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The role of HSV amplicon vectors in cancer gene therapy

Review Article

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Summary

Recent progress in tumor biology, virology and immunology has led to new approaches to the gene therapy for cancer. Herpes Simplex Virus (HSV) based vectors are attractive vectors for gene therapy use due to a number of favorable biologic features. Several characteristics render HSV suitable for gene therapy, including high transduction efficiency, ability to transduce non-dividing cells, high packaging capacity, wide cellular tropism and the ability to package multiple copies of the same gene or several genes. Newer HSV vectors differ in replication potential, sensitivity to anti-viral agents, neurotoxicity, tumor-specific cytotoxicity and persistence in the host cell. So-called "oncolytic" HSV based vectors demonstrate selective replication in tumor cells relative to normal tissue. HSV amplicon based vectors allow genetic transfer of multiple transgene copies in the absence of viral genes. This degree of flexibility, relative to other viral vector systems, has allowed for the use of HSV vectors in a variety of antitumor strategies including oncolytic, as well as immune-based strategies. Successful immune based strategies in animal models have included transfer of cytokines, costimulatory molecules and/or chemokines. Phase I/II clinical trials using HSV based vectors have been initiated.

I. Introduction

Current advances in the understanding of the viral structure and function, mechanisms of viral entry, and replication have resulted in the use of engineered viruses for the treatment of cancer. Although effective gene transfer based on the murine retrovirus vectors was developed in the 1980s, the use of retroviral vectors in cancer gene therapy was hampered by low titers, poor transduction efficiency and limited expression of the desired gene products. As a result, other viruses such as adenovirus, adeno-associated virus and herpesviruses have been explored as viral vectors for gene therapy of cancer.

Herpes simplex virus type 1 (HSV-1) is one of the most extensively characterized of all the herpes viruses and has several advantages over other viruses used as gene therapy vectors. Among the advantages are a wide host range, high efficiency of gene transfer and ability to replicate in dividing and non-dividing cells. Multiple genes can be simultaneously expressed due to the large genome size that can be replaced with the desired gene(s) up to ~30 kb (Mocarski et al. 1980). The HSV genome consists of a linear double stranded DNA of ~150 kb size coding for over 83 genes (Sears and Roizman.1996). The genome is divided into long and short segments each flanked by their own terminal repeat sequences . Following entry of HSV into a susceptible cell, the viral capsid is transported to the nucleus and the viral genome released in the nucleoplasm where it is transcribed. Transcription of HSV genes is regulated in a tightly controlled manner. Immediate early (IE) or α-genes which are not dependent on the viral protein synthesis are the first to be expressed. The second set of genes, early genes (E) or β-genes are expressed in the presence of IE gene products and encode enzymes

required for DNA synthesis and virus genome replication. Other genes, late or γ-genes specify products for virus assembly, tegument and envelope glycoproteins. HSV replicates through a "rolling circle" mechanism and the viral genome is packaged into assembled capsids (Sears and Roizman.1996). The mature capsids acquire envelope proteins at the nuclear membrane and the virus particles exit from the cell by exocytosis (Locker et al. 1979). During the natural course of infection, HSV may be a lytic virus or can remain latent in the infected cells of neural origin (Ward et al. 1994). During HSV latency, lytic genes are silenced and a set of stable processed intronic RNA transcripts known as latency associated transcripts (LATs) are expressed (Stevens et al. 1987; Stevens. 1989). The ability of HSV to remain latent in the infected host, can be advantageous for vector development if long-term gene expression is desired.

II. HSV in cancer gene therapy

The development of HSV vectors for gene therapy of cancer coincided with efforts to create a prototype HSV recombinant vaccine for HSV-1 and HSV-2. Mutants of HSV-1 were generated by making deletions in certain regions to eliminate virulence and toxicity of the virus (Post et al. 1981; Jenkins et al. 1985). HSV derived deletion mutants can be divided into either replication competent or replication defective vectors and generally fall into four categories:

A. Amplicons

HSV amplicons are plasmids containing only an HSV origin of replication ('ori') and packaging sequence ('pac'). The recognition that such amplicons could be constructed grew out of the observation that defective interfering particles accumulating in HSV stocks passaged at high moi were simple reiteration of 'ori' and 'pac' sequences (Spaete et al. 1982; Kwong et al. 1995; Sears and Roizman. 1996). Such defective viral particles are comprised of amplified head-to-tail concatemers, hence plasmids designed for propagation in HSV-1 have been called "amplicons". These amplicon plasmids are engineered to contain the HSV sequences for 'ori' and 'pac' in cis, which allow them to be packaged as a concatemeric repeated unit into the viral capsid (Frenkel et al. 1994). These sequences represent < 1% of the ~150 kb HSV genome. Since the 'ori' and 'pac' sequences together measure less than 1 kb, amplicon vectors can be used to clone large fragments of foreign DNA and can serve as excellent vectors for delivery of the desired genes into the host cells. Several genes can be cloned into a single amplicon vector and different amplicon vectors can be mixed during packaging to generate vector stocks containing multiple combinations of different genes. Since the HSV amplicons only include cis signals for replication and packaging and do not encode any viral proteins ('ori' and 'pac' are non-coding) , they must be supplied with both viral structural and nonstructural proteins for viral DNA synthesis and exocytosis.

