Oncolytic Herpes Simplex Virus Type 1 and Host Immune Responses

Hiroshi Fukuhara[1] and Tomoki Todo[2,*]

[1]*Departments of Urology and* [2]*Neurosurgery, The University of Tokyo, Tokyo, Japan*

Abstract: The use of oncolytic herpes simplex virus type 1 (HSV-1) is a promising strategy for cancer treatment. Accumulating evidence indicates that, aside from the extent of replication capability within the tumor, the efficacy of an oncolytic HSV-1 depends on the extent of induction of host antitumor immune responses. Ways to modify the host immune responses toward viral oncolysis include expression of immunostimulatory molecules using oncolytic HSV-1 as a vector and co-administration of reagents that modulate immune reactions. Viral propagation may be enhanced via temporary suppression of innate immune responses. Elucidation of the role of the host immune system in oncolytic HSV-1 therapy is the key to establishing the approach as a useful clinical means for cancer treatment.

Keywords: Herpes simplex virus type 1, oncolytic virus therapy, antitumor immunity, cancer immunotherapy, innate immunity.

INTRODUCTION

The use of conditionally-replicating herpes simplex virus type 1 (HSV-1) is an effective means for treating cancer. The therapeutic strategy is based on á simple concept that HSV-1 kills the host cell as a consequence of viral replication. Genetically engineered mutations in the genes associated with virulence and/or viral DNA synthesis can limit the virus to replicate only in transformed cells. The first oncolytic HSV-1 with a deletion in the thymidine kinase gene was described in 1991 [1]. Since then, several different oncolytic HSV-1 vectors have been tested in clinical trials [2, 3]. HSV-1 has many advantages over other types of virus for treating cancer [4-6]: (i) It infects a variety of tumor cell types. (ii) A total cell killing can be achieved by a relatively low multiplicity of infection (MOI). (iii) Circulating anti-HSV-1 antibody does not affect cell-to-cell spread of the virus, therefore a vector can be administered repeatedly in immunocompetent hosts. (iv) The large genome (~152 kb) is well characterized and contains many non-essential genes that can be mutated or replaced with large-sized transgenes [7]. (v) Anti-herpes drugs, such as acyclovir and ganciclovir, are available that can be used for termination of therapy [8, 9]. (vi) Virus DNA remains episomal and does not get integrated into the cellular genome. (vii) There are mice and nonhuman primates that are susceptible to HSV-1 and can be used for preclinical evaluation for safety and efficacy.

The cytopathic effect of HSV-1 depends on the extent of tumor cells to support viral replication, and there is a wide range of variation among cell lines. The *in vivo* antitumor effect of HSV-1 further depends on the extent of antitumor immunity induction in the course of oncolytic activity. One way to enhance the efficacy of oncolytic HSV-1 without compromising the safety is to harness the capability of inducing antitumor immune responses. With HSV-1, stimulation of systemic immune responses does not seem to inhibit viral replication or spread within the tumor, despite that it may also cause enhanced antiviral immunity. Dissimilarly, with adenovirus vectors, it has been shown that antiviral immune responses act adversely to the therapeutic efficacy [10, 11]. It has been suggested, however, that the extent of innate immunity is associated with the therapeutic efficacy of oncolytic HSV-1 [12].

In this review, we discuss the immune responses involved with oncolytic HSV-1 therapy and the strategies to improve the efficacy by augmenting the induction of antitumor immune responses.

IMMUNE RESPONSES TO WILD-TYPE HSV-1

The two major mechanisms of protection against HSV-1 via the host immune system are initial antiviral innate defenses and acquired responses. Innate immunity initially acts to destroy pathogenic microorganisms after the physical barriers of the skin, epithelium or mucosa fail to block their invasion. Secondarily, pathogen-specific acquired immune responses are stimulated that result in production of circulating antibodies and T cell responses. Innate immunity against wild-type HSV-1 consists of the following responses [13]:

(1) Activation of the complement cascade.

Mucosa and tears include lysozyme, complement and interferons (IFNs) that may all act to prevent HSV-1 infection [14]. The type 1 IFN response is triggered by HSV-1 infection and mediated by toll-like receptors (TLRs), especially TLR2, TLR3 [15, 16] and TLR9 [17, 18].

(2) Activation of macrophages and recruitment, activation, and maturation of natural killer (NK) cells or T cells.

