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Mathematical model of the primary CD8 T cell immune response: stability analysis of a nonlinear age-structured system

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Abstract The primary CD8 T cell immune response, due to a first encounter with a pathogen, happens in two phases: an expansion phase, with a fast increase of T cell count, followed by a contraction phase. This contraction phase is followed by the generation of memory cells. These latter are specific of the antigen and will allow a faster and stronger response when encountering the antigen for the second time. We propose a nonlinear mathematical model describing the T CD8 immune response to a primary infection, based on three nonlinear ordinary differential equations and one nonlinear age-structured partial differential equation, describing the evolution of CD8

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T cell count and pathogen amount. We discuss in particular the roles and relevance of feedback controls that regulate the response. First we reduce our system to a system with a nonlinear differential equation with a distributed delay. We study the existence of two steady states, and we analyze the asymptotic stability of these steady states. Second we study the system with a discrete delay, and analyze global asymptotic stability of steady states. Finally, we show some simulations that we can obtain from the model and confront them to experimental data.

Keywords Immune response · CD8 T cell · Ordinary differential equations · Delay equations

Mathematics Subject Classification (2000) 34D20 · 34K60 · 35L60 · 35Q92 · 92C37

1 Introduction

Immune response to an infection by a pathogen is supported by different populations of cells (macrophages, B cells, CD4 T cells, CD8 T cells...). Here we focus on a specific response, the CD8 T cell response.

The T CD8 lymphocytes involved in this response are produced by differentiation from hematopoietic stem cells in the thymus, and are maintained in a naive state in secondary lymphoid organs. T CD8 immune response begins when naive CD8 T cells encounter activated antigen-presenting cells that present antigen derived epitopes, signaling the presence of the pathogen. This process leads to an immune response characterized by three phases in the response of T CD8 population: cellular expansion, contraction and memory cell generation (Appay and Rowland-Jones 2004; Murali-Krishna et al. 1998). Indeed, the encounter with the antigen results in differentiation of naive CD8 T cells into an other state, called effector. In this state, CD8 T cells have acquired cytotoxic capacities allowing to kill infected cells (Appay and Rowland-Jones 2004; Hermans et al. 2000). Effector cells proliferate, with a strong and fast increase of T cell count, during the so-called expansion phase. For example, for a lymphocytic choriomeningitis virus infection, effector cell count increases from around 100 cells specific for the epitope encountered in the spleen of a mouse, up to 10^7 cells (Antia et al. 2003; Murali-Krishna et al. 1998). With an Influenza A virus infection in humans, a peak of virus is observed at 2–3 days post-infection, and effector cells are detected at 6–14 days post-infection (Baccam et al 2006; Ennis et al. 1981). These observations give an idea of the time ranges necessary for the beginning of the response, with pathogen recognition by naive cells, followed by their differentiation in effector cells and expansion phase. The expansion phase is followed by a cellular contraction where most of effector cells, about 90% of the population (Murali-Krishna et al. 1998), die by apoptosis: a programmed cell death. Indeed, contraction phase occurs when infection seems to be controlled. For instance, effector cells clear the virus in 7–8 days for lymphocytic choriomeningitis virus infection (Murali-Krishna et al. 1998). With an Influenza A virus infection, effector cells disappear 21 days post-infection (Baccam et al 2006; Ennis et al. 1981). During the response, there is also generation of memory

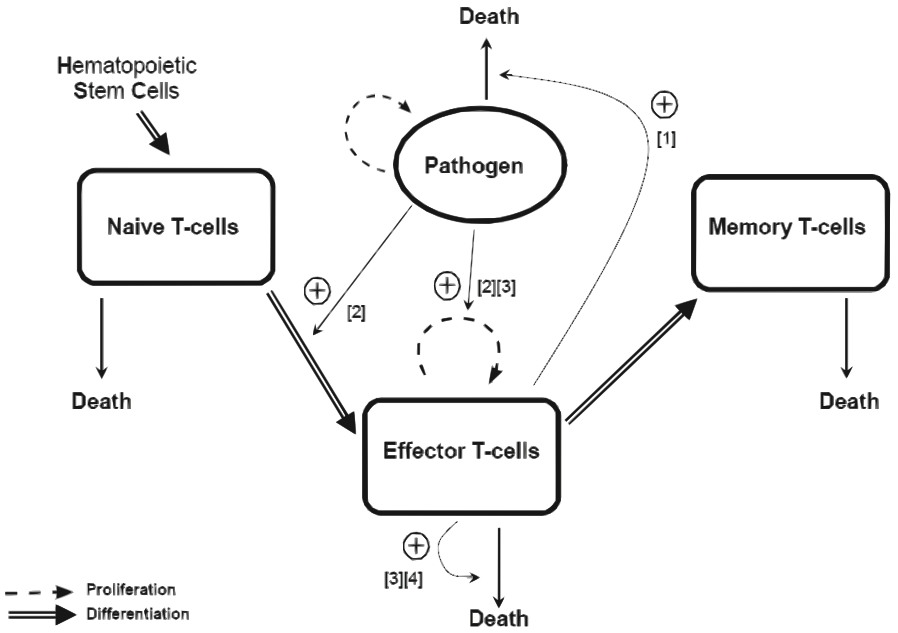

Fig. 1 Schematic representation of the T CD8 immune response mechanisms. Differentiation (of naive cells into effector cells, and effector cells into memory cells) is represented by *thick arrows*, proliferation (of pathogen and effector cells) by *dashed arrows*, and death by *straight lines*. Positive feedback controls are represented by *thin arrows*. Biological justifications of this scheme are mentionned in the beginning of Sect. 2, and referenced here by numbers ([1] Antia et al. 2003, [2] Appay and Rowland-Jones 2004, [3] Kemp et al. 2004, [4] Su et al. 1993)

cells that in numbers amount to 5–10% of the effector population (Antia et al. 2003; Murali-Krishna et al. 1998). These cells are specific of the antigenic epitope and will support a faster and stronger response when re-encountering the antigen in the future (Arpin et al. 2002; Veiga-Fernandes et al. 2000; Wodarz et al. 2000). Different hypotheses are discussed about generation of memory cells. The main hypothesis remains that memory cells are generated from the differentiation of effector cells, previously differentiated from naive cells (Appay and Rowland-Jones 2004; Bannard etal. 2009; Jenkins et al. 2008; Sprent and Surh 2001), see Fig. 1.