HSV amplicons are packaged in the presence of a defective HSV-1 helper virus. Detailed methods for amplicon packaging have been previously described (Geschwind et al. 1994a; Kwong and Frenkel. 1995).The basic components of HSV amplicon packaging systems include: a) helper virus HSV-1 deleted in an essential gene such as (IE3, also known as ICP4), b) a packaging cell line such as RR-1 cells, which is an IE3 expressing BHK derived cell line that complements the deleted function of the helper virus *in trans*, and c) amplicon plasmid DNA. The packaging procedure involves transfection of the amplicon plasmid into the packaging cell line, followed by infection with HSV-1 helper virus (**Figure 1**). The HSV-1 genes present in the helper virus and the packaging cell line allow for the replication and packaging of a concatemeric form of amplicon DNA (containing the gene of interest) packaged in HSV assembled virions. The resultant virus stock obtained therefore is a mixture of helper and vector virions that can subsequently be concentrated, titrated and stored at -70^0C for use. The amplicon titers that can be obtained by the use of helper virus range from 10^8 to 10^9 pfu/ml. Until recently, production of amplicons required repeated passaging of the amplicon/helper preparation to increase amplicon vector titer.

Due to the HSV tropism for tissues of CNS origin *in vivo* in the infected host, amplicon vectors have been tested for treatment of diseases of the central and peripheral nervous system both *in vitro* (Federoff et al. 1992; Battleman et al. 1993; Geller et al. 1993; Geschwind et al. 1994b) and *in vivo* (During et al. 1994; Kaplitt et al. 1994). Significant progress has been made in the use of HSV amplicons for expressing several neuronal genes including nerve growth factor (NGF) (Federoff et al. 1992; Bahr et al. 1994; Geschwind et al. 1994b), brain-derived neurotrophic factor (BDNF) (Geschwind et al. 1996; Garrido et al. 1998), the NGF receptor (*trkA*) for converting NGF non-responsive cells to NGF responding cells (Xu et al. 1994), and neurotropic glutamate receptor (Bergold et al. 1993).

More recently, amplicon vectors have been tested for utility in treatment of cancer in several experimental models in different laboratories including owr own. Amplicons have been used to transduce an immune-stimulating gene (e.g. B7.1[CD80], IL-2, IL-12, and GM-CSF) or drug-susceptibility genes (e.g. HSV-*tk*) for cancer therapy.

Although amplicon use affords great flexibility, persistence of helper virus in amplicon preparations is

currently a potential limitation. Prolonged expression of the desired gene is possible in post-mitotic cells; however, in dividing cells, non-replicating amplicon genomes are segregated which results in progressive loss. One attempt to address this issue was by the inclusion of specific EBV sequences required to maintain the amplicon as an episome in the nucleus of transfected cells (Wang et al. 1997). Epstein-Barr virus (EBV) has been shown to contain a unique latent replication origin (ori P) which directs viral self-replication and maintenance in cells without entering the lytic cycle (Yates et al. 1985). EBV nuclear antigen1 (EBNA-1) is a DNA binding transactivator for ori P. HSV amplicon vectors carrying the combination of EBNA-1 and EBV ori P should be more effective for stable segregation following division of eukaryotic cells.

B. Helper virus free amplicons

Helper virus/amplicon mixture preparations are toxic to most cells because of helper virus expressed IE genes and this has stimulated efforts to produce helper-free amplicon systems. Amplicon preparations that are free of helper virus have been made using cosmid sets containing the entire HSV genome with deletions in the packaging sequences (Cunningham et al. 1993; Fraefel et al. 1996). These cosmids provide the necessary structural and non-structural proteins for amplicon replication and packaging into virus capsids but cannot be packaged themselves due to absence of packaging sequences. Cotransfection of cells with this modified cosmid sets and amplicon plasmid DNA results in the production of amplicon stocks free of detectable helper virus. Helper virus free amplicon preparations have been produced by some groups but virus yield produced by this method is relatively low (~10^7 pfu/ml). More recently, efficient helper free amplicon methods were reported using bacterial artificial chromosomes (BAC) encoding the HSV genome minus the packaging sequences (Stavropoulos et al. 1998). Such helper free amplicon preparations avoid disadvantages related to expression of ICP47 and other HSV encoded genes that may influence immune response to transduced tumor cells.

