Cytotoxic T-lymphocyte memory, virus clearance and antigenic heterogeneity

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Cytotoxic T-lymphocyte (CTL) memory to viruses has traditionally been studied in an isolated setting. However, recent experiments have indicated that the presence of antigenically heterologous challenges can result in the attrition of CTL memory. Here we use mathematical models in order to explore the consequence of these dynamics for the ability of the immune system in controlling multiple infections. Mathematical models suggest that antigen-independent persistence of CTL memory is required in order to resolve and clear an infection. This ensures strong immunological pressure at low loads when the virus population declines towards extinction. If the number of antigenic stimuli exposed to the immune system crosses a threshold, we find that immunological pressure is significantly reduced at low loads and this can prevent virus clearance and reduces overall control of viral replication. Hence, exposure to many heterologous challenges reduces the ability of CTL memory to contribute to virus control. The higher the number of infections present in the host, the higher the overall virus load and the higher the total number of memory CTLs. Beyond a given threshold, addition of new viruses to the system results in accelerated loss of virus control which eventually leads to a reduction in the overall memory CTL population. These dynamics might contribute to the progressively weaker immunity observed as a result of ageing. In this context, antigenically variable pathogens expose the immune system to many heterologous challenges within a short period of time and this could result in accelerated ageing of the immune system. These results have important implications for vaccination and treatment strategies directed against viral infections.

Keywords: cytotoxic T-lymphocyte memory; heterogeneity; infection; virus; mathematical models

1. INTRODUCTION

Following a typical virus infection, the cytotoxic T-lymphocyte (CTL) precursor population expands extensively, differentiates into effector function and suppresses virus load by either lysing infected cells or secreting soluble factors inhibiting viral replication (Kagi *et al.* 1995; Kagi & Hengartner 1996). As virus load declines, a phase of apoptosis is observed within the CD8 cell population (Welsh *et al.* 1995). This reduces the number of virus-specific CD8 cells before the population settles at a given memory level. The population of specific CD8 cells in the memory phase is significantly higher compared to the naive state of the host and has been observed to persist at a stable equilibrium for a long period of time (Zinkernagel *et al.* 1996; Murali-Krishna *et al.* 1999). The role of this CTL memory population has been subject to intense debate. Traditionally, it was thought that persistence of CTL memory protects the host more efficiently against rechallenge with the same virus (Zinkernagel *et al.* 1996). However, experiments with mice have shown that protection against rechallenge might only be efficient in a restricted set of circumstances and this seems to require persisting antigen (Kundig *et al.* 1996a,b; Ehl *et al.* 1997, 1998). Recently, mathematical models have revealed that antigen-independent persistence of memory CTLs (CTL precursors) might also be required to clear the primary virus infection (Wodarz *et al.* 2000b,c). This is because persistence of memory CTL in the face of minimal antigenic stimulation ensures that immunological pressure is maintained on the declining virus population.

Most studies analysing the long-term persistence of memory CTLs have approached this problem by

looking at specific CTLs in the context of a single infection. It has recently been shown that the homeostasis in the memory CTL pool is disrupted when heterologous viruses are introduced (Welsh *et al.* 1995; Selin *et al.* 1996, 1999). Heterologous infections first activate and then reduce the memory CTL pool to earlier viruses. This could be due to a regulatory mechanism involving cross-reactive T-cell activation (Welsh *et al.* 1995; Selin *et al.* 1996, 1999). Since each infection induces the development of high levels of memory CTLs, it would not be possible to maintain lymphoid homeostasis if CTLs specific for previous infections did not decline as new infections occur.

These findings show that CTL responses to individual viruses do not occur in isolation, but influence each other. If long-term persistence of memory CTLs were to serve primarily in protecting the host against secondary viral challenges, then the only implications of these findings would be that protection against rechallenge diminishes over time while the host experiences infection with heterologous viruses. On the other hand, if long-term persistence of memory CTLs is required in order to achieve virus clearance or long-term control of the infection, then the finding that exposure to heterologous viruses can lead to the attrition of previous CTL memory has important implications for the ability of the immune system in dealing with multiple infections at the same time. Here, we use mathematical models to investigate this problem.