In this paper, we are interested in modelling a primary CD8 immune response to an acute infection, that is to say the pathogen has never been encountered by the organism before, and the infection does not result in a chronic infection. For the last 10 years, several models of such an immune response have been proposed. Bidot et al. (2008) focused on activation of CD4 and CD8 T cells, with description of the dynamics of the T cell receptor. They modeled the molecular mechanisms involved in activation and proliferation of T cells, such as production of IL2 and kinetics of expression of co-receptors on T cells, by ordinary differential equations. Hence, they described the beginning of the response, when a T cell encounters an antigen-presenting cell. Yet they did not consider modelling kinetics of a complete population of T cells on the total duration of the response, with their different states, naive, effector and memory.

Other works focused on the modelling of the evolution of infected cells, target cells, and free virus by linear ordinary differential equations. In these models, target cells become infected by the free virus, which is produced in the infected cells (Baccam et al 2006; Saenz et al. 2010). Other mechanisms were studied in this type of models, such as interferon response, effects of a drug and influence of an eclipse phase during which cells are infected but the virus cannot replicate in these cells (Baccam et al 2006; Beauchemin et al. 2008). Adams et al. (2005), Perelson (2001) and Wodarz et al. (2000) considered the same type of mechanisms than Baccam et al (2006), Beauchemin et al. (2008) and Saenz et al. (2010) but they added equations for immune cells, with either a unique state or two states, resting and activated. These different models focused on the virus titer, so that kinetics of immune cells were not considered in detail. The expansion and contraction phases were not modeled, and there was no study of memory cells. Let us mention an other model which took into account a large amount of actors of the immune response, not only virus, target and infected cells, but also dendritic cells, CD4 and CD8 T cells, and B cells (Lee et al. 2009). CD8 T cells could be naive or effector cells in this model, but there was no memory cell. The model focused in particular on the influence of the presentation of antigen and activation of T cells by antigen-presenting cells, such as dendritic cells. Lee et al. (2009) also described migrations of effector cells between tissue and lymphoid compartments with a delay, effects of a drug, and effects of immune cell depletion.

On the contrary, some authors modeled in detail kinetics of different populations of CD8 T cells, naive, effector and memory cells, with linear systems of differential equations. De Boer et al. (2001) proposed two systems of ordinary differential equations. In the first one, they assumed that CD8 T cell response was only driven by the pathogen count, hence defining two threshold times, T_{on} and T_{off}. The parameter T_{on} was taken as a recruitment time, which allowed to not consider explicitly naive cell population, supposed to become activated at T_{on}. The period after the time T_{off} corresponds to the end of the response, as antigen stimulation is assumed to be insufficient to maintain proliferation of effector cells after T_{off}. In the second model, differentiation of T cells depends on a saturation function of the viral load. Moreover, CD8 T cells were not supposed to act on the viral load. Naive cells were explicitly modeled, but only their kinetics of activation were taken into account. However, it seems clear that immune response is not strictly dependent on pathogen amount, since the end of the response does not correspond exactly to the elimination of the pathogen (Antia et al. 2003; Kaech and Ahmed 2001; Stipdonk et al. 2001). It has been observed that even with a brief pathogen encounter, T cells begin a complete programmed response, with the different phases of differentiation, proliferation and generation of memory cells. This process seems to be relevant for efficient generation of memory cells, and protection against a future infection by the same pathogen. It is also relevant for vaccinations, for which only one injection may be needed to allow efficient generation of memory cells. Rouzine et al. (2005) proposed a system of ordinary differential equations, with a viral load parameter depending on time, given by experimental data. This parameter modeled influence of the pathogen on the immune response, such as proliferation of CD8 T cells or activation of antigen presenting cells. Controls between CD8 T cell differentiation and antigen presenting cell count were also modeled. Kim et al (2007) proposed a more complex model, which is however difficult to study and to confront

to experimental data, taking into account CD4 and CD8 T cells, antigen-presenting cells, in the different organs, lymph nodes and tissues, where the response takes place. It can be noticed that none of these models is formed by nonlinear systems, since the different biological rates are taken constant, and do not depend on cell population kinetics.

Here, we will in particular focus on the model of Antia et al. (2003, 2005), which has inspired our model with its structure in age for effector cell equation. They modeled a programmed proliferative response of the CD8 T cells after a pathogen encounter, according to the fact that even with a brief pathogen encounter, a complete response is initiated. They proposed the following model,

$$\begin{aligned} \frac{dN}{dt}(t) &= -bN(t)P(t), \\ \frac{\partial y(t,\tau)}{\partial t} + \frac{\partial y(t,\tau)}{\partial \tau} &= [\rho(\tau) - d(\tau)]y(t,\tau), \\ \frac{dP}{dt}(t) &= rP(t)\left(1 - \frac{P(t)}{c}\right) - hP(t)E(t), \end{aligned}$$

with

$$y(t,0) = bN(t)P(t),$$

where $N(t)$ corresponds to the naive T cell number at time t, $P(t)$ corresponds to the pathogen count, and $y(t,\tau)$ is the effector cell number at time t and age τ. The total numbers of effector cells $E(t)$ and memory cells $M(t)$ at time t are respectively given by

$$E(t) = \int_0^{\tau^*} y(t,\tau)\,d\tau \quad \text{and} \quad M(t) = \int_{\tau^*}^{\infty} y(t,\tau)\,d\tau.$$

Parameter b describes the differentiation of naive cells into effector cells, according to the mass action law, $\rho(\tau)$ is the cell division rate and $d(\tau)$ the apoptosis rate of effector cells with age τ. The amount of pathogen increases with a rate r, with a limitation by carrying capacity c, and pathogen is eliminated according to a coefficient of proportionality h such that death is proportional to pathogen and effector cell counts. This system is formed with two ordinary differential equations and a linear age-structured partial differential equation. In this model, one can first notice that the naive cell population is not supplied, neither continuously nor punctually, by stem cell differentiation. Only a pool of naive cells is considered which is emptied by T cell differentiation under the action of the pathogen $P(t)$. Second, memory cells are produced from "old" effector cells which did not die before reaching the age $\tau = \tau^*$. Finally, no nonlinear dependency of the different rates is considered, only cell age is assumed to act on effector cell proliferation and differentiation.