Primary HSV-1 infection induces inflammation at the site of the infection. The inflammation is characterized by an infiltrate of polymorphonuclear leukocytes, macrophages, lymphocytes and NK cells (reviewed by Broberg *et al.* [19]). Some of these cells, like macrophages, serve to kill virus-infected cells directly, and others express viral antigens in association with major histocompatibility complex (MHC) molecules. Antigens are recognized by T cells that enter the inflammatory lesion by chemokine attraction. Both CD4+ and CD8+ T cells have been reported to be present in the inflammatory infiltration and responsible for the viral clearance or control of recurrent infection [20, 21]. Patients with a history of herpetic keratitis have circulating memory T and B cells in the blood and lymph. Memory T cells reside in the regional lymph nodes to prevent the spread of the recurrent infection to

*Address correspondence to this author at the Department of Neurosurgery, University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan; Tel: +81-3-5800-8853; Fax: +81-3-5800-8655;
E-mail: toudou-nsu@umin.ac.jp

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other tissues [22]. A dense CD4+, CD56+ T-cell infiltrate has been observed in the nasopharynx with HSV-1 infection [23].

(3) Generation and secretion of a variety of cytokines and chemokines.

A variety of cytokines have been detected in the tissues with HSV-1 infection both in humans and in experimental animals which include IFN-α, -β and –γ, IL-1, -2, -4, -5, -6, -10, -12 and -23, and TNF-α (reviewed by Hukkanen *et al.* [24]). IFN-γ production and the presence of CD4+ and CD8+ T cells are key regulators of viral clearance during acute cutaneous infection [25, 26]. CD4+ T cells recognizing integument antigens have been suggested to be the producers [27]. Benecia *et al.* showed that infection of tumors by oncolytic HSV-1 was associated with upregulation of IFN-inducible chemokines and marked infiltration of activated NK and T cells into the tumor [28]. Furthermore, they showed that tumor-associated dendritic cells and monocytes were responsible for producing MIG and IP-10 during oncolytic HSV-1 infection and that this mechanism might be partly mediated by type I IFNs.

THE USE OF ONCOLYTIC HSV-1 FOR IMMUNOTHERAPY

Augmenting the capability of oncolytic HSV-1 to induce antitumor immune responses is a reasonable strategy to improve the efficacy (Fig. 1). Conversely, oncolytic HSV-1 can be a useful tool for immunotherapy. In the following sections, we discuss issues and advantages regarding the use of oncolytic HSV-1 for immunotherapy.

HSV-1 Replication in Tumor Induces CTL-Mediated Antitumor Immune Responses

Multiple studies support the notion that the immune responses to viral antigens presented on the surface of tumor cells are redirected to tumor cell antigens and enhance the efficacy of oncolytic HSV-1 by inducing antitumor immunity [29-31]. In HSV-1-sensitive A/J mice harboring bilateral subcutaneous tumors of syngeneic N18 neuroblastoma, intraneoplastic inoculation of the unilateral tumors with G207, an oncolytic HSV-1 with deletions in both copies of the *γ34.5* gene and an inactivation of the *ICP6* gene [32], caused tumor growth inhibition not only of the inoculated tumors but also of remote, non-inoculated tumors. The antitumor effect on remote tumors was caused via systemic antitumor immunity and accompanied an elevation of cytotoxic T lymphocyte (CTL) activity specific to N18 cells [29]. The mice cured of N18 tumors by the G207 treatment obtained persistent tumor-specific protection against rechallenge with subcutaneous injections of N18 cells [29]. Corticosteroid administration did not reduce the oncolytic activity of G207, but did suppress the CTL-mediated immune responses, which led to delayed regrowth of tumors that once diminished by G207 treatment [33]. G207-induced systemic antitumor immunity was also observed in tumor models using mouse CT26 colon carcinoma [34], mouse M3 melanoma [34] or hamster KIGB-5 gallbladder carcinoma [35]. In mice harboring subcutaneous CT26 tumors,