2. THE MODEL

We constructed a simple model taking into account two basic variables: the virus populations and the memory

CTL populations specific for the respective viruses. We assumed that the host can be infected by n different viruses during its lifetime. Virus of type i is denoted by v_i and the memory CTL response specific to that virus is denoted by z_i , where $i = 1 \dots n$. The model is given by the following set of differential equations:

$$\dot{v}_i = r_i v_i \left(1 - \frac{v_i}{k_i}\right) - p_i v_i z_i \quad (1)$$

and

$$\dot{z}_i = c_i v_i z_i - b_i z_i - z_i \sum_{j=1, \dots, n}^{j \neq i} \alpha_j v_j. \quad (2)$$

Some mathematical details and derivations based on this model will be available as electronic Appendix A on The Royal Society Web site.

The virus replicates at rate r_i . Replication is density dependent and is limited by the carrying capacity k_i . The carrying capacity corresponds to target cell limitation. Virus replication is inhibited by the specific CTL response at rate p_i . The memory CTL response becomes activated and proliferates in response to its specific antigen at rate c_i . In the absence of antigen, the CTL response has an average life span of $1/b_i$. In addition, the model assumes that heterologous antigenic stimuli (v_j) reduce the memory CTL response to virus i (z_i) at rate α_j .

For analytical convenience, the CTL dynamics are modelled in a relatively simple way in the sense that CTL proliferation does not saturate. More elaborate models result in more complex equilibrium expressions (De Boer & Perelson 1998). However, the core results that are relevant for the current analysis remain the same in these more complicated models. Hence, the results derived here are qualitatively robust. In addition, note that the model is simplified in that it collapses the CTL response into a single variable. When modelling memory, it is useful to distinguish between CTLps and CTL effectors (CTLes), since memory is manifested mostly at the level of precursors. Previously published more elaborate models (Wodarz *et al.* 2000c) have distinguished between these two subpopulations and assumed that CTLps proliferate and differentiate, whereas CTLes do not proliferate but have an antiviral effector function. The life span of the CTLps would be long and that of the CTLes short. For the present purpose the two subpopulations can be collapsed into one assuming that the effector population is proportional to the precursor population.

Note that the model makes a number of assumptions that are worth mentioning explicitly.

- (i) The n different viruses are assumed not to be in competition with each other, that is every virus has its own set of target cells without significant levels of direct interference. This is a reasonable assumption if the viruses are distinct pathogens. If the antigenically heterologous virus is only a different strain of the same pathogen this assumption is valid if virus load remains low and competition is not the driving force underlying the dynamics (see § 6).

- (ii) For analytical simplicity it is assumed that the impairment by a viral strain is the same for all strains, i.e. the α_j -values are the same for all i .
- (iii) In the model, the CTL response to a given infection is directly impaired by the presence of antigenically heterologous viral stimuli. An alternative mechanism could be that immune responses have an effect on each other. This would be similar to Jerne's (1974a,b) network hypothesis (see also Hoffman 1975; Urbain 1986), according to which immune cell type I can act on cell type II and vice versa. This can be viewed as a reciprocal predator-prey interaction in which a given immune cell type is both predator and prey to another and vice versa. There has been considerable debate and controversy regarding the network hypothesis and the applicability of such a regulatory mechanism could be different in the resting and active immune states (Anderson & May 1991). The network hypothesis is not pursued further here, since previous modelling approaches (Anderson & May 1991) have suggested that it might not be able to account for the observed experimental results fully (see § 6).

3. VIRUS CONTROL AND ANTIGENIC HETEROGENEITY

We start by summarizing the properties of CTL responses specific for a single virus population in isolation (for now we omit subscripts for simplicity). If $r > 0$, then the virus population grows during primary infection. If $ck > b$, virus growth is followed by expansion of the CTL response. The rising CTL response reduces virus load which settles at an equilibrium level described by $v^* = b/c$. The level of CTL at equilibrium is given by $z^* = r(1 - v^*/k)/p$. According to the model, virus load at equilibrium is influenced by two immunological parameters: (i) a low virus load is promoted by a high CTL responsiveness (c), and (ii) a low virus load and long-term control of an infection requires a long life span of the CTL response in the absence of antigen, i.e. a high value of $1/b$. Hence, long-term virus control or clearance requires antigen-independent persistence of CTL memory (Wodarz *et al.* 2000b,c). This is important because antigen-independent persistence of memory CTL ensures that immunological pressure is maintained even if virus load declines to low levels.