In our current work, we model kinetics of the immune response for the populations of CD8 T cells described above, naive, effector and memory cells, and kinetics of

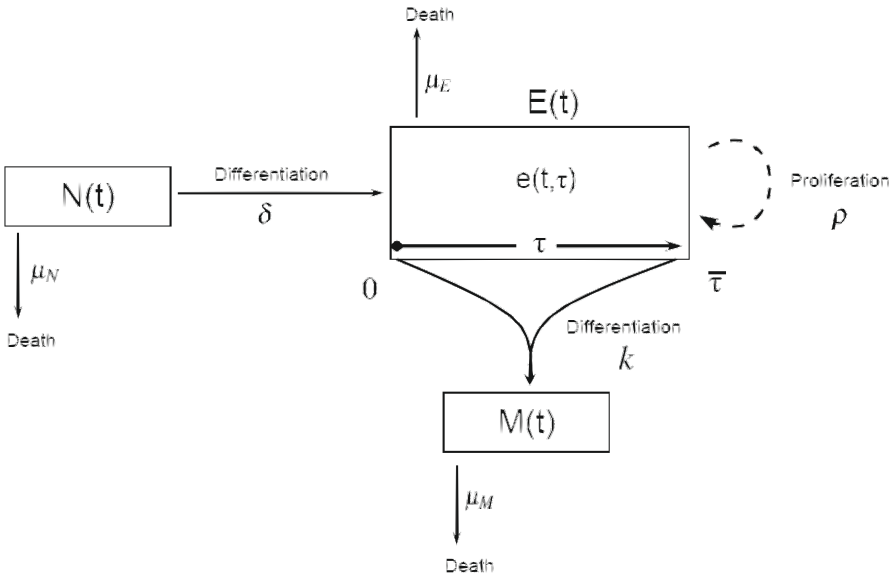

Fig. 2 Model of the T CD8 cell immune response with a distributed delay. Feedbacks were omitted from the figure for clarity

the pathogen (see Fig. 1). Inspired by Antia et al. (2005), our model is based on a system with an age-structured partial differential equation for effector cell population dynamics, and the age represents time since cells have differentiated into effector cells. However, our system is nonlinear since we consider an other mechanism, the regulation of cell dynamics by feedback controls. These controls describe real biological influences of a cell population on differentiation, proliferation and death of the other populations, and on its own fate. For example, the pathogen amount can influence proliferation of effector cells (Appay and Rowland-Jones 2004; Kemp et al. 2004; Kim et al 2007), while effector cell population regulates itself by killing not only pathogen, but also immune cells (Guarda et al 2007; Kemp et al. 2004; Su et al. 1993). These mechanisms influence the kinetics of the expansion and contraction phases and the switch between these two stages of the response. We consider also that differentiation of effector cells into memory cells is dependent on effector cell age, increasing with cell age, as differentiation of an effector into memory cell is progressive (see Fig. 2). As a result, the two populations can be present at the same time during the response, which is more realistic than to model a period with only effector cells and then a period with only memory cells.

In the next section, we present the model, which is formed by three nonlinear ordinary differential equations and one age-structured partial differential equation. Then we reduce this age-structured equation to a nonlinear delay differential equation using the method of characteristics. In Sect. 4, we study existence and uniqueness of solutions for this system, and we determine steady states of our model. Then, in Sect. 5, we analyse the local asymptotic stability of these steady states. Our model can be simplified considering the delay as an average time since effector cells have differentiated. With this modification, we study global asymptotic stability of the system. Finally, we

illustrate on some numerical simulations how the model is able to reproduce a CD8 T cell response, before discussing our work in a conclusion.

2 Mathematical model of the CD8 immune response

This section is devoted to the presentation of a mathematical model of the T CD8 immune response to a primary infection. We consider three types of cells involved in the response, naive T cells, that are resting CD8 T lymphocytes able to react to the stimulation by an antigen; effector cells, that are antigen-specific cells able to eliminate infected cells; memory cells, that are resting cells specific of an antigen, generated during the CD8 immune response. We also consider a pathogen amount. These populations interact, so cell fate (here, cell differentiation, proliferation and death) is strongly controlled by feedback loops. They appear with dependencies in the different variables for the functions presented below. Hence these dependencies are based on real biological phenomena, this yields more realistic mechanisms in the model.

We denote by $N(t)$ the naive cell number at time t. These cells are regularly produced by differentiation of hematopoietic stem cells, with a flow H assumed to be constant and positive. Naive cells die with a constant rate μ_N, positive, and differentiate in effector cells with a rate $\delta(P(t))$ which depends on the pathogen amount denoted by $P(t)$ (Appay and Rowland-Jones 2004).

We denote by $e(t, \tau)$ the effector cell number at time t, with age τ. We consider a limit $\bar{\tau}$ for effector cell age, at which cells necessarily become memory cells, so $\tau \in [0, \bar{\tau})$. Effector cells are killer cells which eliminate not only pathogen but also cells of immune system as soon as they express the antigen and are then recognized as targets. Hence effector cells die with a rate μ_E which depends on total effector cell number (Kemp et al. 2004; Su et al. 1993)

$$E(t) = \int_0^{\bar{\tau}} e(t, \tau)\, d\tau, \tag{1}$$

they proliferate with a rate ρ which depends on pathogen amount $P(t)$ (Appay and Rowland-Jones 2004; Kemp et al. 2004; Kim et al 2007), and finally, effector cells differentiate into memory cells with a rate $k(\tau)$ which depends on cell age, in agreement with the hypothesis of a linear model of differentiation, in which cells become effector before differentiating into memory cells (Appay and Rowland-Jones 2004; Bannard etal. 2009; Jenkins et al. 2008; Sprent and Surh 2001).

We consider the pathogen amount $P(t)$ at time t. As pathogen may reproduce within the organism, we denote by $I(t)$ the production rate of pathogen. Later, we will consider the particular case of a nonproliferating pathogen, as in a vaccine injection for example, so we will no longer consider the parameter I in the system. Pathogen is eliminated with a rate μ_P which depends on the total number of effector cells $E(t)$ (Antia et al. 2003).

We denote by $M(t)$ the memory cell number at time t. These cells die with a rate μ_M assumed to be constant and positive, and are produced by differentiation of effector cells.