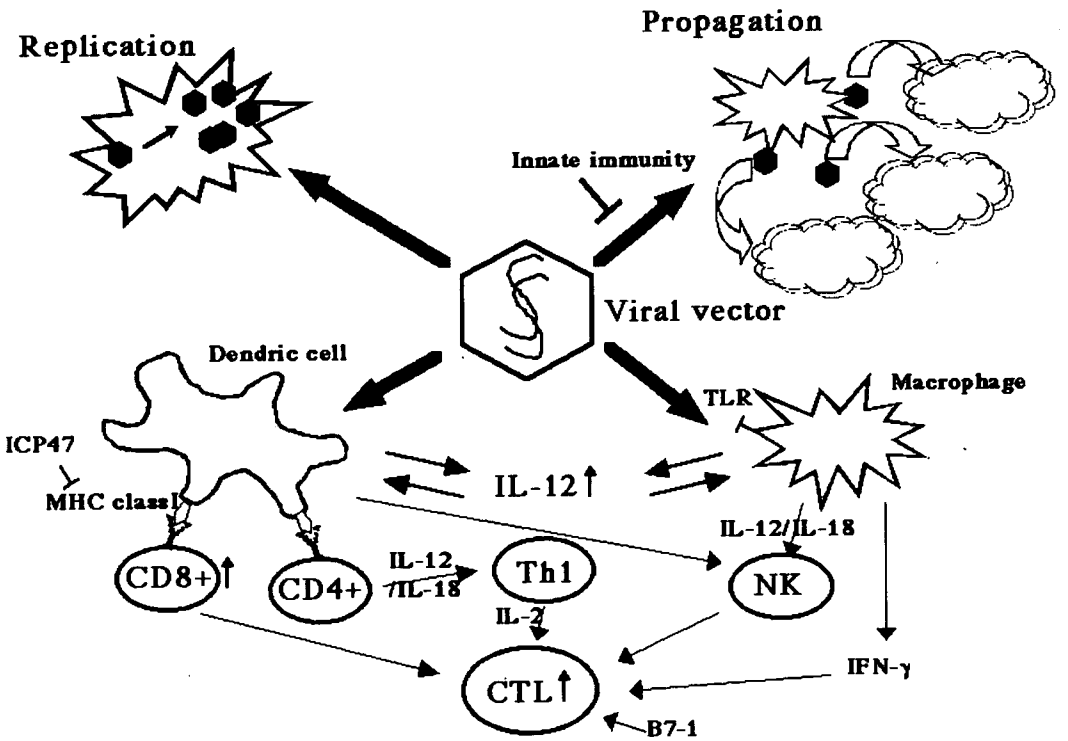

Fig. (1). Ways to enhance the antitumor efficacy of oncolytic HSV-1 therapy without compromising the safety. Approaches include: (1) improving the viral replication within the tumor, but not in the normal tissue, by additional manipulation of the viral genome (such as in G47Δ [Ref. 45]) or the use of tumor-specific promoter to drive a viral gene (such as in rQNestin34.5 [Ref. 76]); (2) improving the viral propagation by transient suppression of innate immunity; and (3) stimulating the induction of specific antitumor immunity by *in situ* expression of immunostimulatory molecules (*e.g.* IL-12, IL-18 and soluble B7-1), co-administration of immunomodulatory reagents, or inhibiting the down-regulation of MHC class I expression of infected tumor cells (as with G47Δ).

G207 treatment was shown to elicit CTL activities specific to the CT26 immunodominant MHC class I-restricted antigen AH1 [34].

In a model using poorly immunogenic murine melanoma cells transfected with the HSV-1 entry co-receptors, Hve A and C, inoculation of intracranial tumors with oncolytic HSV-1 induced tumor-specific cytotoxic and proliferative T cell responses [30]. However, there was no increase following viral therapy in either serum tumor antibody levels or viral-neutralizing antibodies. Therefore, specific T cell responses mediated the prolongation of survival caused by oncolytic HSV-1 therapy. The CTL responses following oncolytic HSV-1 therapy were directed toward both tumor and viral antigens; however, the proliferative response was toward tumor antigens only. Following oncolytic HSV-1 treatments, the lysis of tumor cells recruits CD4+ T cells to the tumor mass, where the cells are triggered to respond to tumor antigens and not viral antigens. This may be partly due to the ability of the virus to block immune recognition of infected cells through modulation of both MHC classes I and II [36-38]. CD4-/-, CD8-/- and NK-/- mice were unable to mount an immune response to prolong survival after oncolytic HSV-1 therapy. The recruited CD4+ T cells secrete cytokines and, in turn, recruit and activate CD8+ T cells. CD8+ T cells, along with NK cells, lyse both infected and uninfected tumor cells, leading to tumor destruction. Induction of the tumor-specific CTL activities together with proliferative T cells can lead to recognition and destruction of remote, untreated metastases.