These considerations apply as long as a given virus population is cleared before the host is infected with an antigenically heterologous virus. However, the situation becomes more complicated if the host is faced with more than one virus infection at the same time. There might be multiple acute infections simultaneously present or the host might harbour a collection of persistent infections that can be immunologically controlled. In such a setting, a given virus population can influence immune responses to the other pathogens. If the CTL response has to deal with more than one infection at the same time, the characteristics of the response required to result in virus control or clearance can be altered. The equilibrium virus load for a given infection v_i in the presence of the specific CTL response z_i is described by

Figure 1. Dynamics of two antigenically heterologous infections. Assume that virus population v_1 is controlled by the CTL response z_1 . The schematic diagram demonstrates possible outcomes when adding a second virus infection, v_2 . Either both infections are controlled by their respective CTL memory responses or one or both responses are driven extinct, resulting in uncontrolled virus growth. For a detailed explanation, see the text.

$$v_i^* = \frac{b_i + \sum_{j=1 \dots n, j \neq i} \alpha_j v_j^*}{c_i}. \quad (3)$$

Thus, the abundance of virus i also depends on the collective abundance of the other viruses in the host. More importantly, if

$$\sum_{j=1 \dots n, j \neq i} \alpha_j v_j^* > b_i, \quad (4)$$

then a long life span of memory CTLs in the absence of antigen (low value of b_i) loses its ability to contribute to virus control. In this case, a reduction in virus load is promoted only by a high CTL responsiveness (c_i). Thus, although in principle the CTL memory response still has the capacity to persist in the absence of antigen (low b), interference by the heterologous antigenic stimuli renders this memory ineffective in maintaining strong immunological pressure at low virus loads. At low loads, the degree of interference from heterologous stimuli is stronger than the amount of specific antigenic stimulation, resulting in a reduction in CTL-mediated pressure. Hence, overall immunity is compromised and the chances of virus eradication as well as the level of control is reduced.

4. TWO HETEROLOGOUS INFECTIONS

Here we assumed that a given virus population v_1 has established persistent infection and is efficiently controlled by a CTL memory response z_1 . Failure to clear the infection could have a variety of reasons. Examples are infection of sites that are difficult to access by the immune response or viral latency. We investigate the consequence of infection with a heterologous virus v_2 for the degree of antiviral immunity to both infections (figure 1). The CTL response against the heterologous virus z_2 can expand if $c_2k_2 > b_2 + \alpha_1b_1/c_1$. If this condition is fulfilled and the CTL response z_2 does invade, then the outcome of infection is as follows. If $c_1k_1 > b_1 + \alpha_2b_2/c_2$ then both infections are controlled by their respective CTL responses. In the opposite case, the CTL response z_1 becomes extinct and virus population v_1 reaches its carrying capacity k_1 . Since an increased load of virus population v_1 has an increased adverse effect on the CTL response z_2 , immunological control of virus population v_2 is also reduced. If $c_2k_2 > b_2 + \alpha_1k_1$ then the CTL response z_2 is still maintained and can control virus population v_2 to a certain degree. In the opposite case, the CTL response z_2 also becomes extinct and virus population v_2 reaches its carrying capacity k_2 .

Similarly, if the CTL response z_2 cannot invade in the first place, virus population v_2 will replicate up to its carrying capacity k_2 and reduce immunity to virus population v_1 . If $c_1k_1 > b_1 + \alpha_2k_2$ then the CTL response

Figure 2. Effect of the number of infections n on (a) the total virus load and (b) the total number of memory CTLs in the neutral model assuming that parameters are identical for each infection. An increase in n results in an increase in virus load. This increase is less than exponential if n lies below a threshold defined in the text. If n lies above that threshold, the increase in virus load is faster than exponential. An increase in n also results in an increase in the total number of memory CTLs. If n crosses a threshold, the overall number of memory CTLs decreases and, finally, becomes extinct. For mathematical details, see the text. The parameter values were chosen as follows: $r_i = 0.5$, $p_i = 1$, $k_i = 10$, $b_i = 0.1$, $c_i = 2$ and $a_i = 0.1$.

z_1 is maintained, otherwise it becomes extinct and $v_1 = k_1$.

