Boosting immunity by antiviral drug therapy: A simple relationship among timing, efficacy, and success

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Drug therapies against persistent human infections such as hepatitis C virus, hepatitis B virus, and HIV fail to consistently eradicate the infection from the host. Hence, recent emphasis has shifted to the study of antiviral therapy aimed at boosting specific immune responses. It was argued that structured therapy interruptions were required to achieve this, because such regimes have shown promising results in early HIV infection. Using mathematical models, we show that, contrary to this notion, a single phase of drug therapy can result in the establishment of sustained immunity. We present a simple relationship between timing of therapy and efficacy of the drugs required for success. In the presence of strong viral suppression, we show that therapy should be stopped relatively early, and that a longer duration of treatment leads to failure. On the other hand, in the presence of weaker viral suppression, stopping treatment too early is detrimental, and therapy has to be continued beyond a time threshold. We discuss our modeling results primarily in the context of HCV therapy during chronic infection. Although the therapy regimes explored here also have implications for HIV, virus-mediated destruction of specific immune cells renders success unlikely during the chronic phase of the infection.

Several human pathogens have the ability to suppress immune responses, allowing them to establish a persistent and productive infection that eventually results in pathology. A potent strategy is to impair virus-specific CD4 T helper cell responses (directly or indirectly), because they are the central component orchestrating antiviral effector mechanisms. According to clinical data, the most prominent examples of this are HIV, hepatitis C virus (HCV), and hepatitis B virus (HBV) infection (1–7). For all these viruses, drug treatment is not effective in all cases, and for HIV, lifelong therapy is generally necessary to control viral replication (8–13). An alternative strategy is to use drug treatment to boost virus-specific immunity, resulting in sustained viral suppression in the absence of drugs. In HIV infection, this has been explored in the context of early therapy and structured therapy interruptions (4, 14, 15). During the chronic phase of the infection, however, such therapy regimes are less promising, because HIV induces the destruction of specific immune cells. Although HCV and HBV have been shown to result in impairment of the specific helper cell responses, these viruses do not induce the destruction of specific immune cells. With both HCV and HBV, a boost in T cell responses during drug therapy has been observed in some chronically infected patients (5, 6, 16).

In this paper, we use a general mathematical framework to study immune response dynamics during therapy in the context of immunosuppressive infections. Contrary to previous analysis (17, 18), we focus on a single phase of treatment during chronic infection. The model suggests that a single phase of treatment can result in long-term immune-mediated control of an immunosuppressive infection. We describe a relationship between the efficacy of drugs and the timing/duration of therapy. In the presence of strong viral suppression, the model suggests that

therapy should be stopped relatively early and that a longer duration of treatment can lead to failure. In contrast, with weaker viral suppression, stopping treatment too early is detrimental. Instead, therapy should be continued beyond a time threshold. The model further suggests that interruption therapy can be helpful in a restricted parameter region. We describe how timing of therapy can in principle be optimized by monitoring immune response dynamics in patients. Theoretical results are primarily discussed in the context of HCV infection, because specific immune cells are not destroyed by the virus, and a recent study of drug therapy during chronic infection supports our theoretical notions. We also discuss implications for HIV infection. Because HIV destroys immunological specificities, however, drug therapy alone is unlikely to result in a boost of immunity during chronic infection.

The Model

The dynamics between an immunosuppressive infection and antiviral immune responses can be studied at various degrees of complexity. Here we take a very general approach. We consider a model that contains two variables: the virus population, y , and a population of immune cells, z . The exact identity of the immune cells is left open. We assume that the degree of immune expansion depends on virus load, and that the response inhibits virus growth. It could thus correspond to any branch of the adaptive immune system, such as CD8 T cells, antibodies, or CD4 T cells. The model is given by the following pair of differential equations:

