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Clonal Exhaustion as a Result of Immune Deviation

CAN KEŞMIR* AND ROB J. DE BOER
Theoretical Biology and Bioinformatics Group,
Utrecht University,
Padualaan 8,
3584 CH Utrecht,
The Netherlands
E-mail: c.kesmir@bio.uu.nl

An overwhelming virus infection that spreads within a few days throughout the host can cause deletion of the specific cytotoxic T lymphocytes (CTL). This phenomenon is known as 'clonal exhaustion'. Current explanations for this phenomenon are 'clonal', and consider either the terminal differentiation of the virus-specific CTL to an effector phenotype, or the lack of help and antigen presentation for a specific CTL clone. The virus remains controlled by some other form of immunity in the exhausted state. Candidates are innate immunity (especially NK cells and macrophages) and a T helper type 2 based immune response. Surprisingly, the role of this other form of immunity in causing exhaustion has been ignored so far. Developing a mathematical model, we here investigate the possibility that this inter-clonal immunity is responsible for exhaustion by down regulating the CTL response. The model is based on previously published exhaustion data for Lymphocytic choriomeningitis virus as an *in vivo* model. We demonstrate that several complicated experiments on clonal exhaustion are consistent with inter-clonal regulation. By interpreting the available data with a mathematical model, we compare this novel mechanism with the mechanisms suggested previously.

1. INTRODUCTION

Most viral infections induce specific B and T cell responses. Although neutralizing antibodies are very effective in protecting against free virus, the cytotoxic T cell response is essential during a primary response, especially when the virus is noncytopathic (Kagi and Hengartner, 1996). Lymphocytic choriomeningitis virus (LCMV) is used as a classical model system for studying infection with noncytopathic viruses. It is well established that an acute primary infection with this virus is almost always cleared by the $CD8^+$ T cell subset. However, an overwhelming infection of rapidly replicating strains of LCMV induces persistent infections (Moskophidis *et al.*, 1993a,b, 1994b; Tishon *et al.*, 1993; Zinkernagel *et al.*, 1993).

*Author to whom correspondence should be addressed.

These strains invoke a short initial cytotoxic T lymphocyte (CTL) response, which is followed by the disappearance of the specific clone. This chain of events is called 'clonal exhaustion' (Zinkernagel *et al.*, 1993; Moskophidis *et al.*, 1993b; Zinkernagel, 1996; Zinkernagel *et al.*, 1996; Pantaleo *et al.*, 1997; Welsh and McNally, 1999), and is another example of the high dose immune paralysis which was originally demonstrated for B cells in the classical experiments of Mitchison (1964). The clonal exhaustion of specific CTLs has been demonstrated using soluble tetrameric major histocompatibility class I-peptide complexes (Gallimore *et al.*, 1998; Ou *et al.*, 2001).

The induction of exhaustion correlates with the immune state of the host. Overwhelming infection of immunocompetent naive hosts induces exhaustion within 2 weeks (i.e., early exhaustion) (Moskophidis *et al.*, 1993a,b; Tishon *et al.*, 1993). Partially immunodeficient mice (e.g., MHC Class II and IgM knockout, $CD4^+$ T cell depleted) immunized with optimal dose and mild strains of LCMV become exhausted 40–200 days after immunization (i.e., late exhaustion) (Matloubian *et al.*, 1994; Cardin *et al.*, 1996; Thomsen *et al.*, 1996; Planz *et al.*, 1997). Congenital and neonatal infections usually become persistent, irrespective of the virus strain and inoculum size used. These life-time carriers have a higher viral load than adult exhausted hosts (Baldridge *et al.*, 1997). Thus this carrier state might be considered *tolerant* rather than exhausted. The hosts that are immunized with optimal dose at least once do not get exhausted (Zinkernagel *et al.*, 1993, 1996; Zinkernagel, 1996).

Although *in vivo* data give clues about the tropism of viruses, MHC dependence (Moskophidis *et al.*, 1994b), and the role of different molecules (Alexander-Miller *et al.*, 1996; Zimmermann *et al.*, 1996), our knowledge of the underlying mechanisms of clonal exhaustion is limited. Zinkernagel *et al.* explain the cause of early clonal exhaustion in terms of total and sudden differentiation (Zinkernagel *et al.*, 1993, 1996; Zinkernagel, 1996); and argue that in overwhelming infections almost all CTL precursors differentiate into the short-living, nonproliferating effector phenotype and thus they disappear during the second week of immunization [see also Grossman (1986), Grossman *et al.* (1986)]. Other mechanisms considered to explain the phenomenon of clonal exhaustion include: high-dose inhibition, immune suppression (Wodarz *et al.*, 1998), anergy and antigen-induced cell death (AICD) (Bocharov, 1998) and programmed cell division (Kaech and Ahmed, 2001; Van Stipdonk *et al.*, 2001). All these mechanisms ignore the fact that the virus remains controlled in the exhausted state [see Fig. 1(a)–1(c)] and sometimes it is eventually cleared (Ou *et al.*, 2001).

