Oncolytic viruses: a novel form of immunotherapy

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Abstract

Oncolytic viruses are novel anticancer agents, currently under investigation in Phase I–III clinical trials. Until recently, most studies have focused on the direct antitumor properties of these viruses, although there is now an increasing body of evidence that the host immune response may be critical to the efficacy of oncolytic virotherapy. This may be mediated via innate immune effectors, adaptive antiviral immune responses eliminating infected cells or adaptive antitumor immune responses. This report summarizes preclinical and clinical evidence for the importance of immune interactions, which may be finely balanced between viral and tumor elimination. On this basis, oncolytic viruses represent a promising novel immunotherapy strategy, which may be optimally combined with existing therapeutic modalities.

Keywords

adaptive; clinical trial; immune response; immunotherapy; innate; oncolytic virus
The anticancer activity of viruses has been reported throughout the 20th century. Developments in virology, genetic manipulation and molecular biology have led to a surge of research investigating viruses with oncolytic or antitumor properties over the last 15 years. Several oncolytic viruses are currently in Phase I–III clinical trials [1]. Until recently, despite the multitude of studies investigating direct viral effects upon cancer cells, relatively little attention had been paid to the interaction between oncolytic viruses and the immune system. We discuss the evidence supporting the view that the host immune response is critical to the efficacy of oncolytic virotherapy. The potential of oncolytic viruses to break immunological tumor tolerance, generating antitumor immunity, represents a novel avenue of immunotherapy.

Oncolytic viruses: background

Oncolytic viruses are self-replicating, tumor selective and directly lyze cancer cells [2]. They may be tumor selective in wild-type or attenuated forms or may be engineered to provide tumor selectivity. Naturally occurring oncolytic viruses include the double-stranded RNA reovirus and single-stranded RNA Newcastle disease virus (NDV) and vesicular stomatitis virus (VSV). By contrast, human DNA viruses, including adenoviruses, vaccinia and herpes simplex viruses (HSV) have been genetically modified in a variety of ways to provide tumor selectivity. A diverse range of mechanisms provide tumor specificity, including inactivation of antiviral defences, such as type I IFN responses in many cancer cells, viral deletions permitting replication only in tumor cells that can substitute for viral defects, tumor-selective uptake via upregulated or mutated receptors, and targeting to tumor promoters.

In the majority of clinical trials performed so far, oncolytic viruses have been administered via intratumoral injection. A smaller number of studies have examined regional or intravenous delivery. Clinical experience has demonstrated a favorable toxicity and safety profile and a number of tumor responses, although overall antitumor efficacy has been limited [1]. For example, ONYX-015, a modified adenovirus, has been used in clinical trials with response rates of 0–14% following intratumoral administration [3]. In view of the short history of oncolytic virotherapy, along with recent scientific advances in methods of viral delivery and enhancing antitumor potency, these low levels of single-agent clinical responses provide encouragement for the future.

Cancer & the immune system

An increasingly powerful body of evidence supports the ability of the immune system to modify the immunogenicity and behavior of tumors [4]. A host of tumor-associated antigens (TAA) have been characterized [5] and in a single tumor, tumor-infiltrating lymphocytes directed towards multiple TAAs can be identified [6]. Despite these antigenic differences, the antitumor immune response is commonly ineffectual. Tumors can subvert antitumor immunity, generating an immunosuppressive tumor microenvironment by a multitude of mechanisms. These include the induction of Treg cells, secretion of soluble immunosuppressive mediators including nitric oxide, IL-10 and TGF-β and recruitment of myeloid suppressor cells [4]. Matzinger’s ‘danger’ hypothesis proposes that the prime role of the immune system is to respond to cellular or tissue distress as opposed to nonself per se [7]. Several danger signals have been identified, including RNA, DNA, IFN-α, heat-shock proteins, uric acid and hyaluron, providing a mechanistic basis for this hypothesis [8]. On this basis, tumor-associated danger signals are critical to the generation of effective antitumor immunity. In addition to their ability to disrupt immune responses, tumors commonly lack such signals and successful tumor immunotherapy will probably depend upon their provision. Oncolytic virotherapy represents a potent approach to cancer immunotherapy, combining the enhanced release of TAA via tumor cell death, in the context of danger signals (FIGURE 1).
Oncolytic viruses, the innate immune response & danger signals

The role of the innate immune response to cancer is double-edged. Chronic inflammatory changes can promote tumor progression via proliferative and proangiogenic signals [9], while by contrast, the infiltration of activated innate inflammatory cells can mediate tumor regression in vivo [10]. Manipulation of the immune environment within a tumor is a potentially critical strategy towards successful tumor immunotherapy [11].

