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Computational approaches to study oncolytic virus therapy: insights and challenges

Review Article

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Summary

The paper reviews computational models for analyzing the use of replicating oncolytic viruses as therapeutic agents against cancers. The paper highlights viral and host paramters which are crucial for success, and discusses how virus strains can be optimized in order to achieve maximial remission of cancers. The models consider three mechansisms by which oncolytic virus therapy could work: (i) virus-mediated killing of tumor cells. (ii) Induction of immune responses against the virus which can kill infected tumor cells. (iii) Induction of tumor specific immune responses following the release of stimulatory signals as a result of the virus infection. The models further give rise to insights into how virus variants should be tested in vitro in order to determine their therapeutic potential.

I. Introduction

Traditional therapy of tumors and cancers (chemotherapy) is characterized by a relatively low efficacy and high toxicty for the patient. While efforts are under way to design more efficient drugs that target genetic abnormalities only present in cancer cells, advances in genetic engineering have opened up possibilities to use replicating viruses as "biological control agents" to combat tumors (Kirn and McCormick, 1996). Several viruses have been altered to selectively infect cancer cells. Examples are HSV-1, NDV, and adenoviruses (Kirn and McCormick, 1996). A specific example that has drawn attention recently is ONYX-015, an attenuated adenovirus which selectively infects tumor cells with a defect in p53 (Kirn and McCormick 1996; Oliff et al, 1996; Hall et al, 1998; Heise et al, 1999a, b; Dix et al, 2000; Rogulski et al, 2000). This virus has been shown to have significant anti-tumor activity and has proven relatively effective at reducing or eliminating tumors in clinical trials (Kirn et al, 1998; Ganly et al, 2000; Khuri et al, 2000). Yet challenges remain. In particular, it is unclear which virus characteristics are most optimal for such therapeutic purposes. Viruses have been altered in a variety of ways by targeted mutations, but it is not clear what types of mutants have to be produced in order to achieve extinction of the cancer. If tumor eradication does not occur, the outcome is the persistence of both the tumor and the virus infection, and this would be detrimental for patients. Persistence of both tumor and virus has been seen in experiments with a mouse model system by Harrison et al, (2001). The reason for the failure to eradicate the tumor despite ongoing viral replication was left open to speculation.

Mathematical models have been used to address this question. Taking into account the complex interactions between viruses, tumor cells, and immune responses, such models have identified conditions under which oncolytic virus therapy is most likely to result in successful clearance of cancer. This review discusses these insights. The models take into account a variety of mechanisms which can contribute to cancer elimination. On the most basic level, virus infection and the consequent virus-induced death of the cancer cell can be responsible for tumor eradication. On top of this, the immune system is expected to have an effect. In particular, cytotoxic T lymphocytes (CTL) are likely to be important. These are immune cells which can kill cells which display foreign or mutated proteins. They may act in two basic ways. They can recognize the virus on infected cells and kill virus-infected cells. Alternatively, the virus infection may promote the establishment of a CTL response against cancer proteins which would otherwise not develop. Significant immune responses are not normally mounted against cancers. A virus infection might alert the immune system and activate an otherwise silent response. This is known as the danger signal hypothesis in immunology.

This review will discuss mathematical models which consider all three scenarios and discuss optimal strategies to achieve cancer extinction. The models also give valuable insights regarding experimental tests of specific virus mutants in order to evaluate them for treatment. The review will finish with a discussion of these insights.

II. Virus-induced killing of tumor cells

This section investigates the basic dynamics between a growing tumor population and a replicating virus selective for the tumor cells. Various aspects of tumor growth and inhibition have been modeled in a variety of ways (Gatenby 1996; Gatenby and Gawlinski 1996; Adam and Bellomo 1997; Kirschner and Panetta 1998). We concentrate on a simple model, capturing the essential assumptions for analyzing virus-mediated therapy. The model contains two variables: uninfected tumor cells, x, and tumor cells infected by the virus, y. It is explained schematically in **Figure 1**. The tumor cells grow in a logistic fashion at a rate r and die at a rate d. The maximum size or space the tumor is allowed to occupy is given by its carrying capacity k. The virus spreads to tumor cells at a rate β (this parameter can be viewed as summarizing the replication rate of the virus). Infected tumor cells are killed by the virus at a rate a and grow in a logistic fashion at a rate s. This assumes that division of infected tumor cells results in both daughter cells carrying the virus. This would certainly be the case with a virus that integrates into the tumor cell genome, but with a non-integrating virus, the chances of transmission upon cell division should be sufficiently high to justify this assumption. The model is given by the following set of ordinary differential equations (Wodarz, 2001):

$$\dot{x} = rx(1 - \frac{x+y}{k}) - dx - \beta xy$$

$$\dot{y} = \beta xy + sy(1 - \frac{x+y}{k}) - ay$$

In the absence of the virus the trivial equilibrium is attained and is given by E0:

$$x^{(0)} = k(r-d)/r, y^{(0)} = 0$$

The virus can establish an infection in the tumor cell population if $[\beta k(r-d)+sd]/r > a$. In this case, two types of outcomes are possible. The virus can either attain 100% prevalence in the tumor cell population (i.e. all tumor cells are infected), or it may only infect a fraction of the tumor