In this study, we develop a mathematical model that employs the fact that in the exhausted state the virus is controlled by another form of immunity. We hypothesize that in addition to passively controlling the virus in the exhausted state, this other immunity form plays an active role in the early clonal exhaustion. Innate immunity (especially NK cells and macrophages) or T helper type 2 (Th2) based immunities are likely candidates for this, as they can both control the viral load

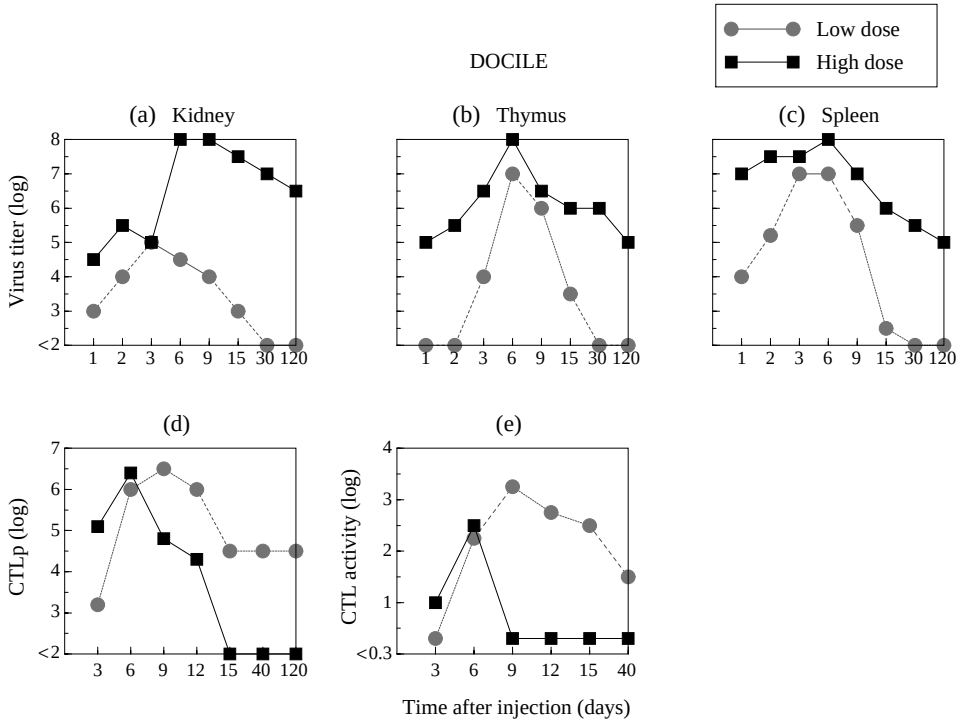

Figure 1. The original exhaustion data for LCMV-DOCILE, reproduced manually from the graphs in Moskophidis *et al.* (1993b). Two different infection doses are shown: 100 PFU (circles) and 10^7 PFU (squares). Panels (a–c) LCMV titre per spleen, kidney and thymus (in PFU g^{-1} per organ); (d) LCMV specific CTL precursor numbers per spleen and (e) CTL activity per spleen. The virus titre and $CD8^+$ T cell numbers are given on a logarithmic scale.

and induce depletion of $CD8^+$ T cells (Zheng *et al.*, 1995; Alexander-Miller *et al.*, 1996; Kos and Engleman, 1996). For the sake of clarity, in the rest of the paper we will refer to the other form of immunity as the 'innate immunity'. This choice is supported by the following experimental evidence. First, $CD8^+$ T lymphocytes are susceptible to direct apoptosis induction via binding TNF-α (Zheng *et al.*, 1995; Alexander-Miller *et al.*, 1996) or Fas/FasL interaction (Zimmermann *et al.*, 1996). The innate immunity, especially activated macrophages and NK cells, produce large quantities of TNF-α (Eigler *et al.*, 1997; Horwitz *et al.*, 1997). Second, when all splenic mononuclear phagocytes are depleted, exhaustion was not observed, although the viral load reached high levels as in high dose immunization (Thomsen and Volkert, 1983; Lehmann-Grube *et al.*, 1987). In these hosts the CTL activity was not altered significantly (Lehmann-Grube *et al.*, 1987). Third, IFN-γ, an essential part of the innate response to viral infection, seems to play a crucial role in the silencing of virus specific $CD8^+$ T cell responses and thus prevention of immune-mediated pathology (Ou *et al.*, 2001). Fourth, CTL exhaustion is perforin dependent (Gallimore *et al.*, 1998), and the cytotoxicity of NK cells is perforin

mediated (Kagi and Hengartner, 1996). In summary these data suggest that the innate immune system can play both the role of controlling the virus in exhausted hosts, and actively inducing exhaustion of $CD8^+$ T cells. However, one should keep in mind that what we call 'innate immunity' is only one of the candidates; humoral immunity is also a likely candidate since in 'exhausted' hosts the virus is eventually cleared by neutralizing antibodies (Ou *et al.*, 2001).

