The importance of lytic and nonlytic immune responses in viral infections

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Antiviral immune effector mechanisms can be divided broadly into lytic and nonlytic components. We use mathematical models to investigate the fundamental question of which type of response is required to combat different types of viral infection. According to our model, the relative roles of the two types of component depend on the cytopathicity of the virus relative to its rate of replication. If the viral cytopathicity is low relative to the rate of viral replication, the model predicts that a combination of lytic and nonlytic effector mechanisms is likely to be required to resolve the disease, particularly if the virus replicates at a fast rate. By contrast, if viral cytopathicity is high relative to the replication rate of the virus, then lytic and nonlytic mechanisms can, in principle, resolve the infection independently. We discuss our findings in the context of specific viral infections and use our model to interpret empirical data.

During viral infections, the host immune system reacts with innate and antigen-specific immune responses. Both types of response can be subdivided broadly into lytic and nonlytic components. Lytic effector mechanisms kill infected cells, whereas nonlytic effector mechanisms inhibit viral replication through soluble mediators. As a part of the innate response, natural killer cells can lyse infected cells and cytotoxic T lymphocytes (CTLs) kill infected cells, whereas antibodies neutralize free virus particles and thus, inhibit the infection of susceptible cells. In addition, CD4+ and CD8+ T cells can secrete cytokines that inhibit viral replication [e.g. IFN-γ and tumor necrosis factor α (TNF-α)]. However, these distinctions are not always clear-cut. For example, cytokines, such as IFN or TNF, have been reported to not only inhibit viral replication but also, contribute to cell death in certain situations [1].

If the viral cytopathicity is low relative to the rate of viral replication, … a combination of lytic and nonlytic effector mechanisms is likely to be required…’

Many experiments have addressed the important question of which types of immune mediator are responsible primarily for controlling specific virus infections [2–5]. Perforin-knockout mice do not lose control of certain cytopathic viruses [3,5]. However, perforin-knockout mice infected with the noncytopathic lymphocytic choriomeningitis virus (LCMV) are compromised severely in their ability to control the infection [3,5]. Based on these observations, Kagi and others formulated the hypothesis that CTL-mediated lysis is an essential immune mechanism for fighting noncytopathic viruses in general, whereas soluble immune factors are sufficient to combat cytopathic viruses [3,5]. However, other and subsequent experiments have demonstrated that the situation is more complicated than this. Soluble factors have been shown to contribute to the resolution of noncytopathic infections [6–15] and some cytopathic infections can be cleared independently by soluble and lytic effectors [16,17].

The relationship between viral replication, cytopathicity and the immune response is very complex. However, because experimental analysis is limited by the conditions under which it is carried out, only select situations, and not the entire spectrum of possible outcomes, are likely to be identified experimentally. In such a context, mathematical...
Box 1. The model

We have constructed a mathematical model describing the basic dynamics of the interaction between susceptible host cells, \( x(t) \), a virus population, \( y(t) \), and immune responses, \( z(t) \). This model is shown in Figure 1. Susceptible host cells are generated at a rate \( \lambda \), die at a rate \( \delta x(t) \) and become infected by virus at a rate \( \beta x(t)y(t) \). Viral replication is inhibited by the immune response at a rate \( qz(t) + 1 \). This corresponds to nonlytic antiviral activity. Infected cells die at a rate \( ay(t) \) and are killed by the immune system at a rate \( py(t)z(t) \). This corresponds to lytic effector mechanisms. The immune response is assumed to get stronger at a rate proportional to the number of infected cells, \( cy(t) \), and also, decays exponentially at a rate proportional to its current strength, \( bx(t) \). Note that the variable \( x(t) \) represents the total immunity that can be generated in response to virus infection. The parameter \( p \) expresses the strength of the lytic component, whereas the parameter \( q \) expresses the efficacy of the nonlytic component. The model details the changes in host-cell number and strength of the immune response as the infection develops over time. This is described by the following set of differential equations.

\[
\frac{dx(t)}{dt} = \lambda - \delta x(t) - \beta x(t)y(t) + ay(t) - py(t)z(t)
\]

\[
\frac{dy(t)}{dt} = \frac{\beta x(t)y(t)}{qz(t) + 1} - ay(t) - py(t)z(t)
\]

\[
\frac{dz(t)}{dt} = cy(t) - bx(t)
\]

Note that we have not written down explicitly the dynamics of free virus. This is because we assume that the turnover of free virus is much faster than that of infected cells. This allows us to make a quasi steady-state assumption, whereby the amount of free virus is simply proportional to the number of infected cells. Hence, the variable \( y \), describing the number of infected cells, can be considered also a measure of virus load.