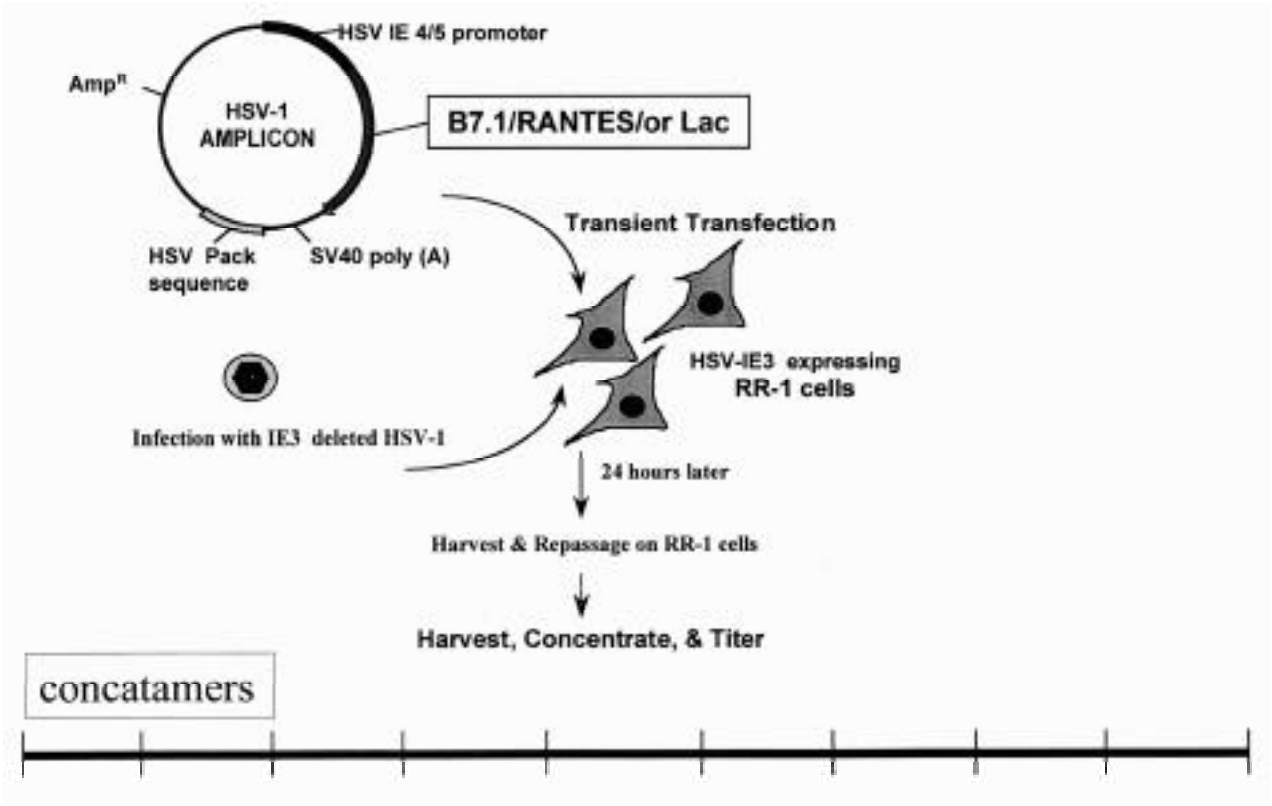

Figure 1. Generation of HSV-1 Amplicon Virus : HSV amplicon plasmids are transfected into RR1 cells and 24 hours later IE3 deleted HSV-1 virus is added to the monolayer. Once cytopathic changes are observed, the cells are harvested, freeze-thawed and sonicated. Viral supernatent is repassaged on RR1 cells to increase titer, and then harvested concentrated and titered. The amplicon plasmid is packaged in a concatemeric form.

C. Replication defective HSV vectors

In an attempt to decrease toxicity, recombinant replication defective vectors have been designed with deletions in the ICP4, ICP22, ICP27, or ICP0 genes (Wu et al. 1996; Samaniego et al. 1997; Samaniego et al. 1998). An example of this is the disabled single-cycle virus (DISC-HSV). DISC-HSV lacks the gene for the essential glycoprotein H (gH) and can be grown to high titers in complementing cells expressing the gH gene. DISC-HSV particles have been shown to transduce a variety of human cell types. Murine pre-B cell leukemia cells transduced with DISC-HSV encoding GM-CSF had an improved survival in mice with the tumor cells acting as a potent vaccine (Dilloo et al. 1997). These vectors are less toxic than replication competent vectors since infected cells do not produce infectious virus.