Cell population numbers $N(t)$, $e(t,\tau)$, $M(t)$ and pathogen count $P(t)$ satisfy the following system, for $t > 0$ and $\tau \in [0, \bar{\tau})$:

$$\frac{dN}{dt}(t) = H - \mu_N N(t) - \delta(P(t))N(t), \tag{2a}$$

$$\frac{\partial e(t,\tau)}{\partial t} + \frac{\partial e(t,\tau)}{\partial \tau} = [\rho(P(t)) - \mu_E(E(t)) - k(\tau)]e(t,\tau), \tag{2b}$$

$$\frac{dP}{dt}(t) = I(t) - \mu_P(E(t))P(t), \tag{2c}$$

$$\frac{dM}{dt}(t) = \int_0^{\bar{\tau}} k(\tau)e(t,\tau)\,d\tau - \mu_M M(t). \tag{2d}$$

One can note that the term $k(\tau)e(t,\tau)$ expresses number of effector cells with age τ that differentiate in memory cells at time t. Hence, the first term in the right hand side of Eq. (2d) corresponds to the total number of cells differentiated from effector to memory cells at time t and these cells supply the memory cell compartment.

System (2) is completed with the following initial conditions:

$$\begin{cases} N(0) &= N_0, \\ e(0,\tau) &= e_0(\tau), \quad \tau \in [0, \bar{\tau}), \\ P(0) &= P_0, \\ M(0) &= M_0, \end{cases}$$

with $N_0 \geq 0$, $e_0(\tau) \geq 0$, $P_0 \geq 0$, $M_0 \geq 0$, and the following boundary conditions:

$$e(t,0) = \delta(P(t))N(t), \quad t > 0, \tag{3a}$$

$$e(t,\bar{\tau}) = 0, \quad t > 0. \tag{3b}$$

Boundary condition (3a) describes naive cell differentiation into effector cells due to the presence of pathogen, whereas condition (3b) describes the fact that all effector cells have already died or differentiated into memory cells at age $\bar{\tau}$, so there are no more effector cells with age $\bar{\tau}$.

Let us discuss properties of the functions δ, ρ, μ_E, k and μ_P defined above. First, regarding naive cells, we can assume that the more pathogen, the stronger the differentiation of naive into effector cells (Appay and Rowland-Jones 2004), so the function $\delta(P)$ is assumed to be increasing. As it has been observed that cellular expansion is not completely dependent on pathogen amount (Antia et al. 2003; Kaech and Ahmed 2001; Stipdonk et al. 2001), the hypothesis that differentiation is not completely dependent on pathogen is also discussed, but remains a more complex mechanism. Indeed, differentiation of naive cells into effector cells is the main process following the encounter of the pathogen by naive cells, so differentiation is released by pathogen and seems to be greatly dependent on its presence. Hence we assume here that, if there is no pathogen, there is no differentiation of naive into effector cells, so $\delta(0)$ is assumed to vanish.

Second, regarding effector cells, the more pathogen, the more effector cell proliferation (Appay and Rowland-Jones 2004; Kemp et al. 2004; Kim et al 2007), so the function $\rho(P)$ is assumed to be increasing. We also suppose $\rho(P)$ nonnegative,

for all P. As told above, cellular expansion is not completely dependent on pathogen amount (Antia et al. 2003; Kaech and Ahmed 2001; Stipdonk et al. 2001). In particular, the end of the response does not correspond strictly to elimination of pathogen. Hence we can assume that $\rho(0)$ is positive, that is to say proliferation of effector cells can occur even if pathogen has been removed. Regarding effector cell death, the more effector cells, the more important their action of killer cells on their own population (Guarda et al 2007; Kemp et al. 2004; Su et al. 1993) and the more important the death rate $\mu_E(E)$, so $\mu_E(E)$ is assumed to be increasing. We also define the natural death rate of effector cells as a positive constant μ_E^0, so that even in absence of cytotoxic activity, effector cells can die, that is to say $\mu_E(0) = \mu_E^0$. This yields $\mu_E(E) \geq \mu_E^0$ for all E. In addition, older cells are more enclined to differentiate in memory cells. This follows the hypothesis of a linear model of differentiation, in which cells become effector before differentiating into memory cells (Appay and Rowland-Jones 2004; Bannard etal. 2009; Jenkins et al. 2008; Sprent and Surh 2001). Hence we suppose the function $k : \tau \in [0, \bar{\tau}) \mapsto k(\tau)$ positive and increasing on $[0, \bar{\tau})$. All effector cells should have died or differentiated in memory cells at age $\bar{\tau}$, so we also suppose

$$\int_0^{\bar{\tau}} k(\tau)\, d\tau = +\infty.$$

Finally, regarding pathogen amount, the more effector cells, the more important their action of killer cells on pathogen (Antia et al. 2003), so the function $\mu_P(E)$ is increasing. We also define the natural death rate of pathogen as a positive constant μ_P^0, so that even in absence of effector cells, pathogen is eliminated, that is to say $\mu_P(0) = \mu_P^0$. This yields $\mu_P(E) \geq \mu_P^0$ for all E.

System (2) is formed with three nonlinear ordinary differential equations and one nonlinear age-structured partial differential equation. Contrary to the model of Antia et al. (2005), these nonlinearities model the regulation of cell dynamics by real biological feedback controls. As Antia et al. (2005), we consider that differentiation of effector cells into memory cells is dependent on effector cell age, even though the dependency is not completely similar.

In the following, we reduce Eq. (2b) to a delay differential equation with a distributed delay. Thus we will work on a system based on evolution of total number of cells, in particular for effector cells. Such a reduction is relevant, since total number of cells corresponds to quantities which can be measured experimentally. Hence the reduced model will be better confronted with experimental results. We can also notice that Eq. (2d) describing evolution of memory cells is not coupled with other equations and its dynamics have no influence on dynamics of the other cell populations. Hence we will not consider it in the following study and we will only focus on Eqs. (2a), (2b) and (2c).

3 Reduction to a delay differential system

We use the method of characteristics (Webb 1985) to reduce Eq. (2b) to a delay differential equation. We integrate Eq. (2b) over the age, with boundary conditions (3), to obtain:

$$\frac{dE(t)}{dt} = [\rho(P(t)) - \mu_E(E(t))]\,E(t) + \delta(P(t))N(t) - \int_0^{\bar{\tau}} k(\tau)e(t,\tau)\,d\tau, \quad (4)$$

where $E(t)$ is expressed by (1).