$$\dot{y} = yg_r(y) - yz, \quad [1]$$

$$\dot{z} = zf(y). \quad [2]$$

The virus population grows at a rate described by the function $g_r(y)$. This is a function that depends on the amount of virus, y , and on the parameter, r , denoting the viral replication rate. The virus population becomes inhibited by the immune response at a rate yz . Immune expansion is determined by virus load, y , and is described by the function $f(y)$. The generic shape of functions $g_r(y)$ and $f(y)$ is presented in Fig. 1. Positive values of these functions indicate growth, and negative values correspond to decay in the population. Consider the virus growth function, $g_r(y)$. On the trivial side, we make the assumption that the higher the replication rate of the virus, r , the higher the viral growth rate. The only other assumption is that virus growth is density dependent: growth slows down at higher virus loads; when virus load crosses a threshold, growth stops and the virus population declines, corresponding to target cell limitation where the virus runs out of cells to infect.

Now we turn to the function describing immune expansion, $f(y)$. We make the assumption that the presence of the virus can

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Fig. 1. Diagram explaining the behavior of the general mathematical model. The model is defined by the virus growth function $g_r(y)$ and the immune expansion function $f(y)$. As explained in the text, the function $g_r(y)$ depends on the viral replication rate, r , whereas the function $f(y)$ does not. (a) The functions $f(y)$ and $g_r(y)$ are plotted versus y . The function $f(y)$ has two fixed roots, y_1 and y_2 . We show $g_r(y)$ as a function of y for several different values of r . The parameter r determines at which value of $y = y^*$ the function $g_r(y)$ equals zero. If the value of r lies above a threshold so that $g_r(y) = 0$ for $y > y_2$, we are in the bistable parameter region. If the value of r is smaller so that $g_r(y) = 0$ for $y_1 < y < y_2$, the only stable outcome is immune control, S_i . If the value of r is still smaller so that $g_r(y) = 0$ for $0 < y < y_1$, only the virus equilibrium, S_v , is stable. Finally, if the value of r is very small, then $g_r(y) = 0$ for $y < 0$ and the infection cannot become established in the first place, S_0 . (b) This is a schematic diagram that summarizes how the outcome of infection depends on the viral replication rate, r , as detailed above.

both stimulate and impair immunity, depending on virus load, y . If virus load lies below a threshold, the rate of immune expansion is negative. That is, levels of antigen are too low to induce a response. If virus load lies above a threshold, the rate of immune expansion is also negative, because immune impairment outweighs antigenic stimulation. Thus, high virus loads inhibit immunity. Immune expansion is positive for intermediate virus loads, because antigenic stimulation is strong relative to immune impairment. The precise conditions $f(y)$ and $g_r(y)$ have to satisfy for the results to hold true are listed in the *Appendix*. This framework makes minimal assumptions and defines a collection of models. Hence, results derived from this framework are robust and do not depend on the particular form of the equations. For illustrative purposes, we used a particular model that fits into this framework, given in the *Appendix*. Here, we analyze the behavior of the whole class of models and find three outcomes:

- In the trivial case, S_0 , there is no infection and no immune response.
- Alternatively, the virus can establish an infection in the absence of an immune response, S_v . We will refer to the equilibrium S_v as the virus equilibrium, because the virus can grow unchecked and cause pathology directly or indirectly.
- Finally, the virus can establish an infection that is controlled by an immune response, S_i . We refer to this outcome as the immune control equilibrium.

A note of clarification: The virus outcome is characterized by the absence of the immune response, z . This describes failure of long-term control in the model and can correspond to an *in vivo* scenario where suboptimal immune responses are temporarily

maintained and subsequently collapse. Such suboptimal responses are not explicitly included in the model but can be assumed to be implicit in parameters determining virus load (such as the replication rate). To use specific examples, the immune control outcome (S_i) in the model can correspond to the state of long-term nonprogression in HIV infection (19), whereas failure of long-term control in the model (S_v) corresponds to typical HIV disease progression. A similar difference can be seen in HCV infection: a small fraction of patients control the virus (or clear virus from blood) and establish long-term immunity (S_i), whereas most patients fail to do so and eventually may develop disease (S_v) (20).