In this model, the basic reproductive ratio of the virus is given by \( R_0 = \lambda \beta / \delta a \). This ratio describes the average number of newly infected cells generated from one infected cell at the beginning of the infectious process. Thus, it is a fundamental measure, which determines whether a virus spreads within the host or becomes extinct. If \( R_0 > 1 \), the virus can establish an infection. In this case, the immune response expands and the system converges to the following equilibrium.

\[
x^* = \left( ba + cq\delta + pbz^* \right) / \left( c q \delta + b \beta \right)
\]

\[
y^* = bz^* / \alpha
\]

\[
z^* = -\left( \delta q ca + b \beta a + \delta c p \right) + \sqrt{\left( \delta q ca + b \beta a + \delta c p \right)^2 - 4p(\delta q c + b \beta)(da - \lambda \beta)} / 2p(\delta q c + b \beta)
\]

Fig. 1. Schematic representation of the mathematical model.
Opinion

The term for the expansion of the immune response to viral infection, in our study of lytic and nonlytic immunity, we keep the model simple. The aim of the model is to investigate the role of lytic and nonlytic that immunity is accounted for in the model. We test and discuss the model in relation to experimental data in qualitative terms. This is valid and possible because the model is simple enough to understand analytically and our case studies correspond clearly to parameter regions discussed in the model: LCMV is completely noncytopathic; VSV is extremely cytopathic; and influenza virus is characterized by intermediate cytopathicity. Furthermore, strains of LCMV differ clearly in their rates of replication. However, the aim of a mathematical model should also be to measure parameters in specific infections, resulting in more scope for testing. To date, measurements of the important parameters for the respective infections discussed here are not available. Although a first effort has been made to quantify immune responses in LCMV infection more precisely [a], more work needs to be done in this respect.

In summary, our model is very phenomenological and is designed to investigate a very specific question: the role of direct lytic and nonlytic inhibition of the virus by immune cells. For this task, the simplicity of the model is an advantage, because it allows us to gain clear analytical insights. In this article, we do not aim to investigate other additional issues, such as the role of the different branches of immunity or their importance at different times in the infectious process. Although these are important and interesting questions, they will require a separate study to be answered.

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**Box 2. Assumptions underlying the model**

The mathematical model presented in Box 1 is based on a set of assumptions that are shown clearly in the equations. Here, these assumptions are discussed further, with a particular focus on the way that immunity is accounted for in the model.

- **The aim of the model is to investigate the role of lytic and nonlytic effector mechanisms in viral infections.** This is defined clearly in the model. Lytic effectors are responsible for the killing of infected cells by the immune system. Nonlytic effectors are responsible for the inhibition of viral replication by soluble factors secreted from immune cells. Clearly, soluble factors have other functions also: for example, attracting immune cells, signaling and other regulatory functions. We do not aim to include these in the model. Our focus is on the direct antiviral activity of lytic and nonlytic effector mechanisms.

- **In our study of lytic and nonlytic immunity, we keep the model simple and do not distinguish explicitly between different branches of the immune system, such as antibodies, CD4+ T cells and CD8+ T cells.** It is assumed that a general immune response expands against the viral antigen. Furthermore, it is assumed that this immune response will result in the lysis of infected cells at a rate $p$ and nonlytic inhibition of viral replication at a rate $q$. The nonlytic responses will involve several different branches of the immune system, such as antibody responses, nonlytic CD8+ T-cell responses and nonlytic CD4+ T-cell responses. These responses are certainly different in the molecular mechanisms of their activity, the outcome of this activity will be the same – a reduction in the rate of viral replication – and this is captured by the model. The question of which branches of the immune system contribute most to virus control is separate from the question of the relevance of lytic and nonlytic effector mechanisms, and would require a separate model. However, this would go beyond the scope of the present article.