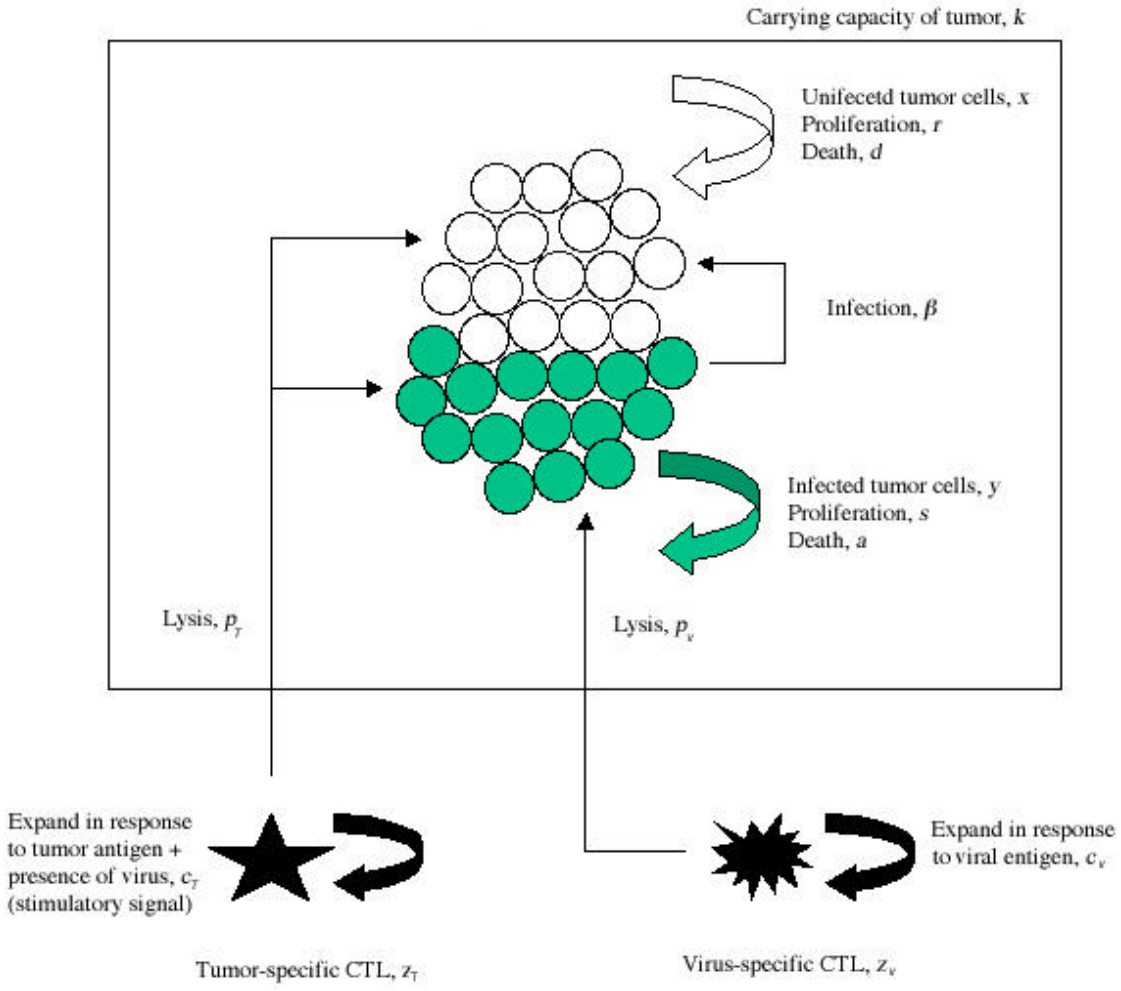

Figure 1: Schematic representation of the assumptions underlying the mathematical models. In the text, the model is built up gradually. It starts with the interactions between the virus and the tumor cells. Then a virus-specific CTL responses is added, followed by including a tumor-specific CTL response.

cells (i.e. both uninfected and infected tumor cells are observed). Hundred percent virus prevalence is described by equilibrium E1:

$x^{(1)} = 0, y^{(1)} = k(s-a)/s$

Coexistence of infected and uninfected tumor cells is described by equilibrium E2:

$$x^{(2)} = \frac{\beta k(a-s)+ar-sd}{\beta(\beta k+r-s)},\ y^{(2)} = \frac{\beta k(r-d)+sd-ra}{\beta(\beta k+r-s)}$$

The virus infects all tumor cells (equilibrium E1) if $a<s(d+\beta k)/(r+\beta k)$. Otherwise, Equilibrium E2 is observed.

With this result in mind, how does viral cytotoxicity influence the size of the overall tumor? The tumor size is defined as the sum of infected and uninfected tumor cells, $x+y$, at equilibrium. Viral cytotoxicity has an opposing influence on tumor load depending on which equilibrium is attained (**Figure 2a**). If all tumor cells are infected, then $x+y=k(s-a)/s$. An increase in viral cytotoxicity results in a reduction in tumor load (**Figure 2a**). On the other hand, if not all tumor cells are infected, then $x+y=k(r-s+a-d)/(\beta k+r-s)$. Now, an increase in the viral cytotoxicity increases tumor load (**Figure 2a**). The reason is that increased rates of tumor cell killing eliminate infected tumor cells before the virus had a chance to significantly spread. This in turn increases the pool of uninfected tumor cells and therefore the tumor load.

Hence, there is an optimal cytotoxicity, a_{opt}, at which the tumor size reaches a minimum. This optimum is the degree of cytotoxicity at which the system jumps from the equilibrium describing 100% virus prevalence to the equilibrium where uninfected tumor cells are also present (**Figure 2a**). The optimal viral cytotoxicity is thus given by $a_{opt}=s(d+\beta k)/(r+\beta k)$. At this optimal cytotoxicity the tumor size is reduced maximally and is given by $[x+y]_{mi} = k(r-d)/(r+\beta k)$.

There are a number of points worth noting about this result. The minimum tumor size this therapy regime can achieve is most strongly determined by the replication rate of the virus, β (**Figure 2a**). The higher the replication rate of the virus, the smaller the minimum size of the tumor. In order to achieve this minimum, the viral cytotoxicity must be around its optimum value. A major determinant of the optimal viral cytotoxicity is the rate of growth of uninfected and infected tumor cells (r and s respectively).

i) If the infected tumor cells grow at a significantly slower rate relative to uninfected cells ($s << r$), the optimal cytotoxicity is low (**Figure 3a**). In the extreme case where the virus abolishes the ability of the tumor cell to divide, a non-cytotoxic virus is required to achieve optimal treatment results. More cytotoxic viruses result in tumor persistence (**Figrue 3a**).

ii) On the other hand, if the growth rate of infected tumor cells is not significantly lower than that of uninfected tumor cells, an intermediate level of virus induced cell death is required to achieve minimum tumor size (**Figure 3b**). If viral cytotoxicity is too weak, the tumor persists. However, if the viral cytotoxicity is too high, the tumor also persists because infected cells die too fast for the virus to spread efficiently (**Figrue 3b**). In general, the faster the replication rate of the virus, the higher the optimal level of cytotoxicity.