Analysis of the Model

We seek to understand the stability properties of these equilibria as the value of r increases from low to high, because the parameter r is influenced by drug therapy. The following regimes are observed (see *Appendix II*, which is published as supporting information on the PNAS web site, www.pnas.org, and Fig. 1b):

- If the replication rate is very small, the virus cannot infect the host, and the system converges to S_0 .
- If the replication rate of the virus crosses a threshold, an infection can be established, but the amount of antigenic stimulation is too low to trigger sustained immunity. The system converges to S_v .
- If the replication rate is higher and crosses another threshold, levels of antigen are sufficient to trigger sustained immunity. The system converges to the equilibrium describing long-term immunological control, S_i .
- If the viral replication rate is still higher and crosses a final threshold, the immune response can be significantly impaired. In this parameter region, both the immune control (S_i) and the virus equilibrium (S_v) are stable, and the outcome of infection depends on the initial conditions.

The dependence on initial conditions in the bistable parameter region (iv) is further explored in Fig. 2a. The domain of attraction for the immune control and virus equilibria is separated by a line we call line L (dashed line in Fig. 2a). This is a separatrix that divides all possible initial states of the system into two classes: above line L , the system will converge to the immune control equilibrium (S_i); below line L , the system will converge to the virus equilibrium (S_v). In biological terms, the initial level of the immune response, z , determines whether we are above or below line L . It is also influenced by virus load, although to a lesser degree. Thus, a naive host (starting with low numbers of specific immune cells) is likely to move to the virus equilibrium. This is also promoted by a high initial virus load. On the other hand, a high initial number of immune cells and also a low initial virus load promote the establishment of immune-mediated control. The reason for the occurrence of this bistability is the assumption that the virus can both stimulate and impair immunity. If virus load is high, immune impairment outweighs antigenic stimulation. If virus load is intermediate/low, antigenic stimulation can outweigh impairment. Therefore, the initial number of immune cells and also the initial virus load determine whether immune responses can expand and get the upper hand, or whether the virus wins this race.

In the following section, we analyze immune response dynamics during drug therapy, assuming that we are in the bistable parameter region (iv). Thus, infection of a naive host will result in the virus/disease outcome. Because the immune control outcome is still stable, however, the model suggests that therapeutic intervention can shift the dynamics toward the immune control outcome. This is explored below.

Fig. 2. Phase-space diagram showing the behavior of the model. The axes show virus load, y , and the immune response, z . Equilibria are indicated, and the arrows show the equilibrium outcomes toward which the system moves over time, depending on the starting conditions. Stable equilibria are indicated by filled circles. Unstable equilibria are indicated by open circles. Shading indicates the biologically irrelevant region where the immune response assumes negative values; it is included only for mathematical completeness. The dashed lines indicate line L , the pretreatment separatrix, which diverges to infinity for y near zero. In the present context, this regime is not relevant because in practical terms it corresponds to absence of infection. (a) The pretreatment regime: behavior of the model in the bistable parameter region. Below line L (dashed line), the system converges to the virus equilibrium, S_v ; above line L , it converges to the immune control equilibrium, S_i . (b and c) Effect of therapy assuming a reduction of the parameter r . The black triangle shows the state of the infection before therapy (at the pretreatment virus equilibrium S_v). To achieve sustained immunological control, therapy has to be stopped while the system is above the separatrix. If therapy is relatively weak (b), the system moves to the immune control equilibrium, S_i , during treatment. For sufficiently weak therapy, this lies above line L . Thus, if therapy is stopped after a time threshold $t > t_{\min}$, immunological control is maintained. On the other hand, if therapy is stronger (c), the system moves to the virus equilibrium, S_v , which lies below line L . It can move only temporarily above line L . In this case, therapy has to be stopped early to achieve sustained immunological control, before the system has reached equilibrium and has fallen back below line L . The flow lines are generated for system (Eqs. 1 and 2) of the Appendix with parameters $k = 10$, $a = 3$, $c = 4$, $\varepsilon = 0.5$ and $q = p = b = 1$. The parameter r takes values $r = 7$ in a, $r = 3.05$ in b, and $r = 3.01$ in c.