Oncolytic viruses represent prime candidates to enhance the immunogenicity of the tumor microenvironment. As detailed below, oncolytic virotherapy may be immunomodulatory via tumor cell death, production of endogenous danger signals, the release of tumor-derived cytokines and direct effects upon cells of the innate immune system. Evidence from preclinical models suggests that an early influx of immune cells, including macrophages and natural killer (NK) cells, occurs in response to tumor viral therapy [12–14]. These changes within the tumor hold the potential to alter the pre-existing immunosuppressive microenvironment, in favor of the generation of therapeutic immune responses. Dendritic cells (DC), the prime antigen-presenting cells and a component of the innate immune response are critical for the subsequent generation of antigen-specific or adaptive immune responses. However, as discussed later, the outcome of the innate response is finely balanced between promotion of tumor clearance and viral clearance limiting efficacy.

Tumor cell death

Virally induced cell death would be expected to enhance the availability of TAA for uptake by DC. Indeed, viral infection of tumors has been reported to enhance the phagocytosis of tumor-derived material [15,16]. The relationship between the mode of cell death and tumor immunogenicity has, however, been controversial; the immunogenicity of tumors has been reported not to be affected by whether tumor cells are alive, apoptotic or necrotic [17]. Even if the mode of cell death is not an immunogenic determinant, the release of intrinsic cell factors, including heat-shock protein [18], uric acid [19] and bradykinin [20], can be identified as danger signals by DC. Oncolytic viral infection may mediate production of these factors. For example, tumor cell infection by a modified oncolytic adenovirus increases intracellular uric acid levels, activating DC [19].

Tumor-derived cytokines

An array of cytokines provides costimulation for T-cell responses, while by contrast, tumor-derived cytokines, including TGF-β and IL-10, have immunosuppressive properties. In addition, the tumor-derived proinflammatory cytokines VEGF, TNF-α and several chemokines have been linked to promotion of tumor growth [21]. Oncolytic viral infection is likely to alter the balance of cytokines produced and the nature of the subsequent immune response. We have investigated the release of cytokines following infection of melanoma cells with reovirus, a naturally occurring double-stranded RNA virus currently in clinical trials [22]. Reovirus was found to induce secretion of IL-8, RANTES and MIP-1α/β, which play a role in the recruitment of DC, neutrophils and monocytes [23], and of IL-6, which can inhibit the immunosuppressive function of Treg cells [24]. Reovirus additionally reduced tumor secretion of the immunosuppressive cytokine IL-10. The immunogenic property of tumor-conditioned media from reovirus-infected tumor cells (filtered to remove viral particles) was confirmed by their ability to activate DC.

DC & the response to viral infection

The immune system is adept at pathogen recognition and a host of receptors specific for pathogen-associated molecular patterns, including the toll-like receptors (TLR), have been identified [25]. Innate viral recognition can center around viral nucleic acids or viral proteins

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[25]. DC play a critical role in the early innate immune responses, reciprocally interacting with other innate immune cells, including NK cells [26]. In this context, oncolytic viruses can influence the nature of the innate tumor response. Reovirus-infected DC, for example, enhance NK cytotoxicity towards tumor cells [27].

The effect of viruses upon DC is virus specific: measles and a vaccinia virus strain impair DC phenotype and function [28,29], an oncolytic adenovirus has a neutral effect [30], while reovirus is directly stimulatory to DC [27]. Although the immunomodulatory effects of oncolytic viruses have been investigated to a limited degree, it follows that the immune consequences of therapy with different viruses will vary widely. In addition, the genetic modification of viruses to confer oncolytic specificity may involve interference with virulence genes whose function is to modify the antiviral immune response, including type I interferon response genes [2,31]; alteration of such immunomodulatory genes will alter the consequences of the immune interactions of these modified viruses.

**Oncolytic viruses & adaptive antitumor immunity**

The innate immune response is thought to provide an important link to the generation of adaptive immune responses. DC are key to this link, taking up TAA, integrating danger signals and presenting antigen in an appropriate costimulatory context to the adaptive arm of the immune system. An adaptive antitumor immune response requires activation of cytotoxic CD8 T cells by DC presenting tumor antigen on MHC class I molecules. The presentation of exogenous antigen in a MHC class I context is termed ‘cross-presentation’. Critically, virally infected cells have been shown to be superior at delivering nonviral antigen for cross-presentation and cross-priming adaptive immune responses in vivo [32]. Intriguingly, recent work has defined a role for TLR-4 receptor ligands (bacterially derived lipopolysaccharide) in enhancing cross-presentation [33]; a similar effect of viral as opposed to bacterial TLR ligands has yet to be explored. Inflammatory stimuli have additionally been shown to enhance antigen processing and the generation of MHC class II complexes, required for CD4+ T-cell help in adaptive immune responses [34,35]; such inflammatory stimuli could be provided by viral tumor infection. Oncolytic virotherapy may therefore enhance immune priming via multiple effects upon DC. There is an emerging body of data from murine and human preclinical research supporting the concept that the efficacy of oncolytic virotherapy is at least partially immune mediated and that antitumor immunity can be generated.