III. Effect of virus-specific CTL

This section expands the above model to include a population of virus-specific CTL, z_v. The CTL recognize viral antigen on infected tumor cells. Upon antigenic encounter, the CTL proliferate with a rate $c_v yz_v$ and kill the infected tumor cells with a rate $p_v yz_v$. In the absence of antigenic stimulation the CTL die with a rate bz_v. The model is given by the following set of differential equations (Wodarz, 2001).

$$\dot{x} = rx(1-\frac{x+y}{k}) - dx - \beta xy,$$

$$\dot{y} = \beta xy + sy(1-\frac{x+y}{k}) - ay - p_v yz_v$$

$$\dot{z}_v = c_v yz_v - bz_v$$

First, we define the conditions under which an anti-viral CTL response is established. This condition is different depending on whether the virus attains 100% prevalence in the tumor cell population in the absence of the CTL. The strength of the CTL response, or CTL responsiveness, is denoted by c_v. If the virus has attained 100% prevalence in the absence of CTL, the CTL become established *if* $c_v>bs/[k(s-a)]$. On the other hand, if the virus is not 100% prevalent in the tumor cell population in the absence of CTL, the CTL invade if $c_v>b\beta(\beta k+r-s)/[r(\beta k-a)-d(\beta k-s)]$.

In the presence of the CTL, we again observe two basic equilibria: we either observed 100% virus prevalence in the tumor cell population, or the coexistence of infected and uninfected tumor cells. Hundred percent virus prevalence in the tumor cell population is described by equilibrium E1:

$$x^{(1)} = 0,\ y^{(1)} = b/c,\ z_v^{(1)} = \frac{kc_v(s-a)-sb}{p_v kc_v}$$

Coexistence of infected and uninfected cells is described by equilibrium E2:

$$x^{(2)} = \frac{r(kc_v-b)-k(c_v d+b\beta)}{rc_v},\ y^{(2)} = b/c_v,$$

$$z_v^{(2)} = \frac{\beta k(rc_v-b\beta-c_v d)-c_v(ar-sd)-b\beta(r-s)}{p_v c_v r}$$

How do the CTL influence the outcome of treatment? We distinguish between two scenarios.

i) If the virus has established 100% prevalence in the tumor cell population in the absence of the CTL response, the presence of CTL can both be beneficial and detrimental to the patient (**Figure 2b**): the virus can remain 100% prevalent in the tumor in the presence of CTL. In this case, overall tumor size is given by $x+y=b/c_v$. At this equilibrium, an increase in the CTL responsiveness against the virus decreases the tumor size (**Figure 2b**). On the other hand, if the CTL responsiveness crosses a threshold given by $c_v > b(\beta k+r)/[k(r-d)]$, the virus does not maintain 100% prevalence in the tumor cell population, and the overall tumor size is given by

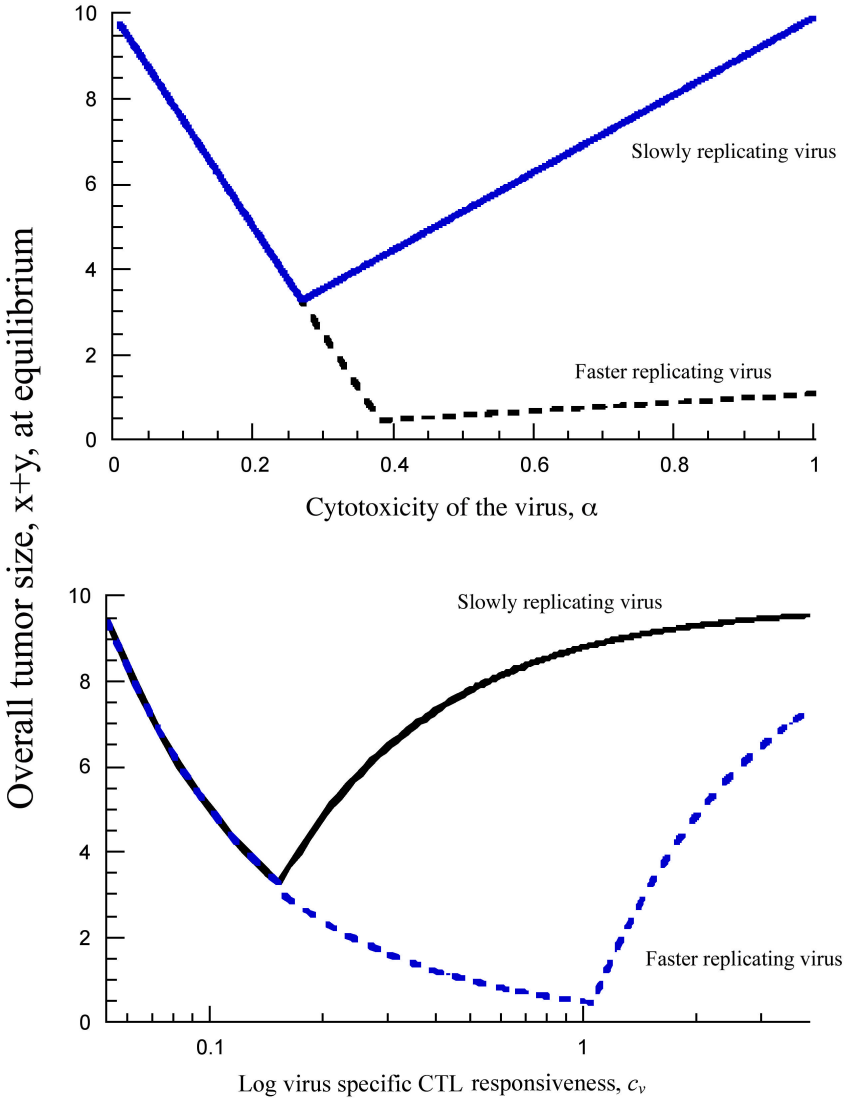

Figure 2: a) Dependence of overall tumor load on the cytotoxicity of the virus. There is an optimal cytotxocity at which tumor load is smallest. This is also the point where the system switches from equilibrium describing 100% virus prevalence in the tumor population to the equilibrium where infected and uninfected tumor cells coexist. The faster the rate of virus replication, the higher the optimal level of cytotoxocity, and the smaller the minimum tumor load. Parameters were chosen as follows: *k=10; r=0.2; s=0.2; d=0.1;* for fast viral replication, β=*1*; for slow viral replication β=*0.1*. **b)** Dependence of overall tumor load on the stength of the virus-specific CTL response. There is an optimal CTL responsiveness at which tumor load is smallest. This is also the point where the system switches from equilibrium describing 100% virus prevalence in the tumor population to the equilibrium where infected and uninfected tumor cells coexist. The faster the rate of virus replication, the higher the optimal strength of the CTL response, and the smaller the minimum tumor load. Parameters were chosen as follows*: k=10; r=0.5; s=0.5; d=0.1; b=0.1; p=1; a=0.2;* for fast viral replication, β=*1*; for slow viral replication β=*0.1*.