A defective HSV-1 vector (dvB7Ig) expressing a soluble form of B7-1, one of the most potent co-stimulatory molecules [39], has been tested in combination with G207 [40]. The soluble B7-1 (B7-1-Ig) was designed as a fusion protein of the extracellular domain of B7-1 and the Fc portion of IgG, so that it was secreted by tumor cells rather than expressed on the cell surface. The *in vivo* efficacy was tested in A/J mice harboring syngeneic, poorly-immunogenic Neuro2a neuroblastoma under the skin or in the brain. Intraneoplastic inoculation of dvB7Ig/G207 at a low titer successfully inhibited the growth of established subcutaneous tumors, despite that the expression of B7-1-Ig was detected in only 1% or less of tumor cells at the inoculation site. The dvB7Ig/G207 treatment also prolonged the survival of mice bearing intracerebral tumors. Inoculation of dvB7Ig/G207 induced a significant influx of $CD4^+$ and $CD8^+$ T cells in the tumor. *In vivo* depletion of immune cell subsets further revealed that the antitumor effect required $CD8^+$ T cells but not $CD4^+$ T cells. The dvB7Ig/G207 treatment conferred tumor-specific protective immunity on cured animals.

The *α47* gene product (ICP47) of HSV-1 inhibits the transporter associated with antigen presentation (TAP) that mediates antigen presentation in the context of MHC class I by translocating peptides across the endoplasmic reticulum [37-38, 41-42]. Upon HSV-1 infection, ICP47 is immediately expressed and causes down-regulation of the MHC class I expression on the cell surface, allowing the infected host cell to escape the host immune surveillance [43]. An *α47*-deleted HSV-1 vector did not down-regulate the MHC class I expression in human melanoma cells [44]. Also, *α47*-deleted replication-competent HSV-1 was less virulent than the parent wild-type virus in the brains of A/J mice, and the attenuation of neurovirulence was dependent on $CD8^+$ T cells [36]. In order to confer an enhanced MHC class I presentation, we have constructed a new generation, replication-competent HSV-1 vector, G47Δ, by further creating a 312 bp deletion within the *α47* gene of double-mutated G207 [45]. G47Δ-infected human melanoma cells showed enhanced MHC class I expression and caused a significantly better stimulation of tumor infiltrating lymphocytes compared to G207-infected cells. The results showed that higher MHC class I expression in cells infected with G47Δ can indeed enhance the antitumor T cell stimulation.

Expression of Immunostimulatory Molecules by Tumor Cells Augments the Efficacy of Oncolytic HSV-1 Therapy

Defective HSV-1 vectors have been used to introduce immunostimulatory genes into tumor cells both *ex vivo* and *in situ* [46]. A replication-incompetent HSV-1 vector expressing interleukin (IL)-2 was effective in inducing antitumor immune responses against head and neck metastases of renal carcinoma in animal models [47]. An HSV-1 amplicon vector expressing murine granulocyte-macrophage colony stimulating factor (GM-CSF) or IL-2 was used in combination with intraperitoneal injections of γ-interferon (IFN-γ) in an animal hepatoma model. The combination therapy was more efficacious than any treatment alone: A complete elimination of the tumor was observed in 4 of 12 animals for GM-CSF/ IFN- γ and 8 of 11 animals for IL-2/ IFN-γ [48]. An HSV-1 amplicon vector expressing IL-2 also showed a significant suppression of the growth of lung squamous cell carcinoma implanted subcutaneously in animals [49, 50]. The treatment with the IL-2 vector caused a retardation of the growth of tumors remote from vector inoculation sites and led to a significant improvement in animal survival. In a bilateral subcutaneous tumor model of murine melanoma, intraneoplastic inoculation of a defective HSV-1 vector encoding murine GM-CSF inhibited the growth of both inoculated and non-inoculated tumors [51].