D. Replication restricted HSV vectors

A variety of replication restricted HSV mutants have been adapted to selectively grow in tumor cells. Replication competent mutants for gene therapy of cancer include viruses that are deleted or attenuated for the ICP-6 and/or $\gamma_1$34.5 genes, ICP0, thymidine kinase, or the US3 protein kinase genes (Glorioso et al. 1995; Kesari et al. 1995; Mineta et al. 1995; Yazaki et al. 1995; Coffin et al. 1996). Most of these genes code for enzymes involved in nucleic acid metabolism (e.g. thymidine kinase, DNA polymerase, ribonucleotide reductase) which are not expressed in post-mitotic neuronal or other differentiated cells such as hepatocytes, but are induced in tumor cells. Therefore, replication restricted mutants have limited ability to replicate in non-dividing cells and preferentially replicate in tumor cells (McKie et al. 1996). The G207 vector (mutated in ribonucleotide reductase and the $\gamma_1$34.5 genes) or the HSV-tk$^-$ mutant replication competent viruses have been successfully tested in brain tumor models (Markert et al. 1993; Kesari et al. 1995; Mineta et al. 1995; Yazaki et al. 1995; Coffin et al. 1996). Use of these vectors is based on the ability of these mutants to replicate in actively growing glioma cells while sparing normal post-mitotic brain cells, thereby reducing the collateral damage. $\gamma_1$34.5 is present in two copies and encodes a 263 amino acid protein in the type 1 herpesvirus. The 70-amino acid carboxyl terminus is highly homologous to the mammalian *g*rowth *a*rrest and *D*NA *d*amage gene *GADD34,* and the carboxyl terminus of GADD34 restores function to $\gamma_1$34.5-deleted virus (Chou et al. 1994a; Chou et al. 1994b). Viral infection normally triggers a host stress response that shuts off protein synthesis and causes apoptosis aborting viral replication. $\gamma_1$34.5 effectively prevents this protein shutoff and allows viral replication to proceed. Specifically, HSV-1 infection activates the host cell PKR kinase and in the absence of a functional $\gamma_1$34.5, this leads to phosphorylation of the α subunit of the translation initiation factor eIF-α2 and total shutoff of protein synthesis (Chou et al. 1995). A second function of $\gamma_1$34.5 is to enable the virus to multiply efficiently in the CNS. Viruses constructed with mutations in this gene are highly attenuated in experimental animals (Chou et al. 1990; Bolovan et al. 1994). This function of $\gamma_1$34.5 appears to be independent of its ability to preclude the protein shutoff. Evidence in support of this is based on the observation that viruses carrying GADD34 in place of $\gamma_1$34.5 show no evidence of protein shutoff but still are attenuated - i.e. replicate poorly in neural tissue (Andreansky et al. 1996). A number of HSV-1 mutants for $\gamma_1$34.5 have been tested as a treatment for CNS tumors in experimental animals with a high degree of success and no incidence of encephalitis (Andreansky et al. 1997). These mutants are still sensitive to acyclovir, thus adding a safety feature to eliminate the virus should an unforeseen complication arise. The success of these mutants prompted others to try them in other solid tumors of non-CNS origin. Kucharczuk et.al. (Kucharczuk et al. 1997) showed the effectiveness of an HSV mutant for $\gamma_1$34.5 in an animal model for mesothelioma. G207 had also been tested in a metastatic breast cancer model with growth inhibition and/or improved survival in mice harboring susceptible tumors (Toda et al. 1998b).

Although replication restricted HSV vectors are engineered to selectively kill tumor cells while sparing the normal host tissues, these vectors have their limitations including lack of efficacy (DNA polymerase mutants), resistance to the two main HSV therapeutics in clinical use, acyclovir and ganciclovir (thymidine kinase negative mutants), retained capacity to induce encephalitis (thymidine kinase and DNA polymerase mutants); lack of an animal model due to significant differences in human and mouse or rat susceptibility to genetically engineered HSV (ribonucleotide reductase mutants); and potential loss of susceptibility of tumor cells previously treated with alkylating agents (ribonucleotide reductase mutants) (Andreansky et al. 1996).

III. Cancer gene therapy using HSV amplicons

Direct activation of cytolytic T cells or immuno-modulatory support for their activation has been extensively applied as a cancer gene therapy strategy. Specifically, these efforts are directed at generating a tumor specific CD8+ T-cell response with the goal of generating systemic and lasting immunity. To generate protective immunity, antigen presenting cells and/or tumor cells need to deliver at least two signals to T cells: 1) an antigen specific signal mediated by the T cell receptor (TCR) recognizing tumor specific peptide bound by molecules of

the major histocompatibility complex (MHC) class I or II proteins and 2) a costimulatory signal mediated by ligand/receptor interaction of CD28 on activated T cells with members of the B7 family (CD80,CD86) of "costimulatory" proteins (Green et al. 1994; Bluestone. 1995). Presentation of the first without the second signal may lead to the induction of T cell anergy and such anergic T cells lack the ability to mount a tumor specific T cell response (Mueller et al. 1989). B7.1 transduction has been shown to be effective in generating anti-tumor specific CD8+ T cell responses (Ramarathinam et al. 1994), and several tumor cell lines are rendered immunogenic upon expression of B7.1 (Chen et al. 1994). B7.1 expression has been shown to have a synergistic effect in combination with the secretory cytokines such as IL2, and IL12 in several tumor models (Gaken et al. 1997). The immune response may be further potentiated by providing chemotactic signals at the site of tumor designed to elicit active effector cell migration to the tumor site. Chemokine expression such as the use of RANTES, a C-C chemokine have been shown to have anti-tumor effects (Mule et al. 1996). Identification of novel chemokines and their receptors, and their biological functions in recruitment of immune T cells and monocytes will allow increased flexibility in the use of these molecules to augment tumor specific immunity.

HSV amplicons have now been used in several solid tumor models. In a murine hepatoma model, Tung et.al., (Tung et al. 1996) have used the HSV amplicon vector for expressing human IL2 to transduce murine hepatoma cells (HEPA1-6) which were irradiated to immunize naïve mice (Tung et al. 1996). Such immunized mice were protected upon intra-portal challenge with viable parental HEPA1-6 tumor cells. In contrast, mice immunized with HEPA1-6 cells transduced with the HSV amplicon for lacZ developed liver tumors upon inoculation of parental tumor cells. In another study HSV amplicons encoding IL12 were used for intratumoral delivery in a murine tumor model. Delayed death was observed by the use of HSV-IL12 amplicon (Toda et al. 1998a). More recently the utility of these vectors in treating hematologic malignancies has been explored by our laboratory and others (see below). The characteristics of amplicon vectors, including ease of packaging, high transduction efficiency and ability to achieve high levels of expression in hematopoietic cells render them potentially useful in gene therapy of a variety of malignancies.