$x+y=k[c_v(r-d)-b\beta]/(c_v r)$. In this case, an increase in the CTL responsiveness to the virus increases tumor load and is detrimental to the patient (**Figure 2b**). This is because the CTL response kills the virus faster than it can spread. Hence, the optimal CTL responsiveness is given by $c_{opt=}b(\beta k+r)/[k(r-d)]$. At this optimal CTL responsiveness, the tumor size is reduced maximally and is given by $[x+y]_{min}=k(r-d)/(r+\beta k)$. The faster the replication rate of the virus, the higher the optimal CTL responsiveness, and the lower the minimum size of the tumor that can be attained by therapy (**Figure 2b**). Note that the minimum tumor size that can be achieved is the same as in the previous case where viral cytotoxicity alone was responsible for reducing the tumor. The effect of the CTL response is to modulate the overall death rate of infected cells with the aim of pushing it towards its optimum value. **Figure 4** shows a simulation of therapy where an intermediate CTL responsiveness results in tumor remission, while a stronger CTL response can result in failure of therapy because virus spread is inhibited.

ii) If the virus is not 100% prevalent already in the absence of the CTL response, a CTL-mediated increase in the death rate of infected cells can only be detrimental to the patient since it increases tumor load. The system converges to an equilibrium tumor size described by $x+y=k[c_v(r-d)-b\beta/(c_v r)]$.

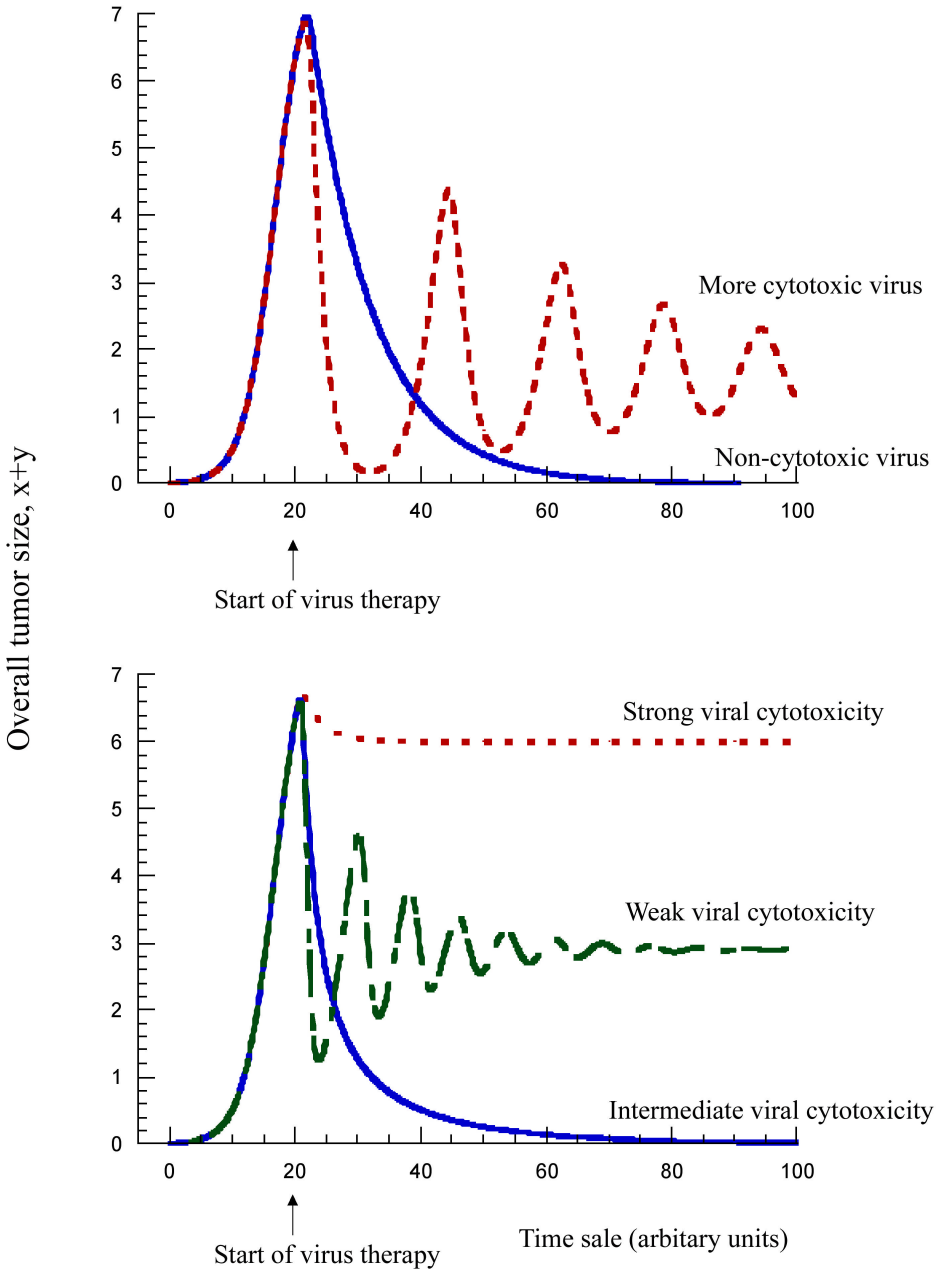

Figure 3: Simulation of therapy using tumor cell infecting viruses in the absence of immunity. (**a**) The growth rate of infected tumor cells is significantly slower than that of uninfected tumor cells. A non-cytotoxic virus now results in tumor eradiation. A more cytotoxic virus results in tumor persistence. Parameters were chosen as follows*: k=10; r=0.5; s=0;* β*=1; d=0.1; a= 0.1* for the non-cytotoxic virus, and *a=0.5* for the more cytotoxic virus. (**b**) The growth rate of infected tumor cells is not significantly reduced relative to that of uninfected cells. An intermediate level of cytotoxicity results in tumor eradication. Weaker or stronger levels of cytotoxicity result in tumor persistence. Parameters were chosen as follows: *k=10; r=0.5; s=0;* β*=1; d=0.1; a= 0.2* for the weakly cytotoxic virus, *a=0.55* for intermediate cytotoxicity, and *a=3* for strong cytotoxicity.