An HSV-1 amplicon can be combined with oncolytic HSV-1 by using oncolytic HSV-1 as a helper virus when generating the defective HSV-1 vector. Several amplicon vectors expressing immunostimulatory molecules have been used in combination with G207 [41, 52-53]. When a mixture of G207 and a defective vector expressing IL-12, for example, is inoculated into the tumor, tumor cells infected with G207 allow the virus to replicate and ultimately destroy the cells, further spreading progeny G207 to surrounding tumor cells. On the other hand, tumor cells infected with the defective vector produce IL-12 and recruit immune cells, which augments the antitumor immune response elicited by the oncolytic activity of G207. Intraneoplastic inoculation of the IL-12 defective vector in combination with G207 showed greater efficacy than G207 alone in a subcutaneous CT26 tumor model [53]. Increases in tumor-specific CTL activity and IFN-γ production by splenocytes were observed [53].

Several replication-competent HSVs-1 have been genetically engineered to contain immunostimulatory trangenes in the viral genome (Fig. **2**). "Armed" oncolytic HSV-1 is advantageous over defective HSV-1 amplicon vector in that, once constructed, it can be generated constantly with a high titer yield by continuous viral passages in culture. Unlimited source and a high titer concentration are essential features for clinical application. The *γ34.5*-deficient HSV-1 containing the murine IL-4 gene displayed a significantly higher antitumor activity and prolonged survival of mice with intracranial tumors compared with its parent virus or the one expressing IL-10 [54].

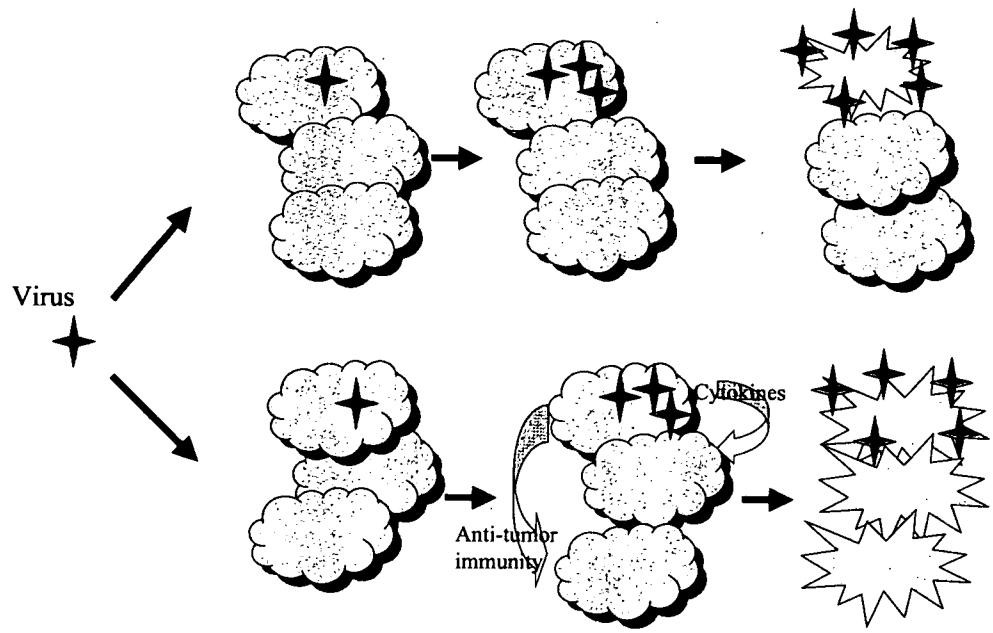

Fig. (2). The use of oncolytic HSV-1 as a vector to express immunostimulatory molecules leads to an enhanced antitumor activity. Oncolytic HSV-1 replicates in and kills the host tumor cell, followed by propagation of progeny viruses to surrounding tumor cells. In addition, when the oncolytic HSV-1 is armed with immunostimulatory transgenes, the infected cell expresses the immunostimulatory molecules before the cell death, which stimulates antitumor immune responses that direct toward infected as well as uninfected tumor cells.

Improvement in antitumor activity was observed in certain mouse tumor models when GM-CSF was expressed by an oncolytic HSV-1 with deletions in the *γ34.5*and *α47* genes [55].