- **The term for the expansion of the immune response to viral infection, cyt(t), means that the response increases at a rate $c$ in the presence of the virus. This is a very simple and phenomenological way of modeling.** However, previous modeling suggests that for the aspects considered in this article, the model results are robust, given that it is assumed that expansion of the immune response is proportional to the level of viral antigen. However, it should be kept in mind that our understanding of how the immune system responds to viral antigen in the acute and persistent phases of an infection is still very incomplete.

- **We test and discuss the model in relation to experimental data in qualitative terms. This is valid and possible because the model is simple enough to understand analytically and our case studies correspond clearly to parameter regions discussed in the model: LCMV is completely noncytopathic; VSV is extremely cytopathic; and influenza virus is characterized by intermediate cytopathicity.** Furthermore, strains of LCMV differ clearly in their rates of replication. However, the aim of a mathematical model should also be to measure parameters in specific infections, resulting in more scope for testing. To date, measurements of the important parameters for the respective infections discussed here are not available. Although a first effort has been made to quantify immune responses in LCMV infection more precisely [a], more work needs to be done in this respect.

- **In summary, our model is very phenomenological and is designed to investigate a very specific question: the role of direct lytic and nonlytic inhibition of the virus by immune cells.** For this task, the simplicity of the model is an advantage, because it allows us to gain clear analytical insights. In this article, we do not aim to investigate other additional issues, such as the role of the different branches of immunity or their importance at different times in the infectious process. Although these are important and interesting questions, they will require a separate study to be answered.

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**Lytic and nonlytic immunity in relation to virus control**

Lytic and nonlytic effector mechanisms influence two basic viral properties: the death rate of infected cells, $a$, and the replication rate of the virus, $\beta$, respectively. To understand the relevance of immune responses, it is useful to consider the effect of these two basic parameters on virus load and the total number of target cells in the absence of immune control. The higher the death rate of infected cells, $a$, the lower the virus load (Fig. 1a). If the value of $a$ lies above a threshold $a_*$, then the basic reproductive ratio of the virus, $R_0\lambda$, will be less than unity and the infection becomes extinct. By contrast, an increase in the viral replication rate, $\beta$, results in an asymptotic increase in viral load (Fig. 1a). If the value of $\beta$ lies below a threshold $\beta_*$, then $R_0<1$ and the infection becomes extinct. Hence, both a decrease in the viral replication rate due to nonlytic effector mechanisms and an increase in the death rate of infected cells due to lytic effectors can contribute to a reduction in $R_0$ and thus, viral load.

The effect of these viral parameters on the total number of host cells is more complex (Fig. 1b). The total number of host cells is not a monotonic function of the death rate of infected cells, $a$. Increasing the parameter $a$ leads first to a decrease in the total number of host cells down to a minimum. A further increase in $a$ results in an increase in the total number of host cells until a crosses a threshold at which $R_0<1$. Then, the virus becomes extinct. The minimum equilibrium number of host cells is given at a death rate $a = (\lambda\beta)^{1/2}$, and it has a value of $[x + y]_{\text{min}} = (2x\beta)^{1/2} - \delta/\beta$. Thus, the higher the rate of viral replication, $\beta$, the higher the death rate of infected cells at which the total number of host cells reaches a minimum, and the lower the value of this minimum (Fig. 1b). For very slow rates of viral replication, the minimum number of host cells approaches a value of $\lambda/\delta$, which is equivalent to the number of host cells in the absence of infection.