5. MULTIPLE INFECTIONS

Here we extend the above analysis in order to account for multiple persistent infections. We investigate how accumulation of persistent infections influences overall immunity and virus control and discuss whether there is a limit to the number of infections the immune system can deal with. We assume that there are a number of n persistent virus infections v_i that can infect the host over time and that can be controlled by their respective specific CTL responses, z_i . We start by considering the simplest case assuming that viral and host parameters are the same for each infection and the respective CTL response (i.e. $r_i = r$, $k_i = k$, $p_i = p$, $c_i = c$, $b_i = b$ and $a_i = \alpha$). Figure 2 shows how the total virus load and the total number of memory CTLs depend on the number of infections present in the host. Increasing the number of infections n results in both an increase in virus load and the total number of memory CTLs. For

$$n > (1 + c/\alpha) - \frac{\sqrt{kb(\alpha + c)}}{k\alpha}, \quad (5)$$

the total number of memory CTLs declines with an increasing number of infections. For

$$n > (1 + c/\alpha) - \frac{b(c + \alpha)}{ck\alpha}, \quad (6)$$

CTL memory collapses and the immune system loses control of all virus infections.

It is interesting to consider the rate of increase in the total virus load when infections are accumulated (when n is increased) (figure 2). For $n < 0.5(1 + c/\alpha)$ the increase in total virus load with the addition of new infections is less than exponential (figure 2). However, this trend is reversed if $n > 0.5(1 + c/\alpha)$. If this threshold is crossed, then the increase in the total virus load becomes greater than exponential when new viruses are added to the system (i.e. when n is increased) (figure 2). Hence, if the number of infections crosses this threshold, CTL memory starts to lose the ability to keep the viruses in check and this culminates in a decrease in the CTL memory population and eventually in extinction of CTL memory.

These basic patterns also underlie the more complicated and realistic case assuming that host and viral parameters differ between individual infections. An important parameter in this respect is the strength of the memory CTL responses. In the model this is described by $c_i k_i$, i.e. it is a combination of the rate of CTL activation and the level of antigenic stimulation provided by the virus. For the purpose of analysis, we rank the CTL specific for the different viruses according to their rate of expansion, so that $c_1 k_1 > c_2 k_2 > c_3 k_3 > \dots > c_n k_n$. We can then test each successive CTL response z_i for its ability to persist in the face of the other virus infections. The CTL response z_i directed against virus infection v_i can persist and control the infection if

$$\sum_{j=1, \dots, n}^{j \neq i} \alpha_j v_j^* < c_i k_i - b_i. \quad (7)$$

Thus, establishment of the memory CTL response z_i is promoted by a low total virus load of heterologous pathogens as well as by a small negative effect of these heterologous infections on the CTL response z_i . If the above condition is not fulfilled, then the CTL memory response z_i is not established and virus population v_i reaches its carrying capacity k_i . This in turn significantly increases the overall virus load and this can have an increased negative effect on all other memory CTL responses present. If the overall virus load is high enough, this can lead to a chain reaction resulting in extinction of further CTL responses (figure 3). In the worst case, this chain reaction culminates in extinction of the entire CTL memory population and uncontrolled replication of all viruses present (figure 3). This would be equivalent to death of the host.

6. APPLICATION AND DISCUSSION

(a) The model and its assumptions

The aim of this paper was to explore the consequences of some basic experimental results concerning heterologous

Figure 3. Effect of the number of infections n on (a) the total virus load and (b) the total number of memory CTLs in the realistic model assuming that parameters differ between the infections. The general pattern is the same as for the neutral model shown in figure 2. The main difference is that, in this more realistic case, not all CTL memory responses necessarily become extinct when the number of infections n cross a certain threshold. If n crosses a threshold, only a fraction of the CTL memory responses are driven extinct. With each additional virus infection added to the system, a higher fraction of the CTL memory responses become extinct until all viruses can grow uncontrolled. The simulation assumes that the main difference between the infections is the CTL responsiveness c_i . Values were assigned according to $c_i = 2 - 0.05i$, where $i = 1 \dots n$. The other parameter values were chosen as follows: $r_i = 0.5$, $p_i = 1$, $k_i = 10$, $b_i = 0.1$, $c_i = 2$ and $\alpha_i = 0.1$.

