *et al.*, 1997) and efficiently treat experimental tumors of melanoma (Randazzo *et al.*, 1997) and mesothelioma origin (Kucharczuk *et al.*, 1997). HSV-1716 was efficacious in the treatment of intraperitoneal (i.p.) human malignant mesothelioma in a severe combined immunodeficient (SCID) mouse model (Kucharczuk *et al.*, 1997), reducing tumor burden and prolonging animal survival in a dose-dependent manner. Administration of the HSV-1716 yielded viral replication only within i.p. tumor nodules. There was no evidence of viral antigen (by immunohistochemistry) or DNA (by polymerase chain reaction analysis) in any mouse organs. The same virus was also used to treat experimental subcutaneous melanoma, yielding similar efficiency and minimal toxicity (Randazzo *et al.*, 1997). Since mRNA for HVEM was readily detected in lung tissue (Montgomery *et al.*, 1996), HSV mutants lacking ICP34.5 were investigated and demonstrated efficacy *in vitro* and *in vivo* against several human lung carcinoma lines (Abbas *et al.*, 1998).

A second generation of multi-attenuated viruses were engineered stemming from a parental ICP34.5-deleted virus, R3616, which is based on the wt HSV-F strain (Chambers *et al.*, 1995). R3616UB was generated by interrupting the uracil DNA glycosylase (UNG) gene in the parental HSV-R3616 mutant (Pyles *et al*., 1997). This viral strain did not show any replication in primary human neuronal cultures *in vitro* and did not spread to normal murine CNS but exerted a direct oncolytic activity *in vitro* and *in vivo* against human CNS tumor cell lines and brain tumor xenografts. Moreover, this mutant demonstrated a hypersensitivity to the anti-herpetic drug ganciclovir. G207 is also a derivative of the ICP34.5-deleted mutant, R3616, in which β-galactosidase is inserted into ICP6 gene, which encodes the large subunit of the ribonucleotide reductase gene (Mineta *et al.*, 1994). This mutant was also found to be efficacious in the treatment of various CNS tumors (Mineta *et al*., 1994; Mineta *et al.*, 1995; Yazaki *et al*., 1995). Both these doubly deleted HSV mutants appear promising for extra-CNS applications. G207 demonstrated efficacy against some tumor cell lines of breast origin both *in vitro* and *in vivo* (Toda *et al.*, 1998b). In our laboratory, a single i.p. administration of HSV-G207 to SCID mice bearing i.p. human ovarian carcinoma tumors (SKOV3 cell line) led to significant reduction in tumor volume four weeks later (**Table 1**). Immunostaining of tumors harvested from HSV-treated animals demonstrated the presence of HSV-1 antigens in multiple scattered areas throughout the tumor nodules, demonstrating the ability of the virus to replicate and penetrate in depth within the tumors (not shown). Extensive necrosis was observed adjacent to the areas that were positive for HSV particles. An emerging strategy for engineering replication selective HSV oncolytic agents involves replication-targeted HSV mutants, achieved through the insertion of tissue-specific promoters regulating HSV replication. To demonstrate the feasibility of this system, an expression cassette containing a heterologous eukaryotic promoter (albumin) regulating ICP4 expression was inserted into an ICP4^{-} mutant (Miyatake *et al.*, 1997). The authors observed that these viruses replicated 10-fold better in albumin-expressing hepatomas than in cells which did not express albumin.

	Pre-treatment	**Control (Media)**	**HSV-G207**
Tumor Weight	12.5±4 mg	278±45 mg	48±7 mg *****

Table 1. To assess the efficacy of HSV-G207 in treating epithelial ovarian cancer *in vivo*, SCID mice (n=10/group) were administered a single intraperitoneal (i.p.) injection of 5x10^6 SKOV3 cells, which led to the establishment of i.p. tumors two weeks later. HSV-G207 was administered directly i.p. to a group of animals at that time. Control animals received media only. Animals from each group were sacrificed four weeks following treatment. A separate group of animals was sacrificed prior to viral administration at two weeks. Tumors were dissected and weighed. Weights are expressed in mg and values are expressed as the mean ± standard error (M±SE). (***** =p<0.001 vs. control animals).