We can explicitly write the term $e(t,\tau)$ in (4) as a function of $E(t)$, $N(t)$ and $P(t)$, by using the method of characteristics and Eq. (2b). Characteristic curves of Eq. (2b) are given by

$$\begin{cases} \dfrac{d\tau}{dt}(t) = 1, \\ \\ \tau(0) = \tau_0, \quad \tau_0 \in \mathbb{R}. \end{cases}$$

We set

$$v(t) = e(t,\tau(t)) = e(t,t+\tau_0), \quad \text{for} \quad t \geq t_0 := \max\{0,-\tau_0\}.$$

Then, using Eq. (2b),

$$\frac{dv}{dt}(t) = [\rho(P(t)) - \mu_E(E(t)) - k(t+\tau_0)]\,v(t).$$

We solve this equation to obtain

$$v(t) = v(t_0)\exp\left(\int_{t_0}^{t} [\rho(P(s)) - \mu_E(E(s)) - k(s+\tau_0)]\,ds\right),$$

where, when $\tau_0 = \tau - t > 0$,

$$v(t_0) = e_0(\tau_0),$$

and when $\tau_0 = \tau - t \leq 0$, from (3a),

$$v(t_0) = e(-\tau_0, 0) = \delta(P(-\tau_0))N(-\tau_0).$$

Since $\tau_0 = \tau - t$ and using the change of variable $s \rightsquigarrow s + \tau - t$ in the first integral term, we finally obtain, for $t < \tau$:

$$e(t,\tau) = e_0(\tau - t)\exp\left(\int_0^t [\rho(P(s)) - \mu_E(E(s))]\,ds - \int_{\tau-t}^{\tau} k(s)\,ds\right),$$

and for $t \geq \tau$:

$$e(t,\tau) = \delta(P(t-\tau))N(t-\tau)\exp\left(\int_{t-\tau}^{t}[\rho(P(s))-\mu_E(E(s))]\,ds - \int_0^{\tau} k(s)\,ds\right). \tag{5}$$

We deduce the equation satisfied by $E(t)$ depending only on the total counts of populations $E(t)$, $N(t)$, $P(t)$, from (4),

$$\begin{aligned}\frac{dE(t)}{dt} &= [\rho(P(t)) - \mu_E(E(t))]E(t) + \delta(P(t))N(t)\\ &\quad - \begin{cases} \displaystyle\int_0^t \delta(P(t-\tau))N(t-\tau)\exp\left(\int_{t-\tau}^{t}[\rho(P(s)) - \mu_E(E(s))]\,ds\right) f(\tau)\,d\tau \\ \quad \displaystyle + \exp\left(\int_0^t [\rho(P(s))-\mu_E(E(s))]\,ds\right)\int_t^{\bar\tau} e_0(\tau-t)K(t,\tau)\,d\tau, & \text{if } 0 \le t \le \bar\tau, \\ \displaystyle\int_0^{\bar\tau} \delta(P(t-\tau))N(t-\tau)\exp\left(\int_{t-\tau}^{t}[\rho(P(s))-\mu_E(E(s))]\,ds\right) f(\tau)\,d\tau, & \text{if } \bar\tau \le t, \end{cases}\end{aligned} \tag{6}$$

where f is defined for $\tau > 0$ by

$$f(\tau) = k(\tau)\exp\left(-\int_0^{\tau} k(s)\,ds\right),$$

and K is defined by

$$K(t,\tau) = \begin{cases} f(\tau)\exp\left(\displaystyle\int_0^{\tau-t} k(s)\,ds\right), & \text{if } t < \tau, \\ f(\tau), & \text{if } t \ge \tau. \end{cases}$$

One can note that f is a density with support $[0, \bar\tau]$.

In Eq. (6), differentiation in memory cells of effector cells with age τ at time t is expressed by the last term on the right hand side. When $t \le \bar\tau$, initial number of cells $e_0(\tau)$ is consumed to generate memory cells, so memory cells are produced both by differentiation of the initial condition $e_0(\tau)$ and differentiation of "new" effector cells at the same time (this latter event produces the delayed term $\delta(P(t-\tau))N(t-\tau)$). However, when $t \ge \bar\tau$, initial condition is totally consumed and memory cells can appear only from differentiation of other cells, that is effector cells coming from the differentiation of naive cells. We can also note that the exponential term acts as a survival rate, and effector cells differentiate with a distribution $f(\tau)$.

Finally, $N(t)$, $E(t)$ and $P(t)$ satisfy the following system:

$$\frac{dN}{dt}(t) = H - \mu_N N(t) - \delta(P(t))N(t), \tag{7a}$$

$$\frac{dE(t)}{dt} = [\rho(P(t)) - \mu_E(E(t))]E(t) + \delta(P(t))N(t) - \int_0^t \delta(P(t-\tau))N(t-\tau)\exp\left(\int_{t-\tau}^t [\rho(P(s)) - \mu_E(E(s))]\,ds\right) f(\tau)\,d\tau - \exp\left(\int_0^t [\rho(P(s)) - \mu_E(E(s))]\,ds\right)\int_t^{\bar\tau} e_0(\tau - t)K(t,\tau)\,d\tau, \tag{7b}$$

$$\frac{dP}{dt}(t) = I(t) - \mu_P(E(t))P(t), \tag{7c}$$

if $0 \le t \le \bar\tau$, and

$$\frac{dN}{dt}(t) = H - \mu_N N(t) - \delta(P(t))N(t), \tag{8a}$$

$$\frac{dE}{dt}(t) = [\rho(P(t)) - \mu_E(E(t))]E(t) + \delta(P(t))N(t) - \int_0^{\bar\tau} \delta(P(t-\tau))N(t-\tau)\exp\left(\int_{t-\tau}^t [\rho(P(s)) - \mu_E(E(s))]\,ds\right) f(\tau)\,d\tau, \tag{8b}$$

$$\frac{dP}{dt}(t) = I(t) - \mu_P(E(t))P(t), \tag{8c}$$

if $\bar\tau \le t$, with initial conditions

$$N(0) = N_0, \quad E(0) = E_0 := \int_0^{\bar\tau} e_0(\tau)\,d\tau, \quad P(0) = P_0. \tag{9}$$

In the following, we will mathematically study this system, to verify existence and uniqueness of solutions and to determine existence and stability of steady states.

4 Existence and uniqueness of solutions and steady states

We now introduce mathematical results for the system (7)–(8). First, we can verify existence and uniqueness of solutions for this system.