IV. Expression of immunogenes using the HSV amplicon in murine and human cells

To examine the infectivity of HSV amplicons, murine EL4 lymphoma cells, as well as human leukemic cells (CML or AML) were infected at different multiplicity of infections (MOIs) with the HSVlac amplicon. 24 hours after transduction cells were stained for beta-galactosidase using X-Gal staining. High levels of expression of the cell associated beta-galactosidase (> 95% cells were positive for beta-galactosidase) were observed at an estimated moi of 0.5 with the use of HSVlac amplicon vector in EL4 cells.

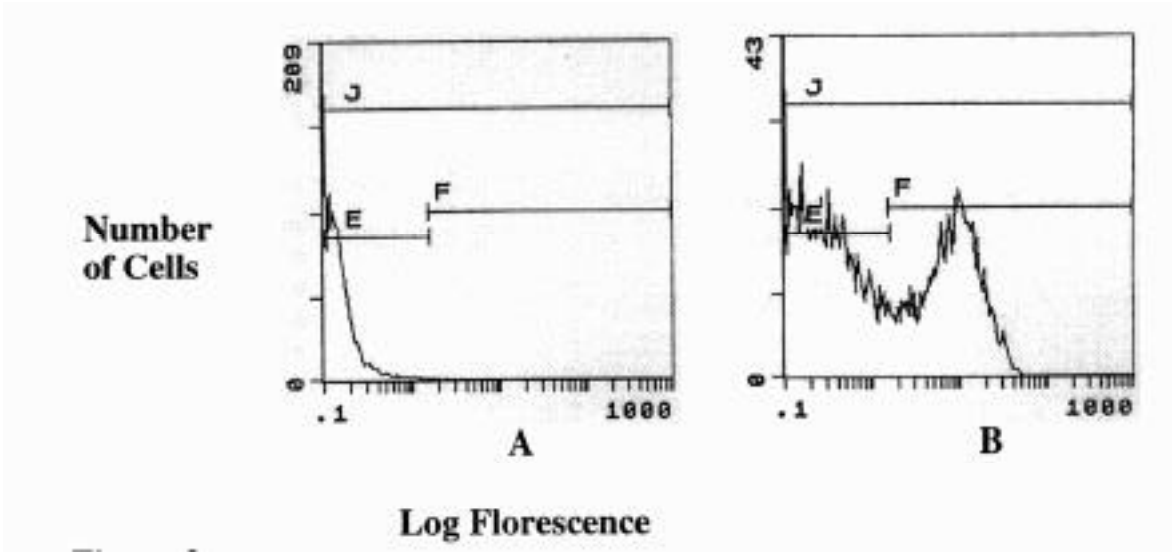

Figure 2a. Expression of B7.1 in primary human CML cells. CML cells are isolated from peripheral blood of a patient in blast crisis by Ficoll-hypaque density centrifugation. Cells are infected with HSVB7.1. 24 hours later, control cells (A) or transduced cells (B) are stained with PE-conjugated anti-B7.1 and samples analyzed by flow cytometry.

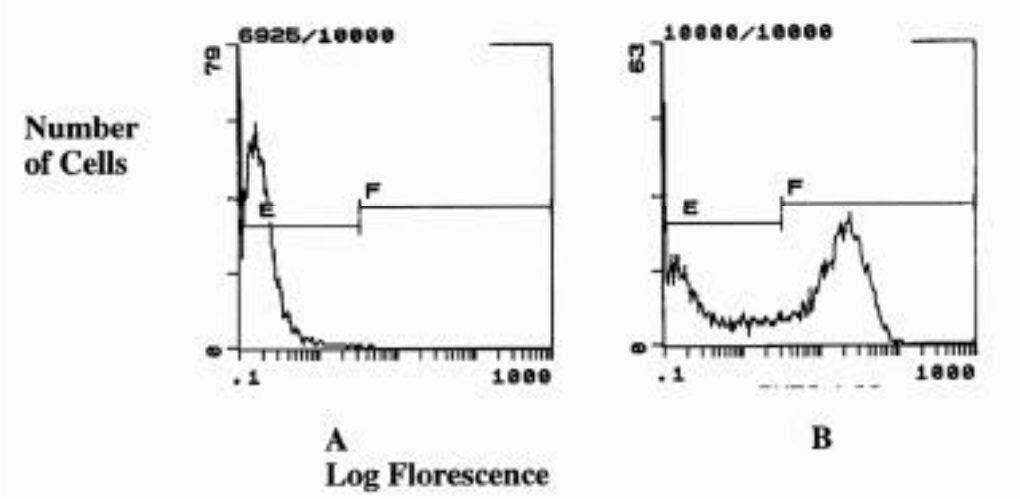

Figure 2b. Expression of B7.1 in primary human AML cells. AML cells are isolated from peripheral blood of an AML patient by Ficoll-hypaque density centrifugation. Cells are infected with HSVB7.1. 24 hours later, control cells (A) or transduced cells (B) are stained with PE-conjugated anti-B7.1 and samples analyzed by flow cytometry.