We study exhaustion as competition between an efficient CTL response and a less efficient innate immunity. Each type of immune response is inhibitory for the other, either directly, e.g., by inducing apoptosis, or indirectly, e.g., by eliminating the antigenic stimulus. Experimental data on LCMV infections are analysed by means of different model experiments in order to demonstrate that many of the complicated *in vivo* results, including late exhaustion, are consistent with our conjecture that a switch of response type (immune deviation) is the underlying mechanism for clonal exhaustion.

2. Materials and Methods

2.1. ***Data.*** The experimental data displayed in Figs 1 and 4(a)–4(c) were taken from original exhaustion data of Moskophidis *et al.* (Moskophidis *et al.*, 1993b; Zinkernagel *et al.*, 1993). We reproduced the graphs manually. Mice were injected intravenously (i.v.) with LCMV-DOCILE and LCMV-WE with both high and low dose. Virus titres in different organs, CTL activity, and LCMV specific CTLp frequency are given in the graphs.

2.2. ***Model.*** We study the competition between the two types of immunity by means of systems of ordinary differential equations. To illustrate our main point with maximum clarity, we keep the model as simple as possible and consider the cytotoxic T cells as a single population; i.e., we do not differentiate between the short- and long-living T cells. Similar results can be obtained, however, with a more detailed model with two distinct sets of $CD8^+$ T cells (i.e., activated/short-living and resting/long-living, results not shown). Regarding the LCMV specific $CD8^+$ T cell clone we consider a source from the thymus (σ), proliferation due to interaction with the antigen, inhibition by the innate immunity and death at constant rate δ. Mathematically this translates into

$$\frac{dT}{dt} = \sigma + \alpha T - dIT - \delta T, \tag{1}$$

where the *per capita* rate of proliferation, α, is defined as

$$\alpha = \frac{pV}{s + V + mT}. \tag{2}$$

The variable T denotes LCMV specific $CD8^+$ T lymphocytes, V the viral titre, and I the innate immunity. $1/\delta$ gives the average lifetime of T cells. Because in early

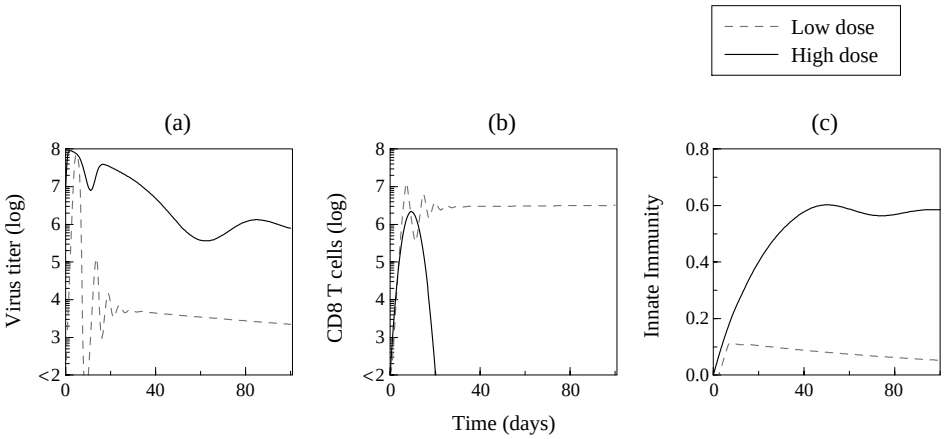

Figure 2. Two time plots generated by the mathematical model. The low initial virus dose ($V(0) = 100$) is shown by dashed lines, and a high initial virus dose ($V(0) = 10^7$) by solid lines. The initial conditions are: $T(0) = 100$, $I(0) = 0$. (a) Virus titre; (b) $CD8^+$ T cell numbers, T; and (c) the innate immunity. The virus titre and $CD8^+$ T cell numbers are given on a logarithmic scale.

exhaustion the CTL response is turned off by deletion (Moskophidis *et al.*, 1993b), we assume that the innate immunity depletes $CD8^+$ T lymphocytes at a rate dI.