IV. Virus infection and the induction of tumor-specific CTL

The above sections explored how virus infection and the virus-specific CTL response can influence tumor load. However, virus infection might not only induce a CTL response specific for viral antigen displayed on the surface of the tumor cells. In addition, active virus replication could induce a CTL response specific for tumor antigens (Fuchs and Matzinger, 1996; Matzinger, 1998). The reason is that virus replication could result in the release of substances and signals alerting and stimulating the immune system. This could be induced by tumor antigens being released and taken up by professional antigen presenting cells (APC), and/or by other signals released from the infected tumor cells. This is known as the danger signal hypothesis in immunology. Normal tumor growth is thought not to evoke such signals, whereas the presence of viruses can does evoke danger signals. Here, such a tumor specific CTL response is included in the model. It is assumed that the responsiveness of the tumor-specific CTL requires two signals: (i) the presence of the tumor

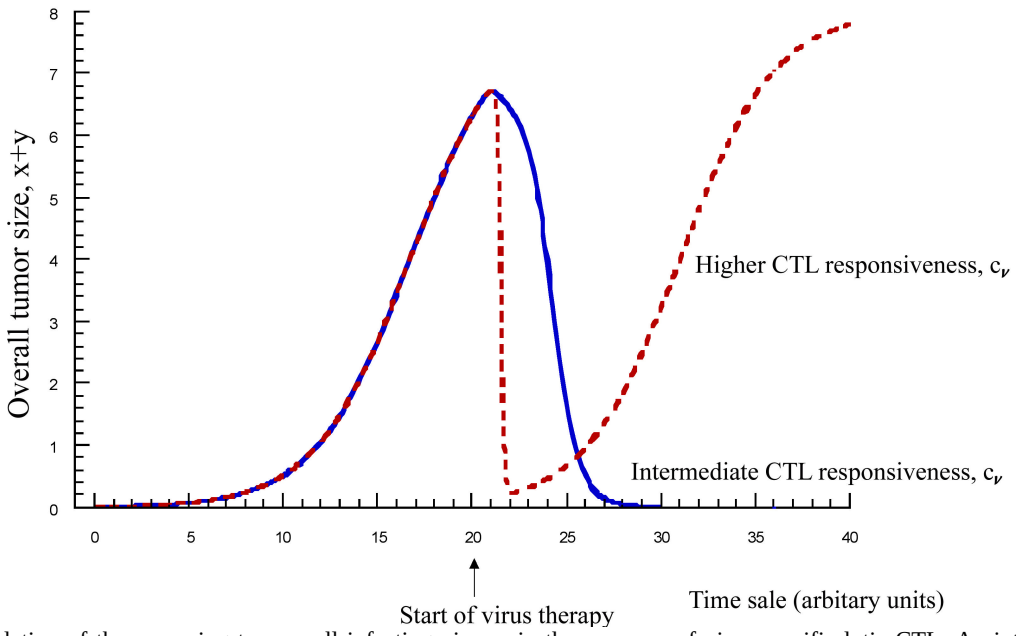

Figure 4: Simulation of therapy using tumor cell infecting viruses in the presence of virus-specific lytic CTL. An intermediate CTL responsiveness results in tumor eradication, while a stronger CTL response results in tumor persistence. Note that with the stronger CTL response, the initial decay of the tumor is faster. However, subsequently the virus is removed from the tumor cell population before the tumor has been driven extinct. Therefore, the tumor cells can start to grow back again. Parameters were chosen as follows: *k=10; r=0.5; s=0.5;* β*=0.1; a=0.2; p=1; b=0.1; b=0.1; d=0.1;* The intermediate CTL responsiveness is characterized by $c_v=0.2625$, while the stronger CTL response is characterized by $c_v=2$.

antigen, and (ii) the presence of infected tumor cells providing immuno-stimulatory signals. In the following, the interactions between the tumor, the virus, and the tumor-specific CTL are investigated.

A model is constructed describing the interactions between the tumor population, the virus population, and a tumor-specific CTL response. It takes into account three variables. Uninfected tumor cells, x, infected tumor cells, y, and tumor specific CTL, z_T. It is given by the following set of differential equations (Wodarz, 2001).

$$\dot{x} = rx(1-\frac{x+y}{k}) - dx - \beta xy - p_T xz_T$$

$$\dot{y} = \beta xy + sy(1-\frac{x+y}{k}) - ay - p_T yz_T$$

$$\dot{z}_T = c_T yz_T (x+y) - bz_T$$

The basic interactions between viral replication and tumor growth are identical to the models described above. The tumor-specific CTL expand in response to tumor antigen, which is displayed both on uninfected and infected cells ($x+y$), at a rate c_T. However, in accord with the danger signal hypothesis, it is assumed that the tumor-specific CTL response only has the potential to expand in the presence of the virus, y. In the model virus load correlates with the ability of the tumor-specific response to expand, since high levels of viral replication result in stronger stimulatory signals. The tumor-specific CTL kill both uninfected and infected tumor cells at a rate $p_T yz_T$.

If the virus has reached 100% prevalence in the absence of CTL, the tumor-specific CTL response becomes established if $c_T > bs^2/[k(a-s)]^2$. If infected and uninfected tumor cells coexist in the absence of CTL, the tumor specific CTL response becomes established if $c_T > b\beta\,(s-r-\beta k)^2/\{k\,[\beta k(r-d)-ra+sd](r-s+a-d)\}$.