Single Phase of Therapy

We now consider the dynamics during a single phase of drug therapy in the chronic phase of infection. We assume that the infection is in the bistable parameter region (iv), and that the virus equilibrium has been attained. We ask whether and how a single phase of therapy can establish sustained immune-mediated control of the infection. During therapy, the replication rate of the virus, r , is reduced in the model. The amount of reduction corresponds to the efficacy of the drugs. On cessation of therapy, the parameter r is reset to its pretreatment value. We

Fig. 3. Simulation of therapy assuming relatively weak and relatively strong therapy. Phases of treatment are indicated by shading. (a) With weaker therapy, immune responses are boosted and are maintained during the phase of treatment. It takes the response a relatively long time to expand, and therapy should be stopped after a time threshold, once the level of immune responses has significantly risen. If therapy is stopped too early, immunological control is not achieved. (b) With stronger therapy, virus load is reduced quickly to low levels. On initiation of treatment, the immune response expands temporarily and declines again to low levels once the amount of antigenic drive has been diminished. To achieve long-term immunological control, therapy has to be stopped early, before the temporary immune expansion has vanished. If therapy is continued for too long, sustained immune control is not achieved. Parameters were chosen as follows: $r = 3.5$, $k = 10$, $a = 3$, $P = 1$, $c = 12$, $\varepsilon = 5$, $u = 3$, and $b = 0.3$. For a, $r = 3.13$ during therapy; for b, $r = 3.013$ during therapy.

find that a single phase of therapy may indeed result in sustained viral suppression after cessation of treatment. We further find an interesting relationship between the efficacy and the duration of therapy required for success.

During therapy, the system converges toward a new equilibrium that is determined by the efficacy of the drugs. When therapy is stopped, sustained viral suppression will be achieved only if therapy has moved the level of the immune response in the domain of attraction of the immune control equilibrium. In other words, whether the treatment moves the system above line L will determine the success of therapy.

In this context, it is important to point out that there is a tradeoff between the amount of immune impairment and antigenic stimulation in the expansion of immune responses. Treatment has to be efficient enough to sufficiently reduce the degree of immune impairment, allowing the immune response to expand. However, if treatment is too efficient, not only immune impairment but also the degree of antigenic stimulation is diminished, reducing the amount of immune expansion. The following cases of increasing drug efficacy are discussed (the dynamical behavior of the model is summarized in Fig. 2, and the corresponding time series are shown in Fig. 3).

For treatment to have any effect, the drugs must have a minimum level of efficacy so that the viral replication rate is reduced from region iv into region iii. In this case, the only stable equilibrium during treatment is the establishment of an immune

response, S_i (Fig. 2*b*). The system will move from the pretreatment equilibrium toward the treatment equilibrium. During this process, the immune response expands (Fig. 3*a*). After a time $t > t_{\min}$, the level of the response moves above line L . Hence, in principle, after the duration of therapy has crossed this time threshold, treatment can be stopped and sustained viral suppression will be achieved (Fig. 3*a*). Note that the time it takes the immune response to cross line L can be long, because the system has to pass the unstable saddle equilibrium S_v (Fig. 2*b*). This time period can be shortened if, in addition to drug treatment, the number of immune cells are augmented by immunotherapeutic approaches. Note in Figs. 2*b* and 3*a* that the approach to the treatment equilibrium is oscillatory. In the *Appendix II*, we define conditions for when such oscillations are expected to occur. If they do occur, they may have different implications depending on the efficacy of the drugs. We have two possible scenarios. If therapy is less efficient, the treatment equilibrium (S_i) lies high above the line L , and the oscillations do not pose a problem. If therapy is more efficient, the treatment equilibrium (S_i) lies above but closer to line L . After the immune response has risen to a peak, the oscillations can take the response temporarily below line L before the treatment equilibrium is reached (Fig. 2*b*). Therapy should not be stopped in the phases when the immune response goes through troughs.