V. HSV mutants used in the immune therapy of cancer

Since HSV-1 and HSV-2 infections are highly prevalent in the adult human population (Whitley, 1996), the effects of the immune response on the efficacy of HSV-based oncolytic or gene therapy in humans is an important issue. To address this issue, the effects of a pre-existing immunity to HSV-1 was tested in a syngeneic rat model. The presence of anti-HSV primed immune response was found to dampen but not

abolish gene transfer by an HSV vector (Herrlinger *et al.*, 1998). However, it should be noted that the clinical significance of pre-existing immunity is still unknown in viral-based oncolytic or gene therapy. In fact, HSV-1 or HSV-2 recurrences occur commonly following a primary infection in the immunocompetent human (Whitley, 1996). Moreover, adenoviral-mediated gene transfer in a phase-1 clinical trial for the treatment of malignant mesothelioma was not blocked by significant anti-adenoviral neutralizing antibody titers or significant T cell proliferation (Molnar-Kimber *et al.*, 1998). Thus, the effect of the immune response on the efficacy of viral therapies will have to be determined in clinical studies.

The interaction of the immune system with HSV-based therapeutic agents could potentially become advantageous. In fact, the utilization of HSV mutants as direct oncolytic agents or as vectors could generate or enhance an anti-tumor immune response. Infection of human cells by wild-type HSV induces an orchestrated immune response, which includes a cellular infiltrate, generation of cytotoxic T lymphocytes (CTL), release of cytokines and induction of an antibody response (Whitley, 1996) and ref. therein). Although ICP47 can decrease the expression of class I major histocompatibility antigens on the cell surface (York *et al.*, 1994), tumor cell infection and death following infection by mutant HSV-1 will most likely induce intratumoral infiltration of lymphocytes and antigen-presenting cells and may lead to unmasking of tumor antigens, triggering an anti-tumor response. This strategy could become particularly advantageous in tumors that down-regulate the immune response or induce a predominant T_H2-like response. Recent experimental evidence supports the concept that HSV-based oncolytic therapy may be followed by an adjuvant tumor-specific immune response (Toda *et al.*, 1998a). In fact, intratumoral administration of HSV-G207 in immunocompetent animals bearing syngeneic tumors led to growth inhibition of distant non-inoculated tumors, likely mediated by an immune response (Toda *et al.*, 1998a).

Cytokines have been shown to enhance the anti-tumor immune response, but their systemic administration has been accompanied by significant side effects. Local administration of cytokines to tumors has led to decreased magnitude of side effects but may be technically challenging (Pardoll 1996). Recent evidence suggests that gene therapy with delivery of cytokine genes into tumors or the generation of cytokine gene-transduced cancer cell vaccines may represent a very powerful tool for augmenting anti-tumor immune responses (Pardoll, 1996). For instance, expression of interferon gamma (INFγ), tumor necrosis factor alpha (TNFα) or GM-CSF in the milieu of the tumor has led to arrest of tumor growth in experimental models *in vivo* through stimulation of local inflammatory and immune responses (Andreansky *et al.*, 1998; Pardoll, 1996; Tepper and Mule, 1994). HSV-1 mutants represent suitable vectors for immunotherapy as they can accommodate large and multiple transgene inserts and efficiently deliver interleukin transgenes into tumors. The administration of a defective HSV vector containing tandem repeats of an amplicon plasmid encoding IL-12 together with a multi-attenuated HSV-1 mutant lacking ICP34.5 and RR (HSV G207) was followed by significant reduction in tumor growth in a syngeneic murine colon carcinoma model (Toda *et al.*, 1998a). Importantly, IL-12 was expressed and secreted by infected tumor cells *in vitro* and *in vivo*. Unilateral inoculation of the virus and amplicon was accompanied by regression not only of the inoculated tumor but also of non-inoculated controlateral tumors. In addition, tumor reduction was significantly greater in animals receiving the amplicon plasmid encoding IL-12 compared to those receiving a control *LacZ*-expressing amplicon plasmid together with the HSV G207 helper. This effect was attributed to the enhancement of tumor-specific CTL activity (Toda *et al*., 1998a). Moreover, a replication-restricted HSV ICP34.5$^{-/-}$ mutant encoding murine IL-4, but not IL-10, was shown to significantly prolong the survival of glioma-bearing mice (Andreansky *et al.*, 1998). Clearly, similar viruses encoding cytokines or immunostimulatory molecules appear very attractive for the treatment of non-CNS tumors as well. Additional support for the potential of HSV-based cytokine-mediated immunotherapy is provided by the observations that amplicons expressing RANTES and B7.1 (Kutubuddin *et al.*, 1998) or IL-2 (Tsuburaya *et al*., 1998) or a multi-attenuated HSV vector expressing GM-CSF (Rees *et al.*, 1998) were showed to augment the efficacy of treatment of lymphoma, metastatic gastric carcinoma or renal carcinoma, respectively, as mentioned above.