Proposition 1 *Suppose that functions μ_E, δ, μ_P, ρ are bounded on $[0, +\infty)$ respectively by $\bar{\mu_E}$, $\bar\delta$, $\bar{\mu_P}$, $\bar\rho$. We also suppose they are Lipschitz functions. Finally*

we suppose $I \geq 0$ *bounded by* $\bar{I}$. *For any initial condition* (N_0, E_0, P_0) *satisfying* (9), *system* (7)–(8) *has only one solution on* $[0, +\infty)$, *denoted by* $(N(t), E(t), P(t))$, *and this solution is bounded.*

Proof From Hale and Verduyn Lunel (1993), for each continuous initial condition, system (7)–(8) has a continuous maximal solution $(N(t), E(t), P(t))$, well-defined for $t \in [0, T)$. We can prove that this solution is bounded.

We consider a solution $(N(t), E(t), P(t))$ of system (7)–(8), defined on $[0, T)$. We can suppose that $T > \bar{\tau}$. Then, it is straightforward, from (8a), that, for all $t \in [0, T)$,

$$|N(t)| \leq |N(0)| + \frac{H}{\mu_N} := C_N.$$

We have also, integrating (8b) between $\bar{\tau}$ and t,

$$\begin{aligned} E(t) = {} & \exp\left(\int_{\bar{\tau}}^{t} \rho(P(\theta))\,d\theta\right) E(\bar{\tau}) + \int_{\bar{\tau}}^{t} \exp\left(\int_{u}^{t} \rho(P(\theta))\,d\theta\right) \delta(P(u))N(u)\,du \\ & - \int_{\bar{\tau}}^{t} \exp\left(\int_{u}^{t} \rho(P(\theta))\,d\theta\right) \mu_E(E(u))E(u)\,du \\ & - \int_{\bar{\tau}}^{t} \exp\left(\int_{u}^{t} \rho(P(\theta))\,d\theta\right) \\ & \left[\int_{0}^{\bar{\tau}} \delta(P(u-\tau))N(u-\tau) \exp\left(\int_{u-\tau}^{u} [\rho(P(s)) - \mu_E(E(s))]\,ds\right) f(\tau)\,d\tau\right] du. \end{aligned}$$

We obtain, for all $t \in [\bar{\tau}, T)$,

$$|E(t)| \leq (|E(\bar{\tau})| + \alpha) \exp((\bar{\mu} + \bar{\rho})(T - \bar{\tau})),$$

where

$$\alpha := \frac{\bar{\delta} C_N}{\bar{\rho}} \left[1 + \int_{0}^{\bar{\tau}} \exp(\bar{\rho}\tau) f(\tau)\,d\tau\right].$$

Finally, from (8c), we get, for all $t \in [0, T)$,

$$|P(t)| \leq |P(0)| + \bar{I} \exp(\bar{\mu_P} T) T.$$

Hence the solutions $(N(t), E(t), P(t))$ of the system (7)–(8) are bounded on $[0, T)$ with

$$\lim_{t\to T}(N(t), E(t), P(t)) < +\infty.$$

Finally, from Hale and Verduyn Lunel (1993), since the maximal solution of the system (7)–(8) is bounded on $[0, T)$ and $\lim_{t\to T}(N(t), E(t), P(t)) < +\infty$, we conclude that this solution is global, and we can prove this solution is unique for $t \geq 0$. □

In the following, we take $I \equiv 0$: we focus on the particular case of a nonproliferating pathogen, as in a vaccine injection for example. System (8) is now autonomous and we can study existence and stability of steady states for this system.

A solution $(\bar{N}, \bar{E}, \bar{P})$ of system (8) is a steady state if and only if

$$\frac{d\bar{N}}{dt} = \frac{d\bar{E}}{dt} = \frac{d\bar{P}}{dt} = 0.$$

So, from (8), $(\bar{N}, \bar{E}, \bar{P})$ is a steady state if and only if

$$(\mu_N + \delta(\bar{P}))\bar{N} = H, \tag{10a}$$

$$\left[\rho(\bar{P}) - \mu_E(\bar{E})\right]\bar{E} = \left(\int_0^{\bar{\tau}} \exp([\rho(\bar{P}) - \mu_E(\bar{E})]\tau) f(\tau)\, d\tau - 1\right)\delta(\bar{P})\bar{N}, \tag{10b}$$

$$\mu_P(\bar{E})\bar{P} = 0. \tag{10c}$$

From (10c), $\mu_P(\bar{E}) = 0$ or $\bar{P} = 0$. Since we supposed that $\mu_P(\bar{E}) > 0$, then $\bar{P} = 0$.

We also supposed $\delta(0) = 0$. Then (10b) becomes:

$$(\rho(0) - \mu_E(\bar{E}))\bar{E} = 0.$$

Hence, in a first case, $\bar{E} = 0$. In a second case, $\mu_E(\bar{E}) = \rho(0)$. We assumed $\mu_E(E) > 0$ for all E and μ_E is increasing, so there exists a unique $E^* > 0$ such that $\mu_E(E^*) = \rho(0)$ if and only if

$$\mu_E^- > \rho(0) > \mu_E(0). \tag{11}$$

In all cases, we determine $\bar{N}$ from (10a). Since $\bar{P} = 0$ and $\delta(0) = 0$, then $\bar{N} = H/\mu_N$. Finally we obtain the following result,

Proposition 2 *If $\rho(0) \leq \mu_E(0)$, system (8) has a unique steady state, $(\bar{N}, \bar{E}, \bar{P}) = (H/\mu_N, 0, 0)$, and if $\rho(0) > \mu_E(0)$, system (8) has two steady states, $(\bar{N}, \bar{E}, \bar{P}) = (H/\mu_N, 0, 0)$ and $(\bar{N}, \bar{E}, \bar{P}) = (H/\mu_N, E^*, 0)$, where $E^* = \mu_E^{-1}(\rho(0)) > 0$.*

We assume the first inequality in (11), $\mu_E^- > \rho(0)$, is always satisfied. Indeed, if $\rho(0) > \mu_E^-$ then proliferation always exceeds apoptosis for effector cells, hence it

becomes impossible to observe an immune response with its typical contraction phase and the model's behavior is not biologically realistic.