The *per capita* rate of proliferation, α, is a saturation function that was derived previously (De Boer and Perelson, 1995). It allows for a true maximum proliferation rate p of $CD8^+$ T cells. In equation (2), m is the degree of T cell competition and s is a saturation constant. The competition term, mT, in α allows for regulation of T cell proliferation under continuous antigenic stimulus; the *per capita* rate of proliferation decreases with increasing number of T cells. The main advantage of this competitive form of the proliferation function, α, is the stability it induces. Without the mT competition term, T cell response may oscillate strongly. For the sake of simplicity, we do not include the dynamics of $CD4^+$ T lymphocytes in our model. However, the late exhaustion depends heavily on $CD4^+$ T cell help, probably via partial control of the virus (Battegay *et al.*, 1994; Matloubian *et al.*, 1994; Cardin *et al.*, 1996; Thomsen *et al.*, 1996). The implications of this for late exhaustion will be discussed in Section 3.3.

A conventional logistic growth function is employed to model virus dynamics. The maximum viral load (i.e., the carrying capacity of the virus, k) is attained in the absence of any immune response, e.g., in the tolerant state. The virus has a maximum growth rate, r. Assuming a proportional relation between the viral load, V, and the number of infected cells we write that

$$\frac{dV}{dt} = rV(1 - V/k) - c_T TV - c_I IV. \tag{3}$$

Virus declines as a function of CTL response and the innate response at rates c_T, and c_I, respectively.

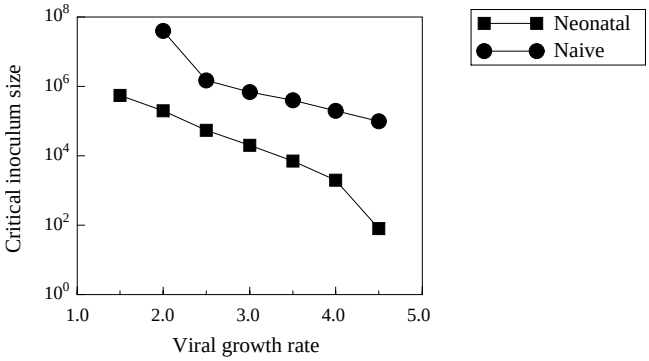

Figure 3. The minimum inoculum size inducing exhaustion as a function of the viral growth rate. The effect of the initial $CD8^+$ T cell numbers is demonstrated by starting the simulations with 100 LCMV specific $CD8^+$ T cells (naive host, circles), or 10 LCMV specific $CD8^+$ T cells (neonatal host, squares). In the simulations the carrying capacity of the virus was set to 10^8. If the carrying capacity is larger, it is possible to find an 'exhaustive' inoculum size even with slowly growing strains (i.e., for $r < 1.5$).

To model the active inhibitory effect of the innate system we choose nonproliferative dynamics. The results do not depend on this choice, because it is possible to obtain the same behaviour with proliferative dynamics (results not shown). Consider the innate immunity as a large pool of cells that may become activated by the antigenic stimulus and revert to the resting state at a constant rate, δ_I. Let the variable I denote the activated cells in this pool. These assumptions are translated into

$$\frac{dI}{dt} = (1 - I)\frac{aV}{s_I + V} - \delta_I I, \tag{4}$$

when $(1 - I)$ is the fraction of the nonactivated cells. The parameter s_I is a saturation constant for the antigenic stimulus, and a is the rate of activation. Although the innate immunity probably plays a stimulating role early in the response, there is data suggesting that it can also become inhibitory later (Kos and Engleman, 1996). We implement this gradually increasing inhibitory effect as the following: first we choose $a \ll 1$ such that the inhibitory effect of innate immunity is slow. Second we choose $s_I \gg s$ such that the innate immunity becomes inhibitory for CTL response only when the viral load is high.

In general this model can be made much more complicated by including additional mechanisms that are known to play a role in the dynamics of an immune response, e.g., normal homeostatic control of T cells. These additional processes might change the parameter regimes in which the clonal exhaustion is induced, however, the immune deviation still remains as the main reason of the clonal exhaustion in our model.

2.3. ***Steady states.*** By setting equations (1)–(3) to zero one can find the four steady states of our model. First, for the *naive state*, one sets $V = 0$ to obtain