Flow cytometric analyses were performed for the cell surface expression of B7.1 on murine and human leukemic cells following transduction with the B7.1 encoding HSV-B7.1 amplicon vector. Cells were stained for B7.1 expression (anti-CD80 PE, Becton-Dickinson) and analyzed by flow cytometry. Murine EL4 cells or the human leukemic cells of chronic myelogenous (CML), acute myelogenous (AML), or acute lymphocytic (ALL) origin were negative for B7.1 expression. However, following infection with the HSVB7.1 amplicon vector at an moi of 1 pfu/cell, over 95% of EL4 cells were B7.1 positive (Kutubuddin et al. 1999), and 20-60% of AML, CML or ALL cells stained positively as analyzed by flow cytometry (**Figure 2a & 2b**). When dual staining for B7.1 and MHC class I molecules was performed in human leukemic cells infected with HSV amplicons, we observed significant down modulation of the MHC class I expression in the HSVB7.1 or HSVlac amplicon infected cells relative to the uninfected control cells (**Figure 3**). It has previously been shown that ICP47 expressed by HSV-1 is known to interact with the human TAP-1 protein and thereby prevents the loading of processed peptide onto the MHC class I molecule. Down regulation of MHC-1 may be due to effects of ICP47 carried by helper virus used in HSV amplicon packaging.

In addition to costimulation, we wished to use amplicon mediated gene transfer to recruit immune effector cells to tumor. Murine EL4 or human leukemic cell cultures supernatants were tested following transduction with HSV amplicon vectors encoding the CC chemokine RANTES in a sandwich ELISA using anti-RANTES antibody (R & D Systems) for capture and biotinylated anti-RANTES (R & D Systems) followed by alkaline phosphatase conjugated avidin for detection. No RANTES was detected in control culture supernatants. HSVrantes infected cells produced soluble RANTES in the range of 2ng/ml to 60ng/ml depending upon the moi of infection and the cell type. Soluble human RANTES produced by the HSVrantes infected cells was biologically active as demonstrated by the murine T cell migration assay using transwell chambers (Kutubuddin et al. 1999).

V. Intra-tumoral delivery of HSV amplicon in a murine tumor model

EL4, a murine T lymphoma cell line was used to develop tumors in syngeneic adult C57BL6 mice ($H2^b$) by subcutaneous inoculation. It has previously been shown that B7.1 expression in EL4 cells leads to tumor rejection in mice due to the establishment of systemic cytolytic T cell responses (Chen et al. 1994). We had been successful in eradicating a pre-established EL4 tumor by the delivery of HSV amplicons for B7.1 (HSVB7.1) or RANTES (HSVrantes). Although approximately 50% of the mice were tumor free with HSVB7.1 (17/26) or HSVrantes (11/22) treatment alone, when both amplicons were used in combination, an increased number of mice (23/26) were tumor free after one month. Control mice injected with untreated EL4 or those whose tumors were treated with the HSVlac (14/14) showed uniform tumor growth. Immune mice consistently rejected rechallenge with parental EL4 cells (Kutubuddin et al. 1999).