**Murine models**

Adaptive antitumor immune responses following oncolytic virotherapy have been demonstrated in a variety of murine tumor models with several oncolytic viruses. We have demonstrated that effective systemic cellular delivery of VSV in a murine model of melanoma lymph node metastases can generate an antitumor immune response, with adoptive transfer of splenocytes from treated mice providing tumor protection [36]. Additionally, following intratumoral injection of VSV into B16ova tumors, antitumor T-cell responses are generated [12]. Similarly, oncolytic HSV strains have been shown to induce systemic antitumor immune responses in several tumor models [37–40]. In an elegant demonstration of this principle, an attenuated HSV was injected intratumorally into one flank of mice with established bilateral colorectal or melanoma tumors; the contralateral uninjected tumors regressed in association with the generation of TAA-specific CD8 T cells [37]. It should be noted that this study utilized an immunogenic tumor model.

In an intriguing study, NDV was administered locoregionally as therapy for liver metastases from a colon carcinoma cell line that was resistant to the virus in vitro; NDV resulted in a significant delay in tumor growth [41]. Similarly, we have data that B16ova cells are resistant in vitro to reovirus, but sensitive in vivo following intratumoral injection (PRESTWICH ET
These data imply a critical role for antitumor host immune responses in oncolytic virotherapy.

**In vitro human systems**

Similarity between the mouse and human immune systems is limited and human *in vitro* studies are therefore important in this field. Tumor cell lysates induced by an oncolytic virus, parvovirus H-1, stimulate DC maturation and cross-presentation of melanoma antigens to cytotoxic lymphocyte clones, providing ‘proof of principle’ that virus-induced cell death can lead to cross-presentation of TAAs [15]. Greiner *et al.* demonstrated that a melanoma cell line infected with an attenuated vaccinia virus was able to prime an adaptive response towards a candidate TAA, MelanA [16]. Similarly, we have demonstrated that loading DC with reovirus-infected melanoma cells can efficiently prime an antitumor response and cross-prime an expansion of MART-1-reactive CD8 T cells [42].

**Oncolytic viruses & the role of antiviral immune interactions: detrimental or beneficial for virotherapy?**

The interaction between oncolytic virotherapy and the immune system, including innate immunity and adaptive antitumor and antiviral responses, is complex and may be detrimental as opposed to beneficial to therapeutic outcome. Soluble mediators including complement [43,44], preimmune IgM [44] and specific neutralizing antiviral antibodies [45] limit viral efficacy following systemic delivery. Circulating viral particles are additionally susceptible to nonspecific binding to blood cells [46], nontarget tissues and uptake by the reticuloendothelial system. Overcoming these immune and nonimmune barriers is critical to the success of systemic virotherapy and may also be key to harnessing any beneficial immune interactions. A host of promising strategies are under investigation to enhance viral delivery to tumors, shielding viral particles within the circulation. These include:

- Modification of the viral coat by lipid encapsulation, polymer coating [47] and polyethylene glycol [48]
- Bispecific fusion proteins or antibodies [49]
- Serotype switching utilizing multiple viral serotypes to evade specific antibody neutralization [50]
- Delivery utilizing cell carriers to chaperone viral particles in the circulation [51,52]
- Arterial delivery [53]

The cellular antiviral immune response may limit the efficacy of virotherapy by eliminating tumor infection via clearance of infected tumor cells. Alternatively, clearance of infected tumor cells may play a key role in tumor regression, augmenting any direct cytolytic activity. In an intracranial murine model of metastatic melanoma, lymphocytes have been shown to be critical to tumor regression mediated by a neuroattenuated HSV [40]; the lymphocyte response was found to include virally specific cytotoxic activity in addition to antitumor reactivity. In a B16ova melanoma model, the efficacy of intratumorally injected VSV has been shown to be dependent upon NK and CD8 T cells [12]. CD8 T cells were detected towards both viral epitopes and the SIINFEKL epitope of the model TAA, OVA. In this study, it remains an open question whether the CD8 T cells critical for virotherapy were directed towards tumor or viral epitopes. Thus, local immune reactivity towards virally infected cells may be critical for the efficacy of virotherapy in this system.
Overall, the antiviral humoral and cellular immune responses may have contrasting consequences. Methods of enhancing viral delivery to tumors or immunomodulation provide an opportunity to alter this balance in favor of therapeutic benefit.