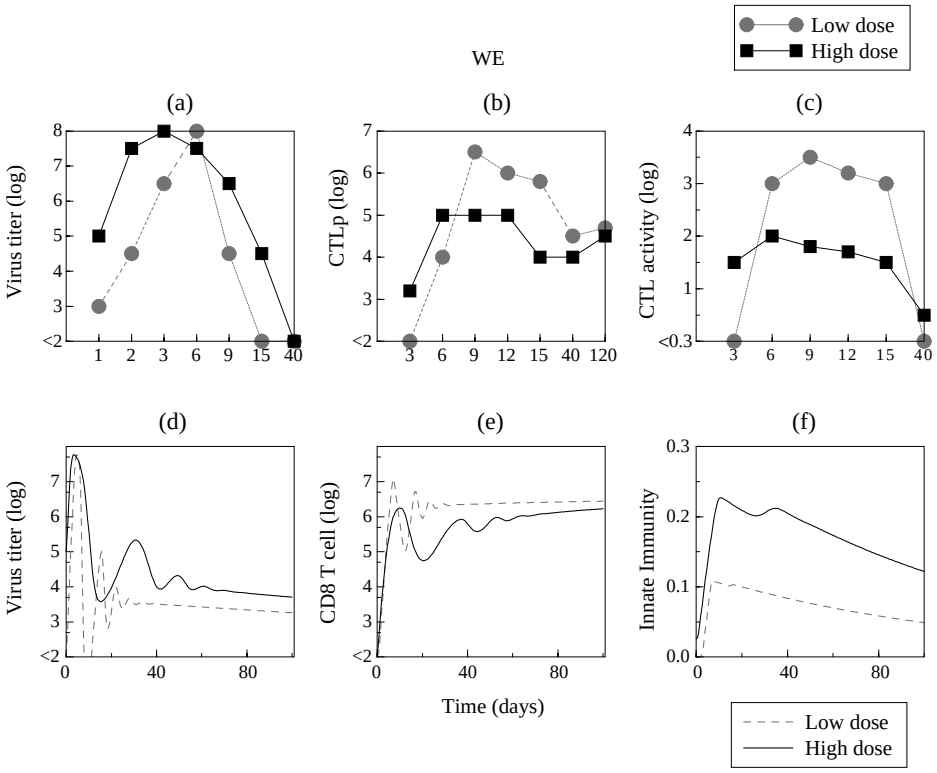

Figure 4. Panels (a–c): dose–response relationship for the WE strain (Zinkernagel *et al.*, 1993). These graphs are reproduced manually from data of Zinkernagel *et al.* (1993). Two different infection doses are shown, 100 PFU (circles) and 10^7 PFU (squares). The units are the same as in Fig. 1. Panels (d–f): results of the mathematical model for $T(0) = 100$, $V(0) = 100$ and $I(0) = 0$ are given by dashed lines (low dose); and for $T(0) = 100$, $V(0) = 10^5$ and $I(0) = 0.025$ by solid lines (high dose). $c_I = 15$ and the other parameters are as given in the main text.

$T = \sigma/\delta$, and $I = 0$. Second, for the *tolerant state*, one sets $\sigma = a = T = I = 0$ to obtain $V = k$. Third, for the *immune state* we approximate $I \cong 0, \sigma \cong 0$ and $s \ll k$ such that the steady-state expressions become:

$$\hat{V} = \frac{\delta(c_T s + mr)}{c_T(p - \delta)}, \qquad \hat{T} \cong \frac{r}{c_T}.$$

Fourth, for the *exhausted state*, we approximate $T \cong 0$ and $S_I \ll k$ such that:

$$\hat{V} = \frac{\delta_I s_I r}{a(c_I - r) - \delta_I r}, \qquad \hat{I} = \frac{r}{c_I}.$$

In both immune and exhausted steady states, the equilibrium value of V is a function of the saturation constants, s and s_I. Thus the basic assumption of $s \ll s_I$ allows us to obtain two different virus levels: one low for the immune state and one high for the exhausted state.

2.4. ***Parameters.*** The parameters in the above equations are chosen such that a time unit corresponds to a day. For the results presented here, we use long-lived CD8$^+$ T cells ($\delta = 0.01$/day). However, the results remain valid for short-living cells (e.g., $\delta = 0.1$/day). The innate immunity reverts to the resting state on a similar time scale: $\delta_I = 0.01$/day. We estimate the net proliferation rate (i.e., $p - \delta$) from the *in vivo* data when antigenic stimulus is at its maximum. Using day 3 and 6 CTL precursor numbers in low dose immunization (see Fig. 1), one obtains: $p - \delta = \ln[10^{5.8}/10^{3.3}]/3 \cong 2$. For $\delta = 0.01$, this sets $p = 2$. The net growth rate of the virus is calculated using the same approach for day 1 and 2 viral load in spleen in low inoculum. We obtain $r = \ln[10^{5.5}/10^4] \cong 3.5$ for LCMV-DOCILE. Interestingly, the 'milder' WE strain has the same growth rate [see Fig. 4(a)]. Since the viral load in spleen can be as high as 10^8 PFU, we set the viral 'carrying capacity' $k = 10^8$. By setting $\sigma = 1$, a naive LCMV specific clone size becomes $\sigma/\delta = 100$ cells. The other parameters which are not possible to obtain from the data are set to: $c_I = 6$, $c_T = 10^{-6}$, $a = 0.03$, $s = 1000$, $s_I = 10^6$, $m = 0.002$; these parameters were chosen such that the behaviour of the system fits best to the *in vivo* data.

3. RESULTS

The four qualitatively different states of a host in the experiments, i.e., the naive, immune, exhausted and tolerant state, can be interpreted as four steady states of our model. In the experiments those four states correspond to four different virus levels: in the naive state $V = 0$; in the immune state the virus is below detectable levels, but might very well persist at low concentrations; in the exhausted state the virus is detectable in any tissue; and in the tolerant state the viral titre is highest. We here briefly discuss the four steady states of the model.