Oncolytic HSVs-1 expressing murine IL-12 (M002 and NV1042) showed improved *in vivo* efficacy in mouse models using neuroblastoma [56], squamous cell carcinoma [57] and colorectal cancer [58]. Immunohistochemical analyses of tumors treated with these HSV-1 mutants revealed a significant influx of CD4+, CD8+ T cells and macrophages. The oncolytic HSV-1 expressing IL-12 (NV1042) was more efficacious than the one expressing GM-CSF (NV1034) in mice with subcutaneous squamous cell carcinoma [57]. The mice cured by NV1042 had a higher rate of rejecting rechallenged tumor cells than those cured by NV1034 [57]. Similarly, the oncolytic HSV-1 expressing IL-12 (NV1042), but not the one expressing GM-CSF (NV1034), showed greater efficacy than the control HSV-1 (NV1023) in two murine prostate cancer models, one with a high MHC class I level (Pr14-2) and the other with a low level (TRAMP-C2) [59]. NV1042, but not NV1023, was effective in reducing the number of hepatic tumors when used to treat microscopic residual tumors after resection of the parent Morris hepatoma established in Buffalo rat livers [60]. In this model, both viruses induced a significant local immune response as evidenced by an increase in the number of intratumoral CD4+ and CD8+ T cells, although the peak of CD8+ T cell infiltration was later with NV1042 compared with NV1023.

We have recently created three oncolytic HSV-1 "armed" with murine soluble B7-1 (B7.1-Ig), IL-12 or IL-18 (designated vHsv-B7.1-Ig, vHsv-IL-12 and vHsv-IL-18, respectively) and a control oncolytic HSV-1 (vHsv-null) [61]. These vHsv vectors have deletions in the *γ34.5* genes and the *ICP6* gene, and the transgenes inserted in the *ICP6* locus. The *in vivo* efficacy was tested in A/J mice harboring subcutaneous tumors of poorly immunogenic Neuro2a neuroblastoma. The triple combination of vHsv-B7.1-Ig, vHsv-IL-12 and vHsv-IL-18 exhibited efficacy greater than any single virus or any combination of two viruses, significantly suppressing the growth of inoculated tumors as well as non-inoculated remote tumors. Studies using athymic mice indicated that this enhancement of antitumor efficacy was mediated by T-cell immune responses.

We have further created four oncolytic HSV-1 vectors expressing murine IL-18, soluble murine B7-1, both of these transgenes, or none, using the triple-mutated G47Δ as the backbone [62] (Fig. 3). The *in vivo* efficacy of the armed oncolytic HSV-1 vectors was tested in two immunocompetent mouse tumor models, TRAMP-C2 tumors in syngeneic C57BL/6 mice and Neuro2a tumors in syngeneic A/J mice. Intraneoplastic inoculation of G47Δ double-armed with IL-18 and B7-1-Ig caused a significant reduction of the tumor growth compared with G47Δ expressing IL-18 alone or G47Δ expressing B7-1-Ig alone [62]. We utilized bacterial artificial chromosome and two recombinase systems (Cre/loxP and FLP/FRT) to develop a method that allows a rapid, reliable and simultaneous construction of multiple "armed" oncolytic HSV-1 vectors using G47Δ as the backbone. Because ICP47 inhibits TAP, *α47*-deficient HSV-1 vectors including G47Δ are especially suited to express immunostimulatory transgenes in human. This system is useful for expedited development of "armed" oncolytic HSV-1.

Efficacy of Oncolytic HSV-1 May be Inhibited by Innate Immunity or Neutralizing Antibodies

Oncolytic HSV-1 therapy has been developed mainly using intraneoplastic inoculation as administration route. One obvious hurdle for intravascular administration is that only a small portion of the virus can reach the tumor. Serological studies have revealed that 50-80% of humans possess neutralizing antibodies against HSV-1 [63]. Yet, the preexisting immunity to HSV-1 does not affect oncolytic HSV-1 therapy