If therapy is even more efficient, the treatment equilibrium lies below line L (greatly reduced virus load cannot maintain immunity during treatment). This occurs if the replication rate of the virus is pushed from parameter region *iv* to region *ii*. As shown in Fig. 2*c*, the only stable outcome during treatment is the presence of the virus (at reduced levels) in the absence of sustained long-term immunity, S_v . Because the treatment equilibrium lies below line L , cessation of therapy when equilibrium has been reached will result in rebound of the virus (Fig. 3*b*). However, on the way to this treatment equilibrium, the number of immune cells can temporarily rise above line L soon after start of therapy. Subsequently, it declines to low levels (Fig. 3*b*). This is because during the initial phase of treatment, immune impairment has been reduced, but levels of virus load are still high enough to stimulate immune expansion. Once virus load is reduced further by the drugs, this initial immune expansion diminishes due to lack of antigenic stimulation. Thus, to achieve sustained viral suppression in this case, treatment must not be continued for too long: treatment has to be stopped early, while the level of immune cells is still high enough and above line L (Fig. 3*b*). Note, however, that the peak of the response during this temporary phase of expansion can lie below line L (Fig. 2). In this case, virus rebound will always be observed when therapy is stopped. Such an outcome is promoted by very strong drug-mediated suppression of viremia and/or the absence of a sufficient number of reactive immune cells.

To summarize, our results have given rise to the insight that a single phase of drug treatment during chronic infection can result in long-term control of the virus. We have described the following relationship among efficacy, duration, and success of therapy (summarized in Fig. 3). Therapy has to be efficient enough to reduce the rate of viral replication r at least from parameter region *iv* to region *iii*. Within this constraint, significant immune responses develop during therapy only if suppression of viremia is relatively weak, because this ensures the presence of sufficient antigenic drive. Control is maintained if therapy is stopped after a defined time threshold, once immunity has peaked and become established. On the other hand, if treatment is stronger, immune responses peak early after initiation of treatment and subsequently decline because the level of antigenic drive is diminished. Long-term control now requires an early stop of treatment, before immunity has significantly declined. Treating too long will result in failure. A single phase of treatment will not lead to sustained immunological control if

drug-mediated suppression of viremia is too strong, or if the number of reactive immune cells is too low.

Why Therapy Interruptions?

An important result from the above discussion is that long-term immune control can be achieved by a single phase of therapy if the combination of timing and efficacy of treatment is optimized. This is in contrast to previous notions that suggested that special regimes, such as structured therapy interruptions, have to be used to boost immunity. According to our theoretical framework, therapy interruptions can be helpful only in a restricted parameter region. Namely, interruptions can be beneficial if the following two conditions hold: (i) drug-mediated viral suppression is too strong to allow sufficient antigenic stimulation, and (ii) the number of reactive immune cells is reduced to very low numbers (however, the number of immune cells must be above a threshold for any therapy to work). In the limited circumstances when interruptions help, the dynamics are as follows.

A single phase of therapy moves the system toward the treatment equilibrium; the immune response increases but is not sufficiently boosted to cross line L . On cessation of therapy, the system moves back to the pretreatment equilibrium. However, as shown in Fig. 4, the trajectory toward the treatment equilibrium on start of therapy is different from the trajectory away from the treatment equilibrium when therapy is stopped. Namely, when treatment is stopped, the immune response expands before it declines again. The reason is as follows. On cessation of treatment, the virus population grows. During this growth phase, virus load first attains levels at which the amount of antigenic stimulation outweighs immune impairment, and this results in immune expansion (see *Appendix II* for a simple mathematical explanation based on Eqs. 1 and 2). Of course, as virus load grows further, immune impairment outweighs antigenic stimulation, resulting in a decline of the response.

On the basis of the above arguments, therapy interruptions can work as follows. The first phase of treatment should be stopped while the immune response is around its maximum value (peak). During the off phase, the immune response will temporarily expand, as described above. When the immune response attains the maximum level during this off phase, therapy should be reapplied, resulting in further immune expansion that can push the response above line L . The second phase of treatment should, however, not be continued for too long: after the response peaks above line L , it will decline and fall back below line L . It is crucial that therapy is stopped while the immune response is still sufficiently high and above line L (Fig. 4). If timing and duration of treatment are suboptimal, the immune response might not be pushed above line L by a single interruption. In this case, repeated therapy interruptions can increase the chances of success if the above scheme is continued.