In the presence of the tumor-specific CTL, the virus can again attain 100% prevalence in the tumor cell population, or we may observe the coexistence of infected and uninfected tumor cells. Hundred percent prevalence in the tumor population is described by equilibrium E1:

$$x^{(1)}=0,\ y^{(1)}=(b/c_T)^{1/2},\ z_T^{(1)} = \frac{k(s-a)-sy^{(1)}}{p_T k}$$

Coexistence of infected and uninfected tumor cells is described by equilibrium E2:

$$y^{(2)} = \frac{b\beta}{ac_T},\ z_T^{(2)} = \frac{1}{p_T}[\,r(1-\frac{x^{(2)}+y^{(2)}}{k}) - d - \beta y^{(2)}\,]$$

$$x^{(2)} = \frac{c_T rk[r-2(d+s+a)] + c_T dk^2[d+2(s-a)] + c_T k^2[s(s-2a)+a^2] + b\beta k[2(s-r)-\beta k] - br^2 + bs(2r-s)}{c_T k(\beta k + r - s)(r-d+a-s)}$$

We investigate how the responsiveness of the tumor-specific CTL, c_T, influences the size of the tumor, $x+y$. The presence of the tumor specific CTL can have the following effects. If the virus achieves 100% prevalence in the tumor cell population, then $x+y=(b/c_T)^{1/2}$. Thus, an increase in the responsiveness of the tumor-specific CTL results in a decrease in tumor load (**Figure 5a**). If $c_T > b(\beta k+r-s)^2/[k\,(r-s+a-d)]^2$, the virus is not 100% prevalent in the tumor cell population. This switch is thus promoted by a high responsiveness of the tumor-specific CTL

relative to the replication rate of the virus (**Figure 5a**). In this case, the size of the tumor is given by $x+y=k(r-s+a-d)/(\beta k+r-s)$. This is the minimum tumor size that can be achieved. Thus, if the CTL responsiveness against the tumor lies above a threshold, tumor load reaches its minimum (**Figure 5a**). Note that it also becomes independent of the strength of the CTL. Hence, a CTL responsiveness that lies above this threshold is not detrimental to the patient. In this situation, tumor size is determined by the replication rate and the cytotoxicity of the virus (**Figure 5a**). The higher the replication rate of the virus and the lower the degree of viral cytotoxicity, the smaller the tumor. The reason is that fast viral replication and low cytotoxicity result in higher virus load which in turn results in stronger signals to induce the tumor-specific CTL. **Figure 5b** shows a simulation of treatment underscoring this result.

A note of caution: the model assumes that the production of immuno-stimulatory signals induced by the virus is proportional to the amount of viral replication. If cellular debris following virus-mediated destruction of cells also contributes to these signals, then the effect of viral cytotoxicity could be more complex.