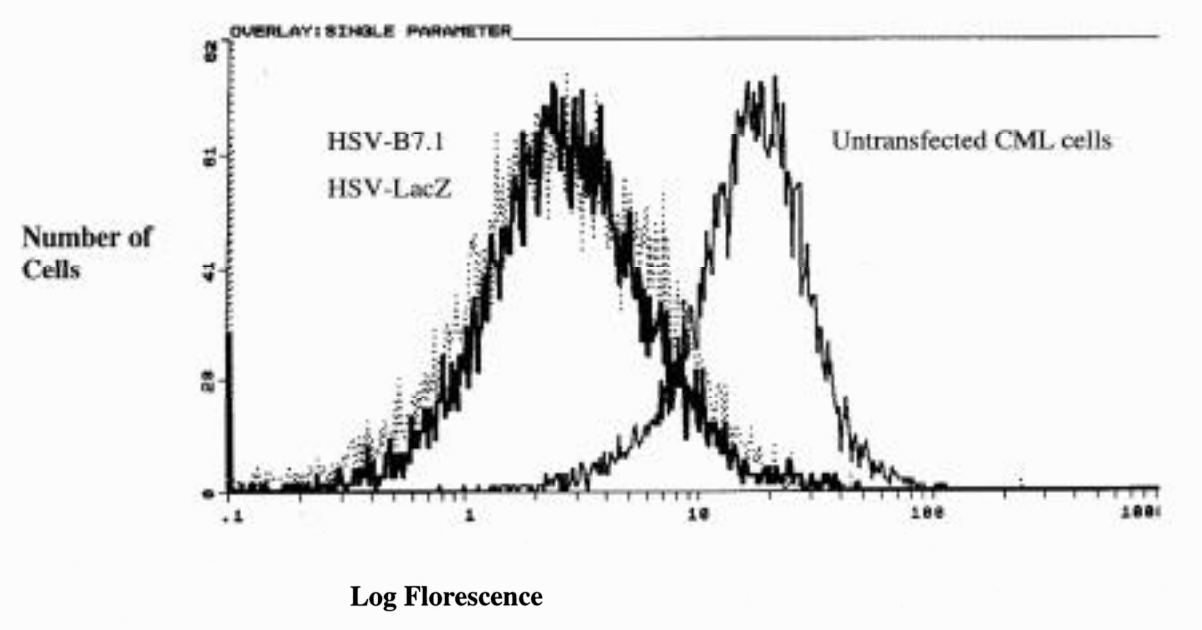

Figure 3. Down-regulation of MHC-I on CML cells transduced with HSV virus coding for B7.1 (CD80) and HSV-LacZ. CML cells are stained with a FITC- conjugated anti-human MHC-I 24 hours after transduction with HSVB7.1 or HSVlac and analyzed by flow cytometry.

In a separate experiment, we tested whether the delivery of HSV amplicon into preestablished tumor resulted in induction of systemic immunity against a contralateral tumor inoculated at the same time on the left hind limb. EL4 cells were inoculated subcutaneously (sc) bilaterally in both the hind limb of mice and tumors allowed to grow to a diameter of 5-6 mm (6-7 days). On day 7 and day 14, HSV amplicons were delivered to the tumor established on the right hind limb (10 mice/group). Complete regression was seen in 5/10 mice inoculated with HSVB7.1, 5/10 inoculated with HSVrantes, and 8/10, in combined HSVB7.1 and HSVrantes treated animals. In animals treated with HSVB7.1 alone (5/5, p=0.0325), or with a combination of HSVB7.1 and HSVrantes (8/8, p=0.0007), regression of the contralateral untreated tumor was consistently observed along with the treated tumor (**Figure 4**). In one HSVrantes treated animal where the treated tumor regressed completely, the contralateral tumor grew at a reduced rate relative to the untreated tumors in control animals. In HSVlac treated animals 10/10 animals demonstrated tumor growth on both the sides, although we noticed that HSVlac treated tumors grew at a slightly reduced rate compared to the control untreated tumors. These experiments demonstrate that systemic immunity generated as a result of intratumoral HSV amplicon injection could prevent progression of the contralateral untreated tumor.

VI. Generation of systemic cell mediated immunity using HSV amplicon vectors

In order to examine the induction of CTL responses in mice transduced intratumorally with HSV amplicon, splenocytes from these mice were harvested and were cocultured *in vitro* along with irradiated stimulator EL4 cells for 6-7 days. *In vitro* primed splenocytes were used at different effector : target ratios and ^{51}Cr release from a fixed number of labeled EL4 cells counted as a measure of CTL activity. EL4 specific CTL activity was seen in splenocytes from mice receiving HSVB7.1 or HSVrantes alone or in combination . CTL responses were only seen in mice in which EL4 tumor regressed following HSVB7.1 and/or HSVrantes amplicon treatment. Control animals which were mock treated or treated with HSVlac amplicon did not show any significant CTL responses. The effector population involved in the observed CTL responses were the CD8+ T cells as lysis was markedly inhibited in the presence of either an anti-T cell monoclonal antibody cocktail (CD4, CD8, or Thy-1), or anti-CD8 antibody, but not by anti-CD4 antibody. Low levels of NK activity were detected when the NK sensitive Yac-1 cells were used as target cells in the lytic assay. Therefore, the predominant effector population appeared to be CD8+ CTLs (Kutubuddin et al. 1999).