Application and Discussion

We discuss our data in the context of HCV infection. Impairment of specific helper cell responses has been clearly documented, and the virus is not thought to destroy the T cells. Therefore, it is an ideal system to consider in light of the theory presented in this paper. Data from treated HCV-infected patients (16) support the immune response dynamics suggested by theory. This study looked at the dynamics of virus and the specific CD4 T cell responses during a single phase of treatment in 15 subjects. These subjects had persistent viremia and lack of significant CD4 T cell responses to the virus before therapy. On cessation of treatment, a fraction of these subjects were characterized by virus rebound, whereas the rest showed long-term control of the infection below detectable levels. In patients with virus rebound, generally CD4 T cell responses temporarily increased and peaked after the start of treatment, followed by a decline to insignificant levels. In the light of our theoretical

Fig. 4. Dynamics of structured therapy interruptions. (a) Phase-space diagram. Solid line indicates treatment; dashed line indicates phases off treatment. We start from the virus equilibrium, S_v , indicated by the black triangle. Therapy is assumed to be strong and the number of reactive immune cells to be low. Thus, during treatment, the dynamics approach an equilibrium which lies below line L , and the number of immune cells does not cross line L during this phase. Stopping and restarting therapy, however, can push the system above line L . Cessation of therapy while the system is above line L results in long-term immunological control. (b) Time series presentation of these dynamics. In this simulation, a single phase of therapy cannot result in long-term immunological control. An interruption, followed by a second phase of treatment, however, can sufficiently boost the level of immune responses. If the second phase of treatment is stopped before the response has significantly declined, long-term immunological control is achieved. In general, treatment should be stopped and restarted while the level of immune responses is around the peaks. Parameter values were chosen as follows: $r = 0.7$, $k = 10$, $a = 0.1$, $P = 1$, $c = 0.6$, $\varepsilon = 1$, $u = 0.075$, and $b = 0.1$. During therapy, $r = 0.101$.

framework, an earlier cessation of treatment, while immune responses were around their peaks, might have resulted in containment of the infection. Patients characterized by long-term control after treatment showed, overall, later peaks of CD4 T cell responses, and therapy was stopped while immune responses were closer to these peak levels. After cessation of therapy, the CD4 T cell responses were, in some cases, boosted to even higher levels, as suggested by our model simulations (Fig. 3). These data confirm that a single phase of treatment can indeed result in long-term immunological control of an immunosuppressive infection, and that the observed dynamics are at least consistent with our theoretical framework. There is indication that similar dynamics can occur in HBV infection (21).

Our theoretical framework is also important to consider in the context of HIV infection. Although there are some indications that with HIV-infected patients, therapy can boost long-term immunity very early after infection (4, 22–25), there are no reports indicating that a single phase of drug therapy during chronic infection could result in long-term immunological con-

trol. Also, therapy interruptions tend to fail during chronic infection (26). The reason is almost certainly that HIV infection results in the depletion of specific immune cells, leaving not enough cells to react.** However, the dynamics of cytotoxic T lymphocyte responses observed during treatment (27) support patterns observed in our model. Among subjects treated relatively early, the average level of HIV-specific CD8 T cell responses during therapy was higher in patients who showed two or more viremic episodes per year compared with patients in whom drug therapy suppressed HIV replication more efficiently. A similar trend was observed in patients treated during chronic infection (27). Therefore, less efficient therapy results in higher levels of immune responses during treatment. The complications associated with immune cell destruction by HIV can be avoided to a certain degree by very early postinoculation therapy in experimental simian immunodeficiency virus infection (14, 15). Treating early ensures that a sufficient level of reactive immune cells is preserved and available. The experiments showed that a single phase of treatment early in acute infection could result in long-term immunological control as well as protection from reinfection. Longer treatment correlated with better immunity. Interestingly, these experiments have been performed with a single drug, PMPA, which is thought to be less efficient at inhibiting viral replication than in combination therapy against HIV. This is in agreement with our theoretical prediction that with weaker viral suppression, treatment has to be continued beyond a time threshold to observe success.