infections by mathematical models. The observation is that a heterologous challenge can result in attrition of otherwise stable memory CTL populations against previous infections. The model assumes that this attrition is mediated by the presence of the heterologous antigen itself. This could be achieved by cross-reactive or non-specific activation of the previous memory CTL populations induced by heterologous antigen. Activation of memory CTLs could also be enhanced by the presence of

certain cytokines such as IL-15 (Zhang *et al.* 1998). This initial activation of previous memory populations could even enhance the response to the heterologous infection to a certain degree if cross-reactive CTL clones are present (Selin *et al.* 1994). Following this, the activated memory CTLs could be eliminated by apoptosis because they are not fully specific for the heterologous antigen and, therefore, do not receive helper or other signals required to maintain the activated CTL responses in the long term. Hence, as the T-cell response to the heterologous virus continues, the T cells with a high affinity to the new infection will be selected over previous CTL populations.

Another possibility, which is not explored in this paper, is that the CTL responses interact directly with each other, resulting in attrition of memory. Such mutual inhibitory effects of the CTL responses would be similar to Jerne's (1974a,b) network models which have been widely explored in the theoretical literature (see also Hoffman 1975; Urbain 1986). However, if direct interactions between the CTLs was the underlying mechanism, theoretical models could suggest that there should also be a negative effect of the previous memory CTL population on the new response in the experiments published (Selin *et al.* 1996, 1999). When the host is challenged with heterologous antigen, it does have memory and, therefore, a high initial number of CTLs against previous infections, whereas the initial number of CTLs against the heterologous infection is low (because the host is still naive to that infection).

The model is based on clear assumptions and makes specific predictions. Further experiments could be devised in order to test aspects of the model. The experiments performed so far could be repeated in a modified form involving two sets of mice. In one set of mice, infection should be such that lymphocytic choriomeningitis virus (LCMV) is cleared and memory is generated. In a second set of mice, LCMV infection should result in CTL exhaustion and persistent replication. This would enable us to test whether antigen alone can result in attrition of other CTL responses or whether direct interactions between the CTLs are required.

(b) CTL memory, virus control and ageing

Experimental observations have indicated that infection with heterologous viruses can lead to attrition of CTL memory to other infections (Welsh *et al.* 1995; Selin *et al.* 1996, 1999). We have used mathematical models in order to explore the consequences of these dynamics for the ability of the immune system in dealing with multiple infections at the same time. The model suggests that the presence of heterologous infections can alter the characteristics of the CTL response required for long-term control or clearance. In the setting of a single virus infection the model predicts that two characteristics of the CTL response contribute to virus control or clearance. A high CTL responsiveness reduces virus load. This has been shown to be the case in persistent infections such as human immunodeficiency virus (HIV) and human T-cell lymphoma virus (HTLV-1) (Carrington *et al.* 1999; Jeffery *et al.* 1999). However, a high CTL responsiveness alone is unlikely to be sufficient for virus clearance or long-term control of the infection. In addition, virus clearance or long-term control requires that the CTL

response is long lived in the absence of antigen—a characteristic of memory CTLs. This has been discussed extensively by Wodarz *et al.* (2000*b,c*). If the life span of memory CTLs in the absence of antigen is short the virus can establish a persistent infection. The reason is as follows. When the virus population declines because of CD8-mediated activity, the CTL population also declines, which in turn reduces the immunological pressure on the virus population and enables the virus to settle at a stable equilibrium. In contrast, with antigen-independent persistence of memory CTLs, the CTL population reaches a stable level while virus load drops. This maintains continuous pressure on the declining virus population and will drive the virus population to extinction (or to an extremely low equilibrium abundance). Hence, CTL memory is required for efficient virus control or clearance. These findings also apply in the presence of antigenically heterologous infections, as long as the adverse effect on CTL memory caused by heterologous stimuli lies below a threshold. If this threshold is crossed, then a long life span of the CTL response in the absence of antigen loses its ability to contribute to virus control. Specifically, the CTL response loses its ability to exert strong immunological pressure on the virus population at low loads. This is because specific antigenic stimulation is weak, while the otherwise stable memory population is diminished by the heterologous infections. Thus, although the CTL response might still be able to reduce virus load, loss of immunological pressure at low loads prevents virus extinction. The exact number of heterologous stimuli needed to reach this threshold is difficult to predict. It depends on the parameter values of the model, such as the efficacy of CTLs against each individual infection and the degree to which a given heterologous stimulus reduces CTL memory to a given infection.