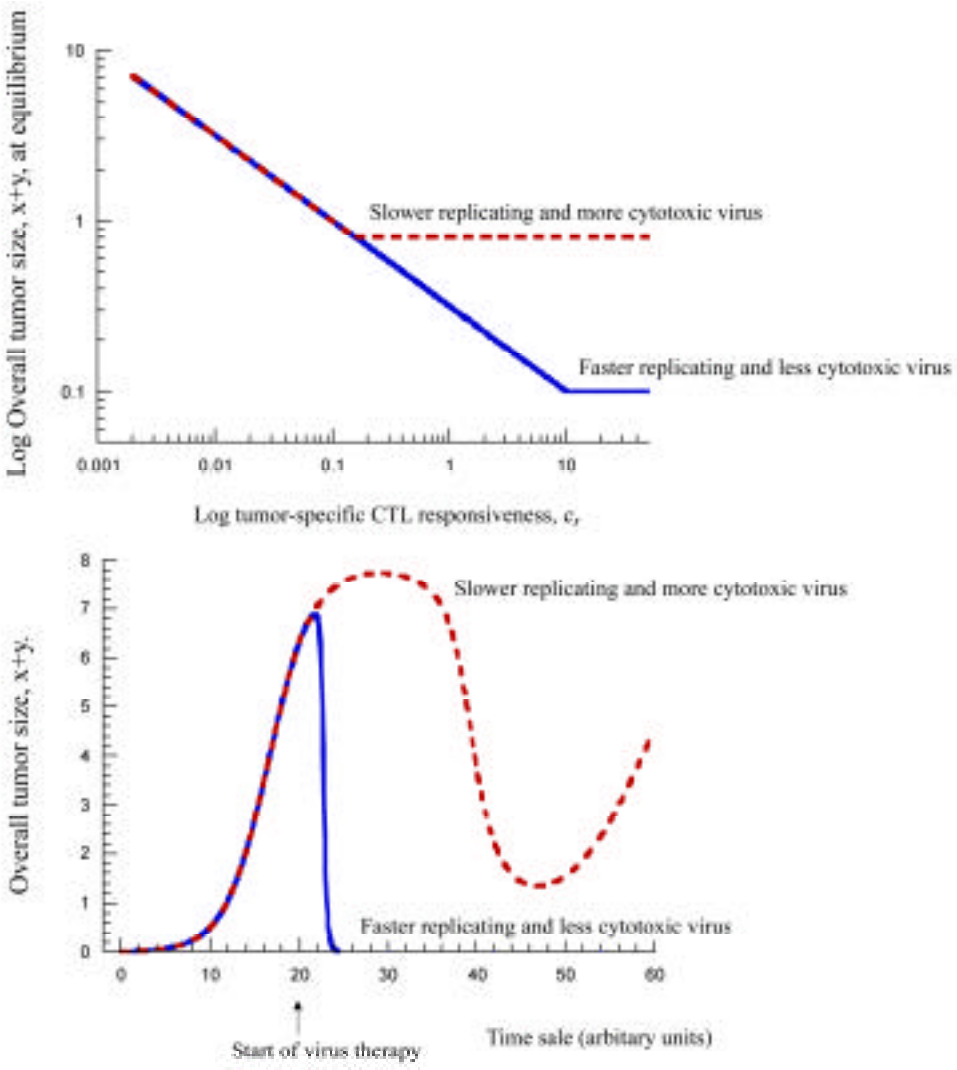

Figure 5: (**a**) Dependence of overall tumor load on the strength of the tumor-specific CTL response. The higher the strength of the tumor-specific CTL, the lower tumor load. If the strength of the tumor-specific CTL crosses a threshold, tumor load becomes independent of CTL parameters. Instead it is determined by the replication rate and cytotoxicity of the virus. The faster the rate of virus replication and the smaller the degree of viral cytotoxicity, the further the overall tumor load can be reduced. The CTL responsiveness at which tumor load becomes independent of CTL parameters is also the point at which the system switches from equilibrium describing 100% virus prevalence in the tumor population to the equilibrium where infected and uninfected tumor cells coexist. Parameters were chosen as follows: $k=10;\ r=0.5;\ s=0.5;\ d=0.1;\ b=0.1;$ The fast replicating and weakly cytotoxic virus is characterized by $\beta=1$ and $a=0.2$. The slower replicating and more cytotoxic virus is characterized by $\beta=0.5$ and $a=0.5$. (**b**) Simulation of therapy using a tumor cell infecting virus in order to stimulate a tumor-specific CTL response. If the virus replicates at a fast rate and is weakly cytotoxic, the level of immun-stimulatory signals is high. Hence the tumor-specific response is strong and drives the tumor extinct. If the virus replicates slowly and is more cytotoxic, the level of stimulatory signals is lower. This compromises the efficacy of the tumor-specific CTL which cannot drive the tumor into remission. Parameters were chosen as follows: $k=10;\ r=0.5;\ s=0.5;\ d=0.1;\ b=0.1;\ c_T=0.2$. The fast replicating and weakly cytotoxic virus is characterized by $\beta=0.5$ and $a=0.2$. The slower replicating and more cytotoxic virus is characterized by $\beta=0.1$ and $a=0.6$.

However, the exact nature and concept of the so called danger signals is still controversial. The model takes into account the simple observation that presence of signals typical of viral replication can enhance immunity to tumors.

V. Interactions between virus- and tumor-specific CTL

In this section, the two types of CTL responses studied above are brought together. That is, both the virus- and the tumor specific CTL responses are taken into consideration. The model is explained schematically in **Figure 1** and given by the following set of differential equations (Wodarz, 2001).

$$\dot{x} = rx(1-\frac{x+y}{k}) - dx - \beta xy - p_T x z_T$$

$$\dot{y} = \beta xy + sy(1-\frac{x+y}{k}) - ay - p_v y z_v - p_T y z_T$$

$$\dot{z}_v = c_v y z_v - b z_v$$

$$\dot{z}_T = c_T y z_T (x+y) - b z_T$$

In this model the virus- and the tumor specific CTL responses are in competition with each other, because both can reduce tumor load and hence the strength of the stimulus required to induce CTL proliferation. In the following these competition dynamics are examined.

If the virus has reached 100% prevalence in the tumor cell population in the absence of CTL, then virus- and tumor specific CTL cannot coexist. If $c_v > (c_T b)^{1/2}$, then the virus-specific CTL response is established. On the other hand, if $c_v < (c_T b)^{1/2}$, then the tumor-specific CTL response becomes established.

If both infected and uninfected tumor cells are present in the absence of CTL, the situation is more complicated. Now, three outcomes are possible. Either the virus-specific response becomes established, or the tumor-specific response becomes established, or both responses can coexist. The virus-specific response persists if $c_v > kc_T(r-s+a-d)/(\beta k+r-s)$. The tumor-specific response persists if $c_T > c_v^2 r/\{k[c_v(r-d)-b\beta]\}$. Coexistence of both CTL responses is only observed if both of these conditions are fulfilled. This outcome is described by the following equilibrium expressions.

$$x^{(1)} = \frac{c_v^2 - bc_T}{c_v c_T}, \quad y^{(1)} = b/c_v,,$$

$$z_T^{(1)} = \frac{1}{p_T}[r(1-\frac{x^{(1)}+y^{(1)}}{k}) - d - \beta y^{(1)}]$$

$$z_v^{(1)} = \frac{1}{p_v}[\beta x^{(1)} + s(1-\frac{x^{(1)}+y^{(1)}}{k}) - a - p_T z_T^{(1)}]$$

If both responses coexist, then the size of the tumor is given by $x+y=c_v/c_T$. Thus, a strong tumor-specific response, c_T, reduces tumor load. On the other hand, a strong virus-specific response, c_v, increases tumor load. The reason is that a strong virus-specific response results in low virus load and therefore in low stimulatory signals promoting the induction of tumor-specific immunity. Note that this last statement only applies to the parameter region where both types of CTL responses co-exist.

VI. Treatment strategies

The above discussion has shown that the outcome of therapy depends on a complex balance between host and viral parameters. An important variable is the death rate of infected tumor cells. In order to achieve maximum reduction of the tumor, the death rate of the infected cells must be around its optimum, defined by the mathematical models. If the death rate of infected cells lies around its optimum, a fast replication rate of the virus and a slow growth rate of the tumor increase the chances of tumor eradication. The death rate of infected tumor cells can be influenced by a variety of factor: *(i)* Viral cytotoxicity alone kills tumor cells. *(ii)* A CTL response against the virus contributes to killing infected tumor cells. *(iii)* The virus helps eliciting a tumor-specific CTL response following the release of immuno-stimulatory signals.

The most straightforward way to use viruses as anti-cancer weapons is in the absence of immunity. If the cytotoxicity of the virus is around its optimum value, minimum tumor size is achieved. It is important to note that the highest rate of virus induced tumor cell killing does not necessarily contribute to the elimination of the tumor. The reason is that a very high rate of virus-induced cell death compromises the overall spread of the infection through the tumor. If a virus specific CTL response is induced, the best strategy would be to use a fast replicating and weakly cytotoxic virus. This is because the CTL will increase the death rate of infected cells. If the overall death rate of infected cells is too high, this is detrimental to the patient, since virus spread is prevented. In addition, a weakly cytotoxic and fast replicating virus may provide the strongest stimulatory signals for the establishment of tumor-specific immunity.

Because the model suggests that a fast growth rate of the tumor decreases the efficacy of treatment, success of therapy could be promoted by using a combination of virus therapy and conventional chemo- or radiotherapy. These suggestions are supported by recent experimental data (Heise et al, 1997; Freytag et al, 1998; Rogulski et al, 2000; You et al, 2000). A combination of treatment with the adenovirus ONYX-015 and chemotherapy or radiotherapy has been shown to be significantly more effective than treatment with either agent alone.