VI. Toxicity considerations

Large amount of pre-clinical data has been accumulated in the rodent model on replication-selective attenuated HSV-1 ICP34.5$^{-/-}$ mutants following intratumoral "stereotactic" inoculations of viral particles within the CNS. In both immunocompetent as well as immunodeficient mice, intracranial administration of viral particles did not lead to encephalitis (Andreansky *et al.*, 1997; Carroll *et al.*, 1996; Chambers *et al.*, 1995; Kaplitt *et al.*, 1994; Kesari *et al.*, 1995; Mineta *et al.*, 1994). HSV-1716 administered intracranially or intraocularly into SCID mice resulted in low or no virulence (Valyi-Nagy *et al.*, 1994). Similarly, HSV-1716 administered i.p. was found to be avirulent in SCID mice in contrast to rapid systemic spread of the wt HSV virus and death of the animals (Kucharczuk *et al.*, 1997). No viral spread was detected beyond the tumor tissue (Kucharczuk *et al.*, 1997). Administration of HSV-1716 to normal human skin in a murine xenograft model was accompanied by no toxicity, while administration of a wild-

type HSV-1 led to rapid destruction of the xenograft (Randazzo *et al.*, 1996). The replication selective hR3, a HSV-1 lacking RR expression, administered systemically (intrasplenic injections) was also found to infect only metastatic human colonic adenocarcinoma tumor nodules within the liver but not the surrounding murine normal liver tissue (Carroll *et al.*, 1996). However, HSV-1 ICP34.5-deleted mutants maintain their ability to infect ependymal cells in the CNS (Kesari *et al.*, 1998; Markovitz *et al.*, 1997). Severe intra-CNS inflammation was observed in some rodent strains after intracranial administration of HSV1716 which expressed *LacZ* (McMenamin *et al.*, 1998). It is possible that additional mutations may significantly decrease any potential for HSV-1 neurotoxicity. Administration of G207, an ICP34.5-deleted/RR- mutated virus was found to be safe following administration to HSV-sensitive primates (Markert *et al.*, 1998). Sufficient toxicity data on the HSV ICP34.5 mutants, HSV-1716, and G207 ($ICP34.5^{-/-}$,RR^{-}), has been presented to the regulatory bodies for initiation of phase I clinical trials. Preliminary results from the dose escalation phase I clinical trials employing HSV-1716 ($ICP34.5^{-/-}$) or G207 ($ICP34.5^{-/-}$, RR^{-}) utilizing intra-CNS administration for the treatment of malignant glioma have reported minimal side effects in humans (Brown *et al.*, 1998; Markert *et al.*, 1998).

VII. Conclusions

Attenuated HSV-1 mutants may represent an emerging powerful tool in human gene cancer therapy. HSV mutants are versatile in that, when partially attenuated, they can function as direct oncolytic agents capable of proliferating within three-dimensional tumors and causing tumor cell death. The advantage of replication-restricted HSV mutants is that they can selectively replicate in tumor cells and thus, potentially express transgenes in a higher percentage of the tumor cells. Alternatively, when super-attenuated or amplicons, they can function as efficient vectors for gene therapy. In that capacity these vectors have the potential to host large and multiple transgenes. A multi-pronged strategy for HSV-based anti-tumor therapy is currently emerging, where multi-attenuated viruses or the oncolytic HSV mutants are used as gene therapy vectors for intratumoral delivery of immunomodulatory or chemotherapy sensitizing transgenes. Based on experimental evidence, HSV-based tumor therapy may induce an anti-tumor "vaccine" effect. This may be due to the immunogenic properties of the virus, as well as to the tumor tissue necrosis. Thus, HSV oncolytic agents and gene therapy vectors show great potential as anti-tumor therapies. Further clinical studies are required to test the clinical efficacy and safety of these agents in extra-CNS malignancies.

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