These observations lead to two basic conclusions: Reducing the replication rate of the virus by nonlytic effector mechanisms is always beneficial to the host, whereas increasing the death rate of infected cells by lytic effector mechanisms can be both detrimental and beneficial to the host. Lytic effectors are likely to
activation of the immune system, resulting in the over-production of cytokines and hence, damage to the host. This is observed when perforin-deficient mice are infected with LCMV [19]. These mice develop severe cytokine-mediated aplastic anemia and can succumb to the infection. Another possible example of this outcome is infection with human T-cell leukemia virus 1 (HTLV-1) [20]. In addition, noncytopathic viruses can induce cell damage without killing, for example by turning off so-called ‘luxury’ functions of the cell [21]. Therefore, evolutionary pressure will have favored lytic effector mechanisms to clear noncytopathic viruses. However, according to the model, it will be hard for lytic effectors to resolve an infection with a noncytopathic virus, particularly if the virus replicates at a fast rate. Lytic effectors increase the death rate of infected cells and thus, can result in the depletion of target cells and immunopathology. The faster the replication rate of the virus, the stronger the CTL response must be to minimize immunopathology and result in resolution of the infection (Fig. 1). According to the model, this difficulty can be overcome by the combined action of lytic and nonlytic effectors. Soluble mediators reduce the replication rate of the virus. If the viral replication rate is decreased, lytic effectors are likely to result in resolution of the infection, rather than immunopathology.

Figure 2 summarizes the roles of lytic and soluble mediators in the clearance of noncytopathic viruses. If the virus replicates at a slow rate, lytic effectors alone are likely to be sufficient to control the infection. Although the model indicates that the absence of soluble factors could result in a slight increase in virus load, the infection is likely to be controlled in the long term. If the virus replicates at a fast rate, soluble immune mediators are required to reduce the replication rate of the virus, enabling the lytic effector mechanisms to have a beneficial effect on the host.

The most extensively characterized noncytopathic virus is murine LCMV. The soluble cytokine IFN-γ has been shown to reduce the rate of viral replication in LCMV infection [13,22]. Yet, the role of IFN-γ in the control of LCMV infection has been established only recently. Early studies suggested that in LCMV infection, IFN-γ did not contribute substantially to virus control [3,5]. However, this conclusion was based on the analysis of mice infected with the slowly replicating Armstrong strain. More recent analysis, by contrast, has provided clear evidence of an important role for IFN-γ and other soluble mediators in the resolution of LCMV infection [6,7,11–14].

Even with the slowly replicating Armstrong strain, our own analysis has revealed that although IFN-γ-deficient mice infected with this strain did not show symptoms of disease, the infection was not controlled completely and significant levels of virus could be detected in spleen and lungs months after infection [6,23]. In these mice, an equilibrium was established
Control of noncytopathic viruses by lytic and nonlytic effector mechanisms. (a) A slowly replicating virus. Lytic effectors alone can achieve a similar level of virus control as a combination of lytic and nonlytic effectors. (b) A fast replicating virus. Lytic effectors alone cannot control the infection and result in immunopathology. Cooperation between lytic and nonlytic effector mechanisms can control the infection, because nonlytic immunity slows down the overall replication rate of the virus. The effect of a nonlytic response alone has not been plotted. This is because we are considering noncytopathic viruses, which do not kill their target cells. Because the life-span of infected cells is not reduced, a nonlytic response is unlikely to result in virus control in the short term. Parameters were chosen as follows: \( i = 10 \); \( d = 0.1 \); \( a = 0.1 \); \( q = 10 \); \( p = 1 \); \( c = 0.1 \); and \( b = 0.1 \). In (a), the viral replication rate, \( \beta = 0.01 \), and in (b), \( \beta = 0.1 \). In (a), the virus replicates at a slow rate, our model predicts agreement with the theory presented here. Because virus load was kept at relatively low levels, pathology was virtually absent (Table 1) [6,7]. This observation is in agreement with the theory presented here. Because the virus replicates at a slow rate, our model predicts that a lack of nonlytic effector mechanisms will only result in a small loss of virus control and lack of severe immunopathology. The situation is different for faster replicating strains of LCMV. IFN-\( \gamma \)-deficient mice infected with LCMV Traub quickly lose control of the infection, despite the presence of efficient lytic effector mechanisms. In contrast to wild-type mice, a relatively large proportion of infected IFN-\( \gamma \)-/\( \gamma \) mice succumbed to immunopathology caused by a lytic CTL response (Table 1) [7]. This observation is in agreement with theoretical predictions also (Fig. 1). Because the virus replicates at a fast rate, soluble factors are required to significantly reduce the replication rate of the virus to avoid immunopathology. The absence of soluble mediators results in CTL-induced tissue damage and death of the host.