From a biological point of view, Proposition 2 indicates that if, in the absence of pathogens, proliferation rate of effector cells is lower than their natural death rate, then the only steady state for system (8) corresponds to extinction of effector cell population. This steady state also exists if, in the absence of pathogen, proliferation rate of effector cells is greater than their natural death rate. Yet, in this second case, an other steady state appears, in which effector cell population is still present and does not completely die out. However, in the two cases, pathogen is completely eliminated and naive cells remain because of a constant production by hematopoietic stem cells. We can finally note that, from Eq. (2d) for memory cells $M(t)$, using (5), we obtain, for $t \geq \bar{\tau}$,

$$\frac{dM}{dt}(t) = -\mu_M M(t) + \int_0^{\bar{\tau}} \delta(P(t-\tau))N(t-\tau) \times \exp\left(\int_{t-\tau}^{t} [\rho(P(s)) - \mu_E(E(s))]\, ds\right) f(\tau)\, d\tau.$$

Hence, since $\bar{P} = 0$ and $\delta(0) = 0$, a solution $\bar{M}$ of this equation is a steady state if and only if

$$\mu_M \bar{M} = 0.$$

This yields that the only steady state for memory cell population is $\bar{M} = 0$, which corresponds to memory cell extinction. It is not a contradiction with generation of memory cells, useful in a second infection by the same pathogen, because despite their long-lived property memory cells die like other cells, at a natural death rate denoted by μ_M here. Hence, on a long term (asymptotically), memory cells are not expected to survive.

In the next section, we analyze the local asymptotic stability of the steady states.

5 Local asymptotic stability of steady states

We can now analyze the asymptotic behavior ot the solutions of system (8) by studying the local asymptotic stability of its steady states. Let $(\bar{N}, \bar{E}, \bar{P})$ be a steady state of system (8), defined in Proposition 2. We assume that all functions in system (8) are continuously differentiable. The linearized system of (8) around $(\bar{N}, \bar{E}, \bar{P})$ is then

$$\frac{dN}{dt}(t) = -\mu_N N(t) - \delta(\bar{P})N(t) - \delta'(\bar{P})\bar{N}P(t),$$
$$\frac{dP}{dt}(t) = -\mu_P(\bar{E})P(t) - \bar{P}\mu'_P(\bar{E})E(t),$$

$$\frac{dE}{dt}(t) = \left[\rho(\bar{P}) - \mu_E(\bar{E}) - \bar{E}\mu'_E(\bar{E})\right] E(t) + \delta(\bar{P})N(t) + \left[\bar{E}\rho'(\bar{P}) + \bar{N}\delta'(\bar{P})\right] P(t)$$
$$- \int_0^{\bar{\tau}} f(\tau) \exp\left((\rho(\bar{P}) - \mu_E(\bar{E}))\tau\right) \Bigg[\delta(\bar{P})N(t-\tau) + \bar{N}\delta'(\bar{P})P(t-\tau)$$
$$+ \delta(\bar{P})\bar{N} \int_{-\tau}^{0} \rho'(\bar{P})P(s-t) - \mu'_E(\bar{E})E(s-t)\, ds \Bigg] d\tau, \tag{12}$$

where we still use, for the sake of simplicity, $N(t)$, $E(t)$ and $P(t)$ instead of $N(t) - \bar{N}$, $E(t) - \bar{E}$ and $P(t) - \bar{P}$. Since $\bar{P}$ is equal to zero for all steady states, and as we assumed $\delta(0) = 0$, then $\delta(\bar{P}) = 0$ in (12). The system (12) can be rewritten as

$$\frac{dX}{dt}(t) = AX(t) - \int_0^{\bar{\tau}} g(\tau) B X(t-\tau)\, d\tau,$$

where $g(\tau) = f(\tau)\exp([\rho(0) - \mu_E(\bar{E}])\tau)$, $X(t) = (N(t), E(t), P(t))^T$, and

$$A = \begin{pmatrix} -\mu_N & -\delta'(0)\bar{N} & 0 \\ 0 & -\mu_P(\bar{E}) & 0 \\ 0 & \bar{E}\rho'(0) + \bar{N}\delta'(0) & \rho(0) - \mu_E(\bar{E}) - \bar{E}\mu'_E(\bar{E}) \end{pmatrix},$$

and

$$B = \begin{pmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & \bar{N}\delta'(0) & 0 \end{pmatrix}.$$

The characteristic equation associated with system (12) is then defined by

$$\det\left(\lambda I_3 - A + \int_0^{\bar{\tau}} e^{-\lambda\tau} g(\tau) B\, d\tau\right) = 0,$$

where $\lambda \in \mathbb{C}$ and I_3 is the identity matrix in $\mathbb{R}^3$. After calculations, this equation reduces to

$$(\lambda + \mu_N)(\lambda + \mu_P(\bar{E}))(\lambda - \rho(0) + \mu_E(\bar{E}) + \bar{E}\mu'_E(\bar{E})) = 0. \tag{13}$$

We recall that the steady state $(\bar{N}, \bar{E}, \bar{P})$ of (8) is locally asymptotically stable if all eigenvalues of (13) have negative real parts, and is unstable when eigenvalues with positive real parts exist (Hale and Verduyn Lunel 1993). All eigenvalues of (13) are

real, defined by

$$\lambda_1 = -\mu_N, \quad \lambda_2 = -\mu_P(\bar{E}), \quad \lambda_3 = \rho(0) - \mu_E(\bar{E}) - \bar{E}\mu'_E(\bar{E}).$$

We assumed $\mu_N > 0$ and $\mu_P(E) > 0$ for all E, hence λ_1 and λ_2 are negative whatever the value of $\bar{E}$.

By Proposition 2, if $\rho(0) < \mu_E(0)$, then $\bar{E} = 0$ is the unique steady state, and $\lambda_{3,0} = \rho(0) - \mu_E(0) < 0$, so the steady state $(\bar{N}, \bar{E}, \bar{P}) = (H/\mu_N, 0, 0)$ is locally asymptotically stable.

On the other hand, if $\rho(0) > \mu_E(0)$, then $\bar{E} = 0$ and $\bar{E} = E^*$ are the two possible steady states. In this case, one gets $\lambda_{3,0} = \rho(0) - \mu_E(0)$ and $\lambda_{3,*} = \rho(0) - \mu_E(E^*) - E^*\mu'_E(E^*)$. Since $\rho(0) > \mu_E(0)$, we have immediatly $\lambda_{3,0} > 0$, so the linearised system about $\bar{E} = 0$ has a positive eigenvalue.