The *naive*, or virgin state, is the normal resting state in the absence of virus. Specific CD8$^+$ T cells are at steady state with a constant thymic influx and a daily turnover (i.e., $T = \sigma/\delta$). Similarly, the innate immunity will be at rest such that $I = 0$. The naive state is unstable to the introduction of virus.

The memory or *immune* state is the steady state that is approached after successful control of the virus by the CTL response. In our model, memory is a stable state because the virus persists at low concentrations, thus maintaining a low level stimulation of the CD8$^+$ T cell clone. The innate immunity is hardly activated, because the virus persists at low concentrations only. In the model, the high frequency of specific T cells is the indicator of the immunological memory. In Section 2.3 we derived the steady-state expressions for V and T in the immune state assuming $I \cong 0$.

The *exhausted* state in the model is attained following the suppression of the CTL response by the innate immunity. Thus, we have only a few CD8$^+$ T cells (i.e., $T \cong 0$), a strong innate immune response and a relatively high virus concentration

controlled by the innate immunity (see Section 2.3). The high virus level maintains the continuous stimulation of the innate immunity, and hence continuous suppression of the $CD8^+$ T cells. Exhaustion is also a form of memory, where the absence of CTL activity is preserved by the ongoing innate response. Establishment of the virus in the thymus might possibly cause deletion of maturing LCMV specific T cells (Zinkernagel *et al.*, 1993), reducing the thymic influx to zero ($\sigma = 0$).

The highest virus levels have been observed experimentally in the *tolerant* state (Baldridge *et al.*, 1997). In our model, tolerance corresponds to an absence of immunity (i.e., to $T = I = 0$), which leaves the logistic growth of the virus as the only equation in the model. Thus, in the tolerant state, the virus is limited only by its carrying capacity k. We obtain realistic model behaviour by setting k larger than the steady-state virus concentration in the exhausted state (see Section 2.3).

3.1. ***Early exhaustion.*** Figure 2 shows the model results for two different initial conditions. Depending on the viral inoculum size, our model does indeed attain the same steady states as are attained in the experiments, i.e., low inoculum dose results in the immune state, and high inoculum dose in the exhausted state. Furthermore, the time scale of the model is in good agreement with the *in vivo* experiments. The oscillations following the acute infection are an artifact of the simplicity of the model. In a model with more detailed T cell dynamics these oscillations disappear (results not shown). It is for reasons of clarity that we show the results of the simple model.

In the model, the steady-state expressions for the immune state and the exhausted state are functions of the viral growth rate (see Section 2.3). If the antigen does not replicate (i.e., if the growth rate is zero), then the naive state is the only steady state of the system. The experimental finding that large amounts of viral peptides induce only temporary exhaustion in euthymic hosts (Aichele *et al.*, 1994, 1995) is thus in natural agreement with the model. Following the disappearance of viral peptides, the LCMV specific $CD8^+$ T cell clone is repopulated by the thymic influx and the naive state is re-established.

In equation (1) the positive terms are the thymic influx, σ, and the proliferation, αT. If these terms are small, [i.e., a small thymic influx and low avidity resulting in a small *per capita* rate of proliferation, α, as defined in equation (2)] it will be easier to induce exhaustion. These model predictions are in agreement with experiments showing that virus persistence is MHC-linked (Moskophidis *et al.*, 1994b), because both thymic influx and the avidity should depend on the host's MHC allele.

The growth rate of the particular strain, and the initial immune state of the host affect the outcome of LCMV infections (Zinkernagel *et al.*, 1993, 1996; Zinkernagel, 1996). In Fig. 3 we plot the minimum inoculum size that causes exhaustion as a function of the viral growth rate, r. An increase of the viral growth rate shifts the exhaustion induction to a lower inoculum size. This was expected since a large r value creates a larger viral load. We may use this result to explain a known fact that IFN-α/β and IFN-γ receptor knockout hosts show exhaustion in almost

any inoculum size (Van den Broek *et al.*, 1995; Planz *et al.*, 1997; Ou *et al.*, 2001). As these IFNs suppress the viral replication, we expect the viral growth rate, r, to be very large in the knockout host, e.g., $r \cong 9$ for LCMV-DOCILE in the IFN-α/β receptor knockout host (Ou *et al.*, 2001). The results shown in Fig. 3 suggest that even small inoculum sizes cause exhaustion when r is large.