### Table 1. Outcome of intravenous challenge with lymphocytic choriomeningitis virus (noncytopathic)\( ^{a,b} \)

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Mouse strain</th>
<th>Weight loss</th>
<th>Virus titer d</th>
<th>Liver</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traub (fast)</td>
<td>Wild-type</td>
<td>≤0%</td>
<td>1.50</td>
<td>2.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IFN-( \gamma )-/( \gamma )</td>
<td>18%</td>
<td>5.50</td>
<td>5.50</td>
<td></td>
</tr>
<tr>
<td>Armstrong (slow)</td>
<td>Wild-type</td>
<td>≤0%</td>
<td>0.75</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IFN-( \gamma )-/( \gamma )</td>
<td>5%</td>
<td>1.50</td>
<td>2.00</td>
<td></td>
</tr>
</tbody>
</table>

\( ^{a} \)Abbreviation: IFN-\( \gamma \), interferon \( \gamma \).

\( ^{b} \)Data are summarized from previously published papers (see Refs [6,7,24,25]). Both weight and organ titer were evaluated by day 10 post-infection.

\( ^{c} \)Virus replication rate in vivo.

\( ^{d} \)Titer values refer to log\(_{10}\) LD50 per 0.03 ml of organ suspension.

**More-cytopathic viruses**

The previous section examined the case of a noncytopathic virus, for which the life-span of an infected cell is equal to that of an uninfected cell. Similar considerations apply to more-cytopathic viruses, provided that the life-span of infected cells lies above a threshold, determined by \( \sqrt[3]{\lambda \beta} \). Below this threshold, an increase in the death rate of infected cells can have a detrimental effect on the host (Fig. 1). However, this does not apply if the death rate of infected cells lies above the threshold, given by \( \sqrt[3]{\lambda \beta} \). In this case, both lytic and nonlytic effector mechanisms always have a beneficial effect on the host. The explanation for this is that above the threshold, an increase in the death rate of infected cells always results in an increase in the total number of host cells (Fig. 1). However, an effect of lytic effectors will only be apparent if the rate of immune-mediated cell killing is high relative to the rate of virus-induced cell killing (i.e. if \( p < q \)) [24]. In this case, there are three possible scenarios regarding the role of lytic and nonlytic effector mechanisms in controlling the infection (Fig. 3). (1) If the lytic effector mechanisms are sufficiently strong, they can resolve the infection on their own. (2) If the nonlytic effector mechanisms are strong enough, they can also resolve the infection on their own. (3) If neither lytic nor nonlytic effectors are sufficiently strong to resolve the disease individually, a combination of both mechanisms is required to overcome the infection.

An example of a virus infection characterized by a very high degree of cytopathicity is VSV infection in mice [5,25–28]. The role of different types of immune effector mechanisms in this extreme case can be investigated in MHC class-I- and -II-deficient hosts (Table 2). Class-II-deficient mice have reduced levels of survival. However, a fraction of the infected animals do survive. In these mice, CD8+ T-cell responses seem to be able to control the infection partially [26,27]. In class-I-deficient mice, CD4+ T-cell-dependent responses can resolve the infection successfully, most probably by the induction of expression of IgG [26,27]. Animals deficient in both class I and class II MHC cannot control VSV infection at all, resulting in the death of all infected mice [26,27].

These results are consistent with the theoretical framework presented here. VSV is extremely
cytopathic, and hence, the rate of virus-induced cell death could be greater than the rate of CTL-mediated lysis. Therefore, although theory suggests that, in principle, both lytic and nonlytic effector mechanisms can, in principle, control the infection independently, lytic mechanisms will have limited capabilities if the rate of viral cytopathicity is very high. Consequently, in class-II-deficient mice, CTLs are expected, at best, to have a limited effect on virus control. However, we must be cautious in the exact interpretation of these data, because CD8\(^+\) T cells could act also in a nonlytic fashion.