In general, an increase in the number of antigenically heterologous infections in the host increases overall virus load. If the number of infections is below a threshold, addition of new viruses results in a smaller than exponential increase in the total virus population. If the number of infections is above this threshold, the rise in total load is faster than exponential with each additional virus that is added. This is the threshold beyond which the immune system not only fails to clear infections but also fails to keep persistent infections at relatively low levels. Despite the fact that accumulations of multiple infections can successively compromise the level of CTL-mediated virus control, the overall number of memory CTLs also increases. This is because higher virus loads induce more CTL proliferation. This observation is in agreement with recent results showing that more efficient CTL-mediated virus control can be associated with lower levels of CTLs at equilibrium (Bartholdy *et al.* 2000; Wodarz *et al.* 2000*a*). If the number of antigenically heterologous infections crosses a threshold, a decrease in the overall number of memory CTLs is observed which eventually results in the absence of CTL memory and uncontrolled viral growth. This is because, above a certain abundance of heterologous infections, the negative effect on CTL memory is very strong and overrides the amount of antigenic stimulation. Note that the model cannot make a statement about the number of different infections the

immune system can deal with. This depends on the exact parameters of the model which are not known. Recent research has argued for a diversity of the memory repertoire of at least 10^5 specificities (Arstila *et al.* 2000).

These dynamics could account for the observation that the efficacy of antiviral immunity decreases with age. With advanced age, people become more susceptible to viral infections and recall responses have been observed to be less efficient compared to younger age groups (Effros & Walford 1983; Fagiolo *et al.* 1993). During the lifetime the host becomes infected with a wide variety of viruses and antigens might persist for prolonged periods of time. Other viruses establish persistent infections. Thus, as the host ages, the number of heterologous antigenic stimuli presented to the immune system could increase, resulting in progressive weakening of CTL memory and antiviral immunity. CTL memory responses might also be required for tumour surveillance and might prevent cancer growth (Xiang *et al.* 1999). In this case, the emergence of clinical tumours could also be the consequence of weakened CTL memory caused by accumulation of too many antigenic stimuli over time.

(c) *Antigenically variable viruses*

Infection with different viruses is not the only mechanism by which the immune system can be exposed to heterologous antigens. It can also be the result of antigenic variation in a single virus population. Hence, accumulation of antigenic diversity can lead to a compromised immune system. Note that this does not require active immune impairment by the virus, as observed for example with HIV (Rosenberg *et al.* 1997; Kalams & Walker 1998; Kalams *et al.* 1999). Nowak *et al.* (1991, 1995), Nowak & May (1991) and Nowak (1992) analysed a mathematically similar model showing that, in HIV infection, there is a diversity threshold beyond which the population of T helper cells collapses, marking the development of acquired immune deficiency syndrome (AIDS). However, in contrast to the work presented here, this diversity threshold was the result of the asymmetric dynamics between HIV and CD4 T cells: HIV can infect and impair any activated CD4 cell, while CD4 cells can only specifically recognize HIV strains. According to the considerations presented here, any antigenically variable virus population could compromise the immune system to a certain degree since heterologous challenges result in attrition of CTL memory. This is the consequence of a mechanism that is intrinsic in the immune system and that might be required for homeostatic regulation of CTL memory. With the above discussion in mind, accumulation of antigenic variation could result in faster ageing of the immune system, since a single virus infection leads to exposure to a large number of antigenically heterologous stimuli. Ageing of the immune system as a result of HIV dynamics has previously been explored by Pilyugin *et al.* (1997). If HIV does induce accelerated ageing of immunity, then it is important to initiate antiviral therapy as early in the disease course as possible, particularly if the aim of therapy is to induce long-term immunological control of the infection (Liszewicz *et al.* 1999; Ortiz *et al.* 1999; Wodarz & Nowak 1999). Empirical data (Liszewicz *et al.* 1999; Ortiz *et al.* 1999) and mathematical models (Wodarz & Nowak 1999; Wodarz *et al.*