Another crucial host parameter in inducing exhaustion is the initial number of $CD8^+$ T cells. In order to study the effect of this parameter we started the simulations in Fig. 3 with two different initial T values, one representing a naive host and the other representing a neonatal host (the latter value having 10-fold fewer LCMV-specific $CD8^+$ T cells). In agreement with the experiments (Zinkernagel *et al.*, 1993; Baldridge *et al.*, 1997) our results suggest that a low initial $CD8^+$ T cell count increases the susceptibility to exhaustion. A host in the *immune* state, i.e., when T cell numbers at the time of infection is several fold higher than the naive host, is resistant to induction of clonal exhaustion (results not shown).

3.2. ***Comparing the responses to WE vs. DOCILE strains.*** High dose immunization with different LCMV strains gives different outcomes. For example, the WE strain seems to be 'milder' than the DOCILE strain, and exhaustion is not observed (Zinkernagel *et al.*, 1993). The WE strain is ultimately always cleared to low numbers. However, when the host is challenged with a high dose of WE, the CTL response is highly suppressed, and the virus clearance is delayed. Although the DOCILE and the WE infections give two different outcomes, their growth rates (as calculated using day 1 and 2 viral titre in spleen) are very similar. The difference between the two strains might therefore be attributed to the host's anti-virus responses.

When compared at day 1 in the spleen [Fig. 1(c) vs. 4(a)] the WE strain attains significantly lower titres than does the DOCILE strain. The lower viral load in spleen could be a consequence of a rapid initial control of the infection by the innate immunity. LCMV-WE might induce earlier activation of the innate immunity because it has a less specific tropism (Ahmed *et al.*, 1984) or it is most susceptible to IFN-γ, whereas DOCILE is resistant (Moskophidis *et al.*, 1994a, 1995). We incorporate this assumption in the model by changing two parameters. First, the initial value of innate immunity is slightly higher than zero, and, second, the rate of elimination of the virus by the innate immunity, c_I, for the WE strain is larger than for the DOCILE. The results are shown in Figs 4(d)–4(f). The response to high dose LCMV-WE shows two distinct phases. In the first phase the innate immunity is dominant, and towards the end of this phase the $CD8^+$ T cell numbers start decreasing. Parallel to this there is a gradual decrease in the viral load due to the innate response. The kinetics of the virus elimination is, however, slower than in the low dose immunization, where the CTL response is dominant. The second phase starts when the antigenic stimulus decreases and the innate immunity is not activated any further. The suppression of $CD8^+$ T cells is lifted and complete deletion is prevented. The system slowly approaches the immune state.

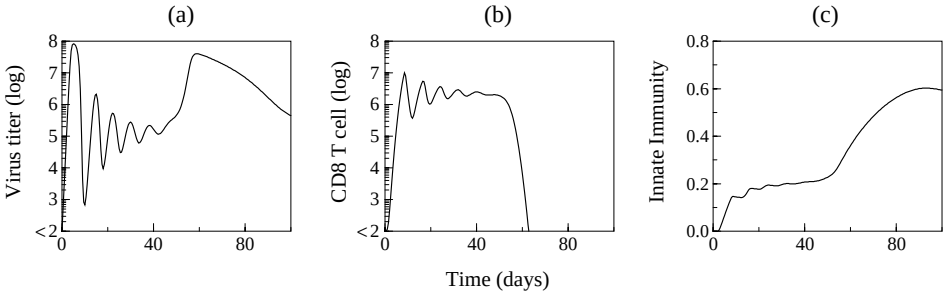

Figure 5. The numerical simulations for late exhaustion. $s = 10^4$ and $V(0) = 100$, $T(0) = 100$, $I(0) = 0$. The primary CTL response is normal. However persistence of higher levels of virus causes the late disappearance of $CD8^+$ T cells and recrudescence of viraemia.

Thus at high dose immunization of LCMV-WE is first controlled by the innate immunity and later by CTL response. This behaviour tends to prevent the high viral concentrations associated with exhaustion.

3.3. ***Late exhaustion.*** Partially immunodeficient hosts (e.g., MHC Class II and B cell deficient, or $CD4^+$ T cell depleted hosts) are more vulnerable to the induction of exhaustion, i.e., the exhaustion can be induced with milder LCMV strains even at a low inoculum dose (Matloubian *et al.*, 1994; Brundler *et al.*, 1996; Cardin *et al.*, 1996; Thomsen *et al.*, 1996; Planz *et al.*, 1997). The influenza virus infections in $CD4^+$ T cell depleted hosts show a similar pattern (D. Wodarz, personal communication). However, the exhaustion of the CTL response occurs a couple of weeks after the termination of the apparently normal primary CTL response. We wanted to test whether we could obtain this behaviour in our model, which was developed originally for early exhaustion.