The principles of the mathematical modeling approaches presented here can help to improve treatment and to attain higher levels of success. In order to achieve this, however, more work is needed. Basic parameters of viruses and virus mutants need to be measured as a first step. Because the optimal death rate of infected tumor cells is crucial, it will be important to precisely measure

the rate at which different viruses kill the tumor cells. Equally important is the quantification of the viral replication kinetics. Once such basic parameters have been measured, it is important to re-consider some model assumptions. The models discussed in this review are only a first approach to use computational methods for the analysis of oncolytic virus therapy, and the models will probably need to be revised and improved. For example, it is unclear whether and how the replication rate of the virus correlates with the rate of virus-induced cell killing. Many possibilities exist, and this is similar to the relationship between pathogen spread and "virulence" in an epidemiological context. Such more detailed information, based on experimental measurements, will be important to incorporate into the models in order to make more solid and reliable predictions.

VII. Evaluating viruses in culture

A central result derived from the mathematical models is that success is promoted by using a virus which induces an optimal death rate of infected cells. Too high a rate of virus-induced cell death is detrimental and leads to the persistence of both tumor and virus, because overall virus spread is impaired. This gives rise to important insights for the methods used to evaluate potential viruses in culture (Wodarz, 2003). The mathematical models suggest that a low multiplicity of infection (MOI, i.e. the initial abundance of the virus relative to the tumor cells) is required to evaluate the virus. The reason is that *in vivo*, the replicating virus has to spread through the cancer cell population, and this has to be mimicked in culture. Using a high MOI can lead to misleading evaluations. These notions are illustrated in **Figure 6** with computer simulations. This figure depicts the dynamics in culture for strongly and weakly cytopathic viruses, using different MOIs. **Figure 6i** shows the dynamics for a high MOI. In this simulation, the strongly cytopathic virus results in quick elimination of the tumor cells, while the weakly cytopathic virus is much less effective. Thus, if viruses are evaluated using a high MOI, the virus with the strongest degree of tumor cell killing receives the highest grades.

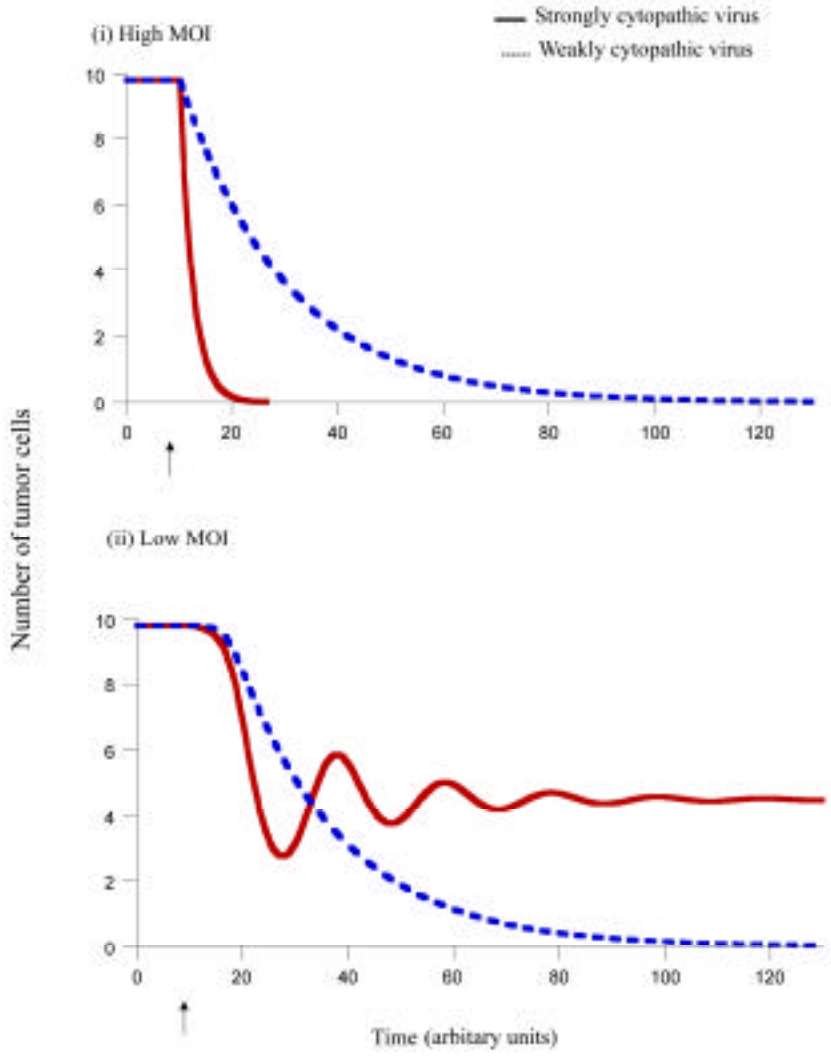

Figure 6: Simulation showing the evaluation of potential replicating viruses in culture. A weakly and a strongly cytopathic virus are compared. Introduction of the virus is indicated by an arrow. **(i)** High multiplicity of infection. In this simulation, the strongly cytopathic virus is more efficient at eradicating the cancer cells than the weakly cytopathic virus. This is a characteristic which will lead to inefficient reduction of tumor load *in* vivo (Figure 3). **(ii)** Low multiplicity of infection. Now the less cytopathic virus results in elimination of the tumor cell, while the virus with higher cytopathicity fails to eliminate the tumor cells. This is how viruses should be tested in culture. Parameters were chosen as follows. *r=0.5; s=0*; *k=10;* β*=1.5; d=0.01; k=0.1; u=1;* For the strongly cytopahtic virus,

a=0.4. For the weakly cytopathic virus, *a=0.04*. Virus inoculum was *y=10* for high MOI and *y=0.01* for low MOI. Note that the replication kinetics of the virus are assumed to have a sufficiently high value so that cancer remission is possible. Obviously if the virus replicates at a significantly slower rate (e.g. a lower value of β), tumor remission is not possible

Importantly, this is the virus which is predicted to be least efficient at reducing tumor load *in vivo*. The situation is different when viruses are evaluated in culture using a low MOI (**Figure 6ii**). The less cytopathic virus results in elimination of tumor cells in culture, while the more cytopathic virus fails to eliminate tumor cells in culture. Therefore, the less cytopathic virus gets the better marks, and this is also the virus which is predicted to be more efficient at reducing tumor load *in vivo*.

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