Figure 4. Co-infection by a virus (v_1) and a bacterial pathogen (v_2). If the amount of CTL memory attrition is significant, co-infection can result in impaired or delayed resolution of both pathogens. Treatment of the bacterial infection (arrow) reduces the amount of bacterial antigen displayed to the immune system. This strengthens the CTL memory response to the viral infection which can now be driven extinct. Treatment of the bacterial infection was modelled by setting $r_2 = 0$ and introducing a term describing removal of the bacteria $\gamma = 0.5$. The other parameter values were chosen as follows: $r_1 = 1$, $r_2 = 0.5$, $c_1 = 0.5$, $c_2 = 0.6$, $k_i = 10$, $p_i = 1$, $b_i = 0.1$ and $\alpha_i = 0.5$.

2000c) have suggested that this could be achieved by structured therapy interruptions or by a combination of drug treatment and vaccination. These regimes could induce improved levels of HIV-specific helper cell responses, resulting in expansion of a sustained CTL memory response. However, if the virus population is already antigenically very diverse, then it could be difficult to induce significant levels of memory CTL, even if enough T-cell help is available. The reason would be that the immune system is faced with too many antigenically heterogeneous stimuli at the same time.

(d) Vaccination

The result that an increase in the number of antigenic stimuli experienced by the immune system at the same time can result in overall compromised immunity has obvious implications for vaccination strategies. Immunization is the most effective way of preventing infectious disease. Traditionally, there are two main types of vaccines: live virus vaccines and inactivated virus vaccines. Live avirulent vaccines have been most successful and have significantly reduced the incidence of several important diseases. The reason for the success of these vaccines is that the live attenuated virus replicates in the recipient. However, this can also have negative effects on overall immunity since, from an immunological point of view, this antigenic exposure is equivalent to a natural infection. Even inactivated virus vaccines could in principle have negative effects on overall antiviral immunity since application of multiple high doses of the antigen could lead to attrition of the memory CTL population against other infections. According to our results it is not a good strategy to vaccinate people against as many infections as possible. Vaccination should be administered if the benefit to the individual achieved by the immunization outweighs the

cost to overall immunity of that individual (due to a possible reduction in previously established CTL memory populations). Hence, patients currently dealing with an acute infection should abstain from vaccination since the additional exposure to heterologous antigens could reduce the efficacy of CTL memory in resolving this acute infection. Similarly, patients harbouring pathogenic persistent infections such as HIV, HTLV or hepatitis B virus (HBV) should consider possible immunizations carefully.

From a broader perspective we could be faced with a potential dilemma when thinking about vaccination strategies. While vaccination could be detrimental to individuals who have already experienced many different antigenic stimuli, vaccinating against as many infections as possible could be beneficial for society as a whole since it would effectively stop the spread of the pathogen within a population. This problem merits further investigation by mathematical models.

(e) Bacterial co-infections

It is often the case that viral infections, particularly with respiratory viruses, are accompanied by bacterial co-infections. According to the model, co-infection can reduce overall immunity and this can result in delayed clearance or in failure to clear either infection (figure 4). This weakened immunity in turn opens up the possibility for other viruses invading the host and deteriorating the situation. Hence, in particular in older patients or in patients with a compromised immune system, it could be helpful to administer antibiotics even if symptoms are caused by a viral infection. Protection from bacterial co-infections ensures that the CTL response can work with highest efficacy in order to resolve the viral disease. If there already is a bacterial co-infection present in the host, inhibition of bacterial growth reduces exposure of the immune system to bacterial antigen and this might enhance the CTL memory response against the virus (figure 4). Hence, inhibition of bacterial growth could result in more efficient antiviral immunity and in faster resolution of the viral disease.

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