**Clinical trials & the immune response**

Although preclinical studies have provided support for the concept that the efficacy of oncolytic virotherapy may be dependent upon the host immune response, there are limited data on the immune response following virotherapy from early clinical trials.

Studies of intratumoral administration have provided direct evidence of a cellular immunological response. In a Phase I trial of a second-generation oncolytic HSV expressing GM–CSF injected into subcutaneous metastases from a variety of tumor types, post-treatment biopsies revealed an extensive immune cell infiltrate [54]. Additionally, suggestive of an immune-mediated antitumor effect, was the observation of inflammation in uninjected tumor deposits in four of 30 treated patients. Similarly, in a study of intratumoral administration of a recombinant vaccinia–GM–CSF virus in patients with melanoma deposits, treated lesions were shown to have a dense immune cell infiltrate. The generation of antitumor immunity was implied by the regression of noninjected regional dermal metastases in association with an immune infiltrate in four of seven treated patients [55]. A Phase I study of injection of JX-594, a targeted poxvirus armed with GM–CSF, into primary and metastatic liver tumors has recently been reported with encouraging evidence of activity, with a partial response in three and stable disease in six of ten evaluable patients by Response Evaluation Criteria in Solid Tumors (RECIST) [56]. Consistent with a possible antitumor immune response was the durability of tumor responses. Notably, there was evidence of functional response in noninjected tumors in three of seven evaluable patients by Choi criteria for reduction in Hounsfield units (n = 2) and by reduced $^{18}$F-fluorodeoxyglucose ($^{18}$FDG)-PET signal (n = 1). There was evidence of viral dissemination to noninjected tumor tissue. The responses in injected and noninjected tumor tissue could therefore have been mediated by direct viral oncolysis, antiviral immune responses towards virally infected cells or antitumor immune responses established in the injected lesions.

Oncolytic viruses have been combined with tumor vaccines in an attempt to exploit viral danger signals. Vaccinia virus–melanoma cell lysate vaccines were used in an adjuvant Phase III study of 700 patients following melanoma resection, with no improvement in recurrence or overall survival [57]. A series of clinical studies has been performed by Schirrmacher et al. using a live autologous tumor vaccine infected by NDV irradiated to render tumor cells nonviable [58]. A significant proportion of patients developed antitumor immune responses as assessed by a delayed-type hypersensitivity response to skin prick tests. Phase II studies have been performed in glioblastoma multiforme, melanoma, breast and colorectal cancer with improvements in overall survival by 20–36% at 2–5-year follow-up compared with historical controls. These studies suggest that oncolytic viruses can break immunological tumor tolerance, although Phase III studies are needed to confirm these findings.

**Oncolytic virotherapy & immunomodulation**

Clinical trials have demonstrated the production of antiviral neutralizing antibodies following both intratumoral and intravenous viral therapy [56,59]. Although neutralizing antibody levels do not consistently appear to correlate with clinical response [3,56], preclinical studies have suggested that suppression of antibody production with cyclophosphamide can enhance viral delivery [60]. Suppression of the innate immune response, including NK cells and macrophages, using cyclophosphamide has also been shown to enhance intravascular delivery of HSV to rat gliomas [44]. Although immunosuppression would not intuitively appear to favor the generation of antitumor immunity, enhanced tumor infection followed by recovery from immunosuppression may be beneficial. In addition, cyclophosphamide may selectively deplete...
Treg cells, which suppress antitumor immunity [61]. The overall immunological consequences of viral therapy with immunomodulating doses of cyclophosphamide remain unclear and clinical trials are currently planned.

**Future directions: combination therapy**

Combination therapy may be the optimal context in which to exploit the immunotherapeutic potential of oncolytic viruses. A rationale exists for combination with existing immunotherapy strategies, along with conventional therapy.

**Adoptive cellular therapy & viral delivery**

The use of cell carriers to chaperone viral particles to the tumor is a promising innovation [51]. Cells of the immune system have proven particularly adept, including cytokine-activated killer cells [52] and T lymphocytes [36]. Adoptive cellular therapy has met with some clinical success, but has been limited by the trafficking to and survival of T cells in the tumor microenvironment [62]. In a mouse model, the combination of oncolytic virus delivery with antigen-specific adoptive T-cell therapy has been shown to improve upon either treatment modality alone [63]. Although yet to be tested in clinical trials, these findings are of significant translational potential.