Finally, although we have concentrated on immunosuppressive infections, we would like to briefly put our results into a broader context. The bistable behavior as described in this paper hinges on the assumption that the virus impairs specific immune responses. Therapy can therefore shift the patient from a disease progression to a control outcome. With viruses that do not impair immunity, there is no bistability. We can still observe two outcomes; for example, with human T-lymphotrophic virus, type I infection, patients can be either asymptomatic carriers, or they may develop diseases such as HTLV-1-associated myelopathy/tropical spastic paraparesis (28). But the two outcomes happen for very different reasons. Which outcome is attained has been shown to correlate with the HLA genotype of the host, among other things (28). Therefore, different outcomes are observed across individuals; within a patient, however, drug therapy will not switch a disease state to a control state. In such cases, different approaches involving therapeutic vaccination will be necessary to improve the degree of immunological control.

Conclusion

In theory, the optimal timing of when therapy should be stopped and/or restarted can be determined by monitoring immune response and virus dynamics. After the start of treatment, we expect the immune responses to rise. The best strategy would be to stop treatment when immune responses are around their maximum value, increasing the chances that the level of immunity is above line L . On cessation of treatment, the system is expected to converge to the immune control outcome if the level of immune responses were above line L (note that the control outcome can be attained by oscillations that can involve a temporary rise in virus load). If the level of immune responses was not above line L when therapy was stopped, the system will converge to the pretreatment equilibrium, which is recognized by a sustained rise in virus load to significantly higher levels. In this case, reapplication of drugs and therapy interruptions can result in long-term virus control, as described above. Therapy should be stopped and restarted at a time when immune

**Mathematically speaking, this corresponds to the fact that at the start of treatment, $z = 0$, so that therapy cannot shift the system above line L .

responses are around their peak values. Such guidelines, however, have currently limited practical meaning, because implementation would require exact knowledge of the identity of relevant responses. In addition, difficulties arise because different assays can capture different subpopulations of immune cells, giving rise to different pictures. Nevertheless, the insights created by modeling give a conceptual framework that will be important for a more rational and targeted design of therapy regimes. The model has provided previously undescribed results regarding the optimal timing and duration of antiviral therapy that have not been considered before. Although with HIV, induction of long-term control will most likely require a combination of drug therapy and therapeutic vaccination, our findings have important practical implications for the treatment of HCV and HBV infections.

Finally, we would like to point out that we have used a very general modeling framework, which makes minimal assumptions. Hence, the results do not depend on the specific forms of equations which describe processes such as virus growth and immune expansion (29). We argue that any system characterized by the basic assumptions defined in our general framework will share the bistable behavior and the therapy results described here. This also applies to higher dimensional systems that have been studied in a different context (17).

Appendix

For our results to hold, the function $g_r(y)$ in Eq. 1 must satisfy the following four conditions (see Fig. 1):

1. $g_r(0) > 0$,
2. $\partial g_r / \partial y < 0 \forall y$,
3. There exists $y^* > 0$ such that $g_r(y^*) = 0$,
4. $\partial g_r(y) / \partial r > 0$ for all r, y .

The function $f(y)$ in Eq. 2 satisfies the following two conditions:

1. There exist only two values $y_1, y_2 > 0$ such that $f(y_1) = f(y_2) = 0$,
2. $\partial f / \partial y > 0$ for $y = y_1$ and $\partial f / \partial y < 0$ for $y = y_2$.

A particular example is given by the system,

$$\dot{y} = ry \left(1 - \frac{y}{k} \right) - ay - pyz, \quad [3]$$

$$\dot{z} = \frac{c y z}{1 + \epsilon y} - q y z - b z, \quad [4]$$

See the *Appendix II* for details.

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