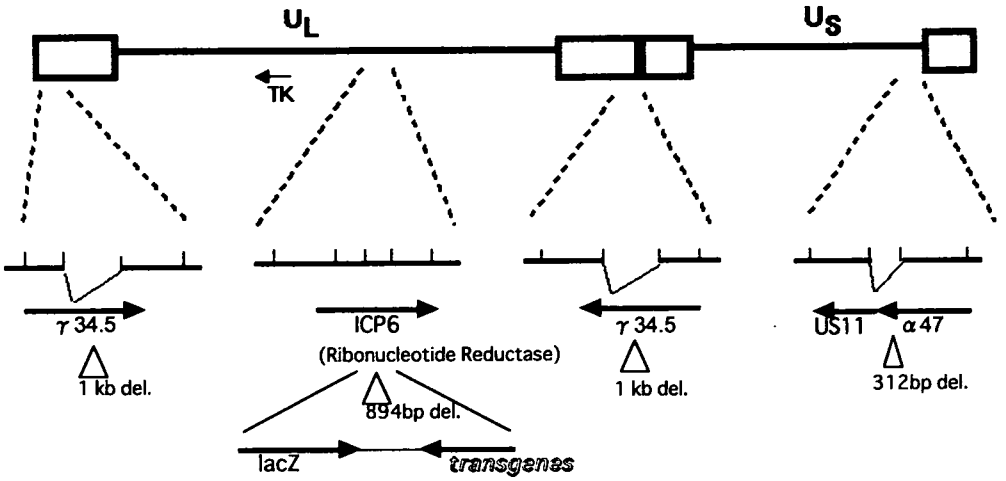

Fig. (3). The structure of the "armed" oncolytic HSV-1 with the G47Δ backbone constructed using the BAC-mediated system. The boxes (top line) represent inverted repeat sequences flanking the long (U_L) and short (U_S) unique sequences of HSV-1 DNA. The armed oncolytic HSV-1 contains 1.0-kb deletions in both copies of the *γ34.5* gene, a 312-bp deletion in the *α47* gene and an 894-bp deletion in the *ICP6* gene. The lacZ gene and the CMV promoter-driven transgenes, placed in opposite directions, are inserted in the deleted *ICP6* locus. Thick arrows indicate transcribed regions.

when the virus is administered intraneoplastically [64]. Further, the presence of neutralizing antibodies and cell-mediated immunity to HSV-1 did not alter the efficacy of an oncolytic HSV-1 after intraportal administration to mice with diffuse liver metastases [65]. However, route of viral administration may influence the efficacy, as oncolytic HSV-1 delivered intravenously produced some detectable attenuation while hepatic arterial therapy remained unaffected in a mouse liver metastases model [66]. The efficacy of oncolytic HSV-1 therapy seems minimally affected by preexisting anti-HSV-1 immunity when the virus is administered at appropriate doses and in reasonable proximity to tumors.

The efficacy of oncolytic HSV-1 may also be affected by the capability of the virus to infect and spread. To exhibit virulence, HSV-1 utilizes glycoprotein C on the envelope to bind or inhibit complement molecules C3, C5 and properdin [67, 68], and glycoproteins E and I to bind immunoglobulin molecules [69]. It has been suggested that the spread of oncolytic HSV-1 within tumor is significantly affected by innate immunity [70]. The innate immune activity against HSV-1 was present in rat and human plasma in which pre-immune IgM and complements were at least in part responsible. The innate immune activity was also present in both naive and previously treated mice. B-cell immunosuppressive agent cyclophosphamide (CPA) can suppress the early innate immune activity and later induction of the specific neutralizing antibodies. In rats bearing intracerebral tumors, intraarterial administration of oncolytic HSV-1 together with RMP-7 (bradykinin analog) in combination with CPA treatment significantly improved the viral propagation within the tumor, leading to prolonged survival.

Virus "Oncolysates" May be Useful for Cancer Immunotherapy

Several non-herpetic viruses have been used to generate "oncolysates" for active cancer immunotherapy. Infection of human tumor cells by an oncolytic virus enhances the antigenicity of the tumor and elicits a systemic immune response against the tumor [71]. Tumor cells infected with vaccinia virus [72], Newcastle disease virus [73] and vesicular stomatitis virus [74] have been developed as immunotherapeutic agents for cancer, and clinical trials are under way [75]. Oncolytic HSV-1 is potentially useful for immune cell therapy for cancer.

CONCLUSION

The oncolytic HSV-1 therapy is particularly attractive in clinical settings, because the viruses exhibit the antitumor activity not only through viral replication and spread, but also through induction of systemic and specific antitumor immunity. The ability of oncolytic HSV-1 to elicit the antitumor immunity can be strengthened by modifying the host immune responses. Such methods include expression of immunostimulatory molecules using oncolytic HSV-1 as a vector and co-administration of reagents that modulate a certain phase of immune reactions. Viral propagation may be enhanced via temporary suppression of innate immune responses. We believe that better understanding of the host immune responses is the key to improving the efficacy and practicality of oncolytic HSV-1 therapy.

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