**Immunotherapy combinations**

Immunotherapy approaches may be logically combined with virotherapy to enhance antitumor responses. For example, IL-2 in combination with Treg cell depletion has been shown to facilitate therapy with systemic VSV [64]. This combination induces a degree of vascular permeability that may increase viral access to the tumor, but also generates ‘hyperactivated’ NK cells with antitumor activity. As an alternative to combination with systemic cytokine therapy, oncolytic viruses have been designed to express cytokines that may facilitate the generation of antitumor immunity. For example, vaccinia viruses have been engineered to express GM–CSF [65] and IFN-β [66]. The in vivo immunological response to these viruses compared with nontransduced viruses has yet to be fully elucidated. Several monoclonal antibodies with immunoregulatory properties, such as anti-cytotoxic T-lymphocyte antigen (CTLA)4 and anti-VEGF antibodies, are currently in clinical trials. There is potential to combine these agents with virotherapy, although this remains to be tested.

**Radiotherapy**

Radiotherapy has been shown to act synergistically with oncolytic reovirus in vitro and in vivo [67]. This combination is also promising immunotherapeutically as radiotherapy has been shown to enhance T-cell trafficking [68], and antigen presentation and T-cell recognition of tumor cells [69]. However, radiotherapy is also locally immunosuppressive, killing lymphocytes, and the optimal combination to enhance antitumor immune responses will require careful consideration of dose fractionation and treatment scheduling.

**Chemotherapy**

Chemotherapy combinations with oncolytic viral therapy may have additive or synergistic effects in terms of direct oncolysis. Post-chemotherapy myelosuppression means that the combination as an immunotherapeutic regimen is problematic, although chemotherapy has been reported to augment immunotherapy [70].

**Clinical trial design**

Clinical trials of oncolytic virotherapy should be designed to assess immunological outcomes in addition to more traditional end points. Importantly, immunologically mediated responses
may take longer to develop than the cytotoxic effects of therapeutic agents. As for other trials of immunotherapy, outcome measures should include appropriate immunological read-outs and appropriate follow-up periods to detect immunologically mediated clinical responses [71]. Patient selection is important, as heavily pretreated end-stage patients may already be immunosuppressed, while patients with rapid disease progression may not have time to generate an effective immune response.

**Expert commentary**

The host immune response will probably be critical to the efficacy of oncolytic virotherapy, although it is a fine balance between rapid viral elimination and innate and adaptive responses, which may mediate tumor regression. The rational design of combination therapy, modulating the immunological outcome, may hold the key to fulfilling the potential of these novel agents. Clinical trials should be designed to include specific assessment of immune responses to both tumor and viral antigens, and recognize the immunotherapeutic potential of virotherapy in terms of clinical end points and patient selection.

**Five-year view**

The next few years will see progress in terms of viral delivery, in particular the use of immune cell carriers that have yet to enter clinical trials. The promising strategy of combining existing antitumor adoptive cellular therapy with oncolytic viral delivery is likely to be explored. Combinations of viral therapy with chemotherapy, radiotherapy, transient immunosuppression and other immunotherapy strategies will probably be tested in early-phase clinical trials. Viruses engineered to enhance antitumor immune responses already appear promising and will enable determination of the consequences of manipulation of the immune response when administered via effective delivery vehicles.

**Key Issues**

- Oncolytic viruses have direct anticancer properties.
- The host immune response is likely to be critical to the efficacy of *in vivo* oncolytic virotherapy, via innate, antitumor and/or antiviral adaptive immunity.
- Tumor infection can induce tumor cell death with release of tumor-associated antigens in combination with endogenous danger signals, cytokines and direct effects on dendritic cells.
- Preclinical and clinical observations suggest that oncolytic virotherapy can induce adaptive antitumor immunity.
- The immune response is finely balanced between viral elimination and antitumor effects.
- Different oncolytic viruses have varying immunotherapeutic potential, some being immunosuppressive and others having immunostimulatory properties.
- Viruses are being engineered to enhance their potential to generate antitumor immunity.
- Clinical trials should be designed with immunological end points in mind.
- The future involves novel methods of viral delivery and combination with immunotherapy or conventional therapy.
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Papers of special note have been highlighted as:

• of interest

•• of considerable interest


Figure 1. Concept of how oncolytic viral infection of tumor cells may lead to the generation of antitumor immune responses
NK: Natural killer; TAA: Tumor-associated antigen; TLR: Toll-like receptor.