An example of a cytopathic infection that is not at the extreme end of the cytopathicity spectrum is influenza infection in mice [29]. Recovery from murine influenza virus infection has been shown to require intact T-cell responses [29–31]. More specifically, experiments have revealed that both CD4\(^+\) and CD8\(^+\) T cells can promote recovery through independent mechanisms [29,30]. In the absence of CD8\(^+\) T cells, the infection can be resolved by a CD4\(^+\) T-cell-dependent antibody response [17]; and the absence of CD4\(^+\) T cells or B cells does not result in loss of virus control either [32,33]. Experiments have shown that CD8\(^+\) T cells can resolve the infection by a lytic mechanism, mediated by either perforin or Fas [16]. The result that both lytic and nonlytic effector mechanisms can clear influenza infection in mice independently is in agreement with the theoretical considerations presented here. Because influenza virus is cytopathic, both a sufficient increase in the death rate of infected cells and a decrease in the rate of viral replication are expected to have a beneficial effect on the host and lead to resolution of the disease. For cytopathic viruses, a collaboration between both types of effector is less likely to be required to ensure resolution of the disease, particularly if the virus challenge is not overwhelming.

‘The relevance of lytic and nonlytic effector mechanisms . . . depends on the viral cytopathicity relative to its rate of replication.’

**Conclusion**

We have used mathematical models to analyze the role of lytic and nonlytic effector mechanisms in viral infections. Theory, complementing experimental data, argues against the simple rule that lytic effectors are required to deal with noncytopathic viruses, whereas soluble factors are sufficient to deal with cytopathic viruses. Instead, we suggest the following pattern. In the present context, the distinction between noncytopathic and cytopathic viruses is not precise. The relevance of lytic and nonlytic effector mechanisms for resolving the infection depends on the viral cytopathicity relative to its rate of replication. If cytopathicity lies below the threshold defined by \( a < (\lambda \beta)^{1/2} \), then a combination of lytic and nonlytic effector mechanisms is likely to be required to resolve the disease. The higher the replication rate of the virus, the greater the extent to which nonlytic effectors are required to resolve the infection. Nonlytic mechanisms reduce the replication rate of the virus. This avoids the occurrence of immunopathology and enables the lytic effectors to clear the infection. However, if the cytopathicity of the virus is high relative to its rate of replication (i.e. if \( a > (\lambda \beta)^{1/2} \)), both types of immune effector are beneficial to the host and can, in principle, independently result in resolution of the infection. We have used this theoretical framework to interpret
experimental data from mice infected with noncytopathic LCMV, more-cytopathic influenza virus and extremely cytopathic VSV. Our discussion has focused on these infections, because aside from covering a wide spectrum in terms of viral cytopathicity, these models are well-characterized. Equally important, the life cycles of the viruses involved allow for rather simple and straightforward virus-host relationships. This is crucial when first testing predictions against real-life observations.

Our theoretical framework has implications for improving our understanding of the mechanisms required for immunological control in a variety of infections. An interesting example is hepatitis B virus (HBV), which appears to be controlled by an intricate balance between a lytic CTL response, nonlytic factors secreted by CTLs and antibody responses [10, 34]. Also, CTL-secreted soluble factors have been reported to ‘cure’ HBV-infected cells (i.e. trigger infected cells to eliminate the viral genome, thus converting an infected cell into an uninfected one). Other viral infections have more-complex life cycles than the ones covered in detail in this article. For example, certain viruses go through cytopathic and noncytopathic phases during the course of infection (e.g. Epstein–Barr virus [35]). Other viruses, such as HIV, are characterized by varying levels of cytopathicity and different replication rates in different cell types (e.g. macrophages versus T cells [36]). Although these infections are characterized by more-complicated life cycles than assumed in our model, the insights from our theoretical framework can still be applied. They can help us to understand how these added complexities influence viral dynamics, as well as the ability of the virus to evade efficient immune-mediated control. For a detailed investigation of specific infections, our framework can be incorporated easily into more-specific models.

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