It is known that the presence of $CD4^+$ T lymphocytes in acute infection is not necessary for obtaining a good primary CTL response (Allan *et al.*, 1990; Mo *et al.*, 1997), especially when rapidly replicating viruses produce larger quantities of available antigen (Zimmermann *et al.*, 1997). However, the absence of $CD4^+$ T cell help would shift the saturation of the *per capita* proliferation rate of $CD8^+$ T cells to higher virus concentrations, i.e., would increase s in equation (2). In other words, at the same viral load $CD8^+$ T cells of $CD4^+$ T cell deficient mice would proliferate slower. In Fig. 5 we show model results obtained by increasing s 10-fold, for a viral inoculum size of 100 units. As a consequence of the larger s, the virus persists at higher levels in the immune state (see Section 2.3), thus enabling continuous stimulation of the innate immunity after the initial CTL response. At some point the inhibitory effect of the innate immunity on $CD8^+$ T lymphocytes exceeds the antigenic stimulation. The number of $CD8^+$ T cells drops, recrudescence of viraemia occurs, and the system approaches the exhausted state, albeit at a long time scale. This is in excellent agreement with the data

reported previously (Cardin *et al.*, 1996; Thomsen *et al.*, 1996; Planz *et al.*, 1997). Our results suggest that the persistence of virus at higher levels plays a crucial role in the exhaustion of some partially immunodeficient hosts.

4. DISCUSSION

We developed a mathematical model based on the assumption that the underlying mechanism of clonal exhaustion is a competition between specific CTL immunity and another, e.g., innate, form of immunity. In other words, a switch of response during an overwhelming infection from a harmful one (CTL) to a less harmful one (e.g., innate) was the main assumption of the model. We demonstrated that the behaviour of this model is consistent with several complicated experiments on clonal exhaustion. The model is able to account for the effect of inoculum size, state of the host, and virus strain on the outcome of an LCMV infection. Although the idea of competition between different forms of immunity originates from data on early exhaustion, we show that late exhaustion can also be explained within the same framework.

In the model we assume that the innate immunity can detect the antigenic stimulus. An initial CTL response might help to trigger an innate response by placing antigen into an appropriate context [e.g., a danger signal (Matzinger, 1994)]. For example, CTL induced activation of macrophages is suggested as an important factor in controlling Hepatitis B infection (Guidotti and Chisari, 1996). Once triggered, the activated state of the macrophages can be preserved by inflammatory cytokines. For reasons of generality, we have not included the CTL mediated triggering of the innate immunity in the model. Due to the simplicity of the current model, we had to assume that in the tolerant state the innate immunity is not triggered by the virus.

As expressed earlier, the immune response controlling the virus growth in exhausted hosts need not be an innate form of immunity. A Th2 dominance can cause $CD8^+$ T cell death via cytokine starvation. This dominance could either be a consequence of higher susceptibility of Th1 cells to AICD (Varadhachary *et al.*, 1997) or could be due to large-scale IL4 production by the activated innate system. Obviously, depending on which other response induces depletion of LCMV specific $CD8^+$ clone, one can elaborate on the dynamics of the second immune response [equation (4)]. We nevertheless expect that our results would remain valid for a wide variety of dynamics.

A recent theoretical study suggests that the main cause of the exhaustion is the lack of antigen presentation and of $CD4^+$ T cell help due to infection of antigen presenting cells and $CD4^+$ T cells (Wodarz *et al.*, 1998). However, it is reported that very few $CD4^+$ T cells are infected with LCMV (Zinkernagel *et al.*, 1993). Moreover, the transfer experiments with transgenic cells suggest that the antigen presentation should be intact in exhausted hosts (Moskophidis *et al.*, 1993b). This is

further supported by the unimpaired CTL response to vaccinia virus injected at the same time as high dose injection of LCMV-DOCILE (Zinkernagel *et al.*, 1993). An alternative mechanism for exhaustion is telomere shortening (Effros and Pawelec, 1997). A simple calculation based on the experimental data [i.e., Fig. 1(d)], however, suggests that the $CD8^+$ T cells are far from their Hayflick limit ($\cong$30 doublings) at the end of the first week. Furthermore, the $CD8^+$ T cell expansion at day 6 is just as large in the immune host as it is in the exhausted host.

Generalizing, we would argue that exhaustion can occur due to a mode switch from a potentially harmful CTL response to a less harmful mode of virus control, when CTL numbers become too large. One could call this immune deviation. Immune deviation is conventionally viewed as a mechanism for avoiding harmful/lethal immunopathology (Matzinger, 1994), as, for example, in the case of tolerance induction. Our immune deviation can be the result of a 'feedback' that is mediated by scores of extracellular chemicals (cytokines) (Segel, 2001a,b). When CTL responses become too excessive, it is indeed protective to switch to a less harmful response. In this way the harm done would be minimized (Segel, 2001b). The idea of immune deviation in high viral burden has also been suggested in a recent review (Zajac *et al.*, 1998). Further analysis of active immune responses in the exhausted hosts should clarify the nature of the immune deviation suggested here, and the possible down regulation of the CTL response by this second form of immunity.

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