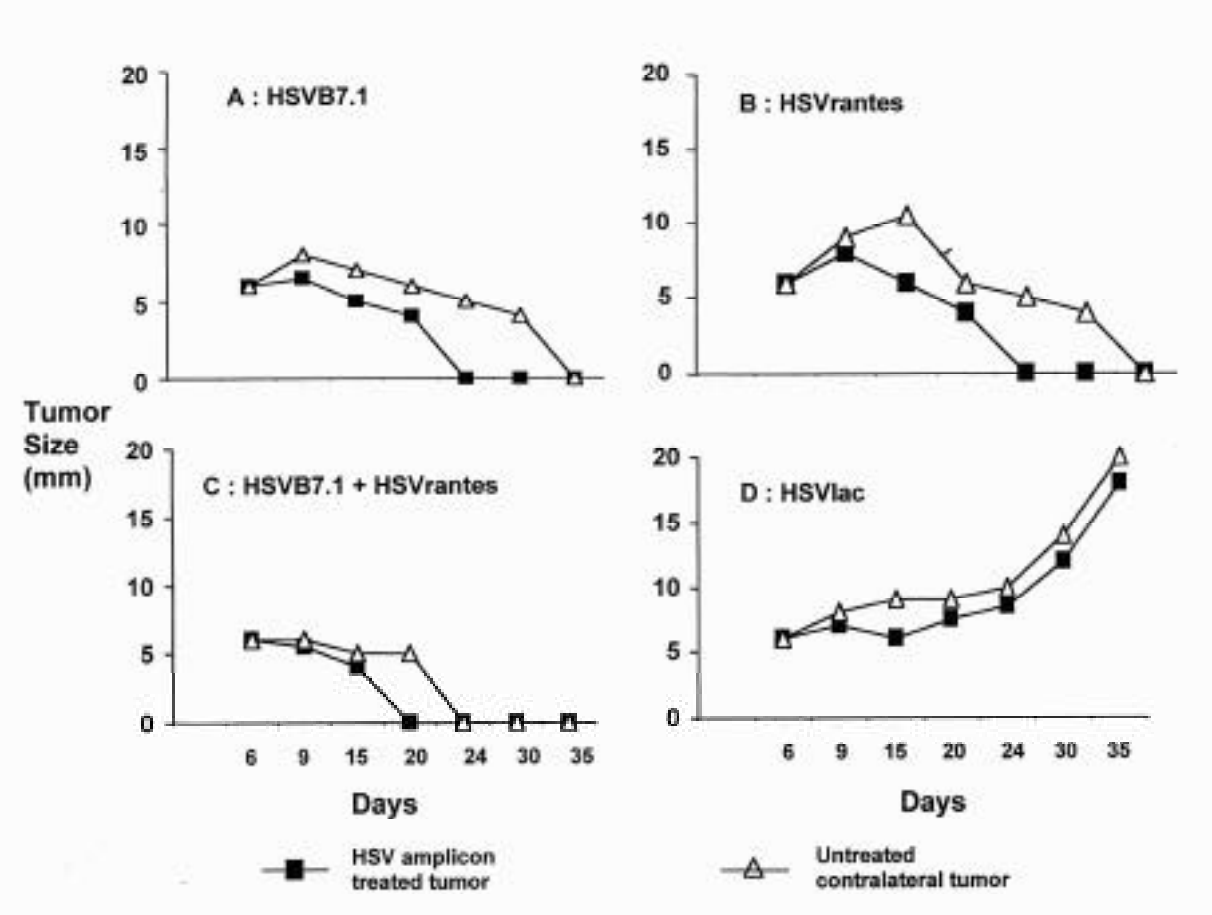

Figure 4. Tumor growth following inoculation of HSV amplicon into preestablished EL4 tumor and growth of parental EL4 cells contra-laterally. Viable EL4 cells (10^6) were implanted sc. on both hind limbs of 8 weeks old C57BL6 mice. Tumors were allowed to develop to a size of 5 to 6mm diameter. HSV amplicon virus (2 X 10^6 amplicon- containing virus particles) was injected into the right tumor on days 7 and 14, and growth of the HSV amplicon- treated and untreated tumors were monitored every 3-5 days.

VII. Future directions

HSV amplicon vectors are safe and effective gene therapy vectors for the expression of immune molecules. High titers of the amplicon virus particles can be generated using a helper virus for packaging, and high levels of expression of the transduced genes can be obtained with the use of the HSV amplicon. HSVB7.1 or HSVrantes amplicons were successfully used to eradicate pre-established tumors in mice. Combined use of the HSVB7.1 and HSVrantes amplicons were more effective than either alone. A tumor specific memory T cell response is established which is effective in preventing tumor growth upon rechallenge. Although HSV amplicon vectors readily infect human cells, including primary leukemic cells, helper virus encoding ICP47 presumably causes decreased MHC-1 expression due to effects on TAP-1. This problem may be overcome using helper free packaging or helper viruses deleted in the ICP47 gene. Current attempts involve packaging with multiple cosmids encoding HSV virion proteins *in trans*. More recently, bacterial artificial chromosomes incorporating a non-replicating HSV genome have been used to provide helper free packaging functions (Federoff H, et al., unpublished). Efforts are also needed to improve the yield of the HSV amplicon when using helper free packaging, as the yield of the HSV amplicon titer using the cosmid system is considerably lower than that achievable with standard helper virus. New generations of HSV amplicons also may encode multiple transgenes as well as genes derived from other virus vectors. One example is the recent incorporation of the adeno-associated virus (AAV) inverted terminal repeats (ITRs) and the AAV rep gene, in addition to the HSV replication and packaging elements (Fraefel et al. 1997; Johnston et al. 1997). AAV rep function can, in theory, recognize the ITRs and allow for subsequent integration in a site specific manner in

chromosome 19 (Berns. 1996). Such an HSV/AAV hybrid vector may be useful for targeted gene delivery to the nucleus of the host cells. In hereditary liver disorders, gene transfer to the liver is highly desirable, and various viral vectors such as adenoviruses, retrovirus, baculovirus have been evaluated. Since HSV amplicon can infect post-mitotic hepatic cells, they are also good candidates for the gene therapy of liver diseases. Further studies are needed to test these vectors in tumor immuno-therapy in the setting of pre-established immunity to HSV. Replication restricted HSV viruses such as the $\gamma_1$34.5 genes deleted G207 virus may preferentially replicate in tumor cells, and can also be used to package amplicons. Whether the lytic function of replication restricted viruses will contribute to tumor antigen uptake and "representation" by antigen presenting cells remains to be tested. HSV vectors have considerable promise as safe, effective vectors for the gene therapy of cancer and other diseases.

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