When $\rho(0) > \mu_E(0)$, we have $\mu_E(E^*) = \rho(0)$, hence $\lambda_{3,*} = -E^*\mu'_E(E^*)$. Furthermore, $\mu_E(E)$ is increasing, so $\mu'_E(E) > 0$ for all E, and we finally obtain $\lambda_{3,*} < 0$. This yields that when $\bar{E} = E^*$, all eigenvalues of (13) are negative.

These results lead to the following conclusion on asymptotic behavior of the solutions of system (8).

Proposition 3 *The steady state* $(\bar{N}, \bar{E}, \bar{P}) = (H/\mu_N, 0, 0)$ *is unstable if* $\rho(0) > \mu_E(0)$*, and locally asymptotically stable if* $\rho(0) < \mu_E(0)$*. The steady state* $(\bar{N}, \bar{E}, \bar{P}) = (H/\mu_N, E^*, 0)$*, which exists only if* $\rho(0) > \mu_E(0)$*, is locally asymptotically stable.*

Biologically, this indicates that if, in the absence of pathogen, proliferation rate of effector cells is lower than their natural death rate, then effector cells and pathogen go to extinction, whereas naive cells reach on a long term a steady state. It can be interpreted as the end of infection, with a return to a healthy organism. On the contrary, if proliferation rate of effector cells is greater than their natural death rate, the previous steady state becomes unstable. Moreover, the new steady state which appears, with effector cells still present despite elimination of pathogen, is locally asymptotically stable. It can also be interpreted as the end of infection, because pathogen is also eliminated in this case, but with an amount of effector cells which does not disappear. It is a case less natural than the previous one, in our context of acute infection, in which specific cells of immune response like effector cells should disappear after elimination of disease.

When $\rho(0) = \mu_E(0)$, then, for $\bar{E} = 0$, $\lambda_{3,0} = 0$ is an eigenvalue. From (13), this eigenvalue is simple, so $(\bar{N}, \bar{E}, \bar{P}) = (H/\mu_N, 0, 0)$ is locally stable in this case, but not locally asymptotically stable. Further analysis would be necessary to analyse the asymptotic stability, yet since this case is unlikely to be biologically realistic, we will not go deeper in such an analysis.

From system (10) and from characteristic equation calculated above, we have existence and stability of steady states for the system (8). This result deals with the local asymptotic stability of system (8) endowed with general initial conditions defined on the interval $[0, \bar{\tau}]$. In the next section, we focus on the global asymptotic stability of system (7)–(8), that is we endow system (8) with particular initial conditions, described

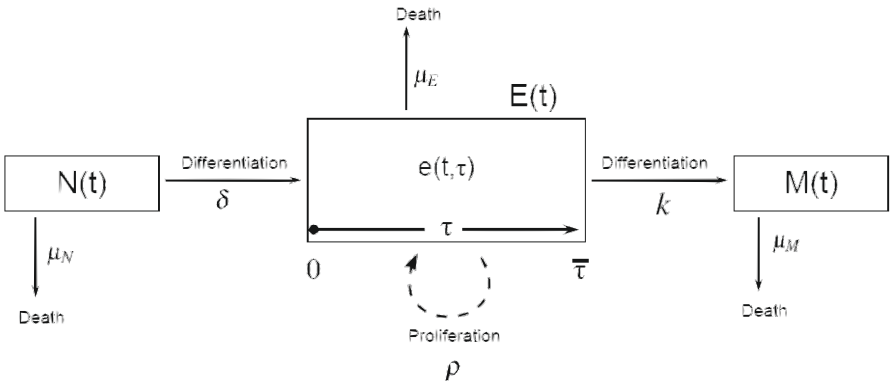

Fig. 3 Simplified model of the T CD8 cell immune response with a discrete delay

in system (7), corresponding to the biological situation we aim at modeling. In the following, since above stability and existence results are independent of the nature of the delay, we simplify the system without losing its properties, considering $\bar{\tau}$ as an average age at which effector cells differentiate and no more as a limit age (see Fig. 3). In the delay differential system, we obtain a discrete delay $\bar{\tau}$ in spite of a distributed delay.

6 Global asymptotic stability

As in the previous analysis, we assume $I \equiv 0$. We modify system (7)–(8) to obtain a system with a discrete delay. Then we determine an expression of $E(t)$ solution of the effector cell equation. Thus we obtain a useful expression to study global asymptotic stability of a steady state of the system, in which effector cells go to extinction after eliminating pathogen. System (7)–(8) with a discrete delay $\bar{\tau}$ becomes

$$\frac{dN}{dt}(t) = H - \mu_N N(t) - \delta(P(t))N(t), \tag{14a}$$

$$\frac{dE}{dt}(t) = \Big[\rho(P(t)) - \mu_E(E(t))\Big]E(t) + \delta(P(t))N(t) - \exp\left(\int_0^t [\rho(P(u)) - \mu_E(E(u))]\,du\right) e_0(\bar{\tau} - t), \quad \text{if} \quad 0 \le t < \bar{\tau}, \tag{14b}$$

$$\frac{dE}{dt}(t) = \Big[\rho(P(t)) - \mu_E(E(t))\Big]E(t) + \delta(P(t))N(t) - \exp\left(\int_{t-\bar{\tau}}^t [\rho(P(u)) - \mu_E(E(u))]\,du\right) \delta(P(t-\bar{\tau}))N(t-\bar{\tau}), \quad \text{if} \quad \bar{\tau} \le t, \tag{14c}$$

$$\frac{dP}{dt}(t) = -\mu_P(E(t))P(t). \tag{14d}$$

with initial conditions (9). One can note that, contrary to system (7)–(8), the condition

$$e_0(0) = \delta(P_0)N_0$$

must be added to obtain continuity of the derivative of E for $t = \bar{\tau}$.

Let $(N(t), E(t), P(t))$ be the unique solution of (9) and (14). Integrating (14b), for all $t \in [0, \bar{\tau}]$, we obtain

$$\begin{aligned} E(t) = &\exp\left(\int_0^t [\rho(P(u)) - \mu_E(E(u))]\,du\right) E(0) \\ &+ \int_0^t \left[\delta(P(s))N(s)\exp\left(\int_s^t [\rho(P(u)) - \mu_E(E(u))]\,du\right)\right. \\ &\left. - \exp\left(\int_0^t [\rho(P(u)) - \mu_E(E(u))]\,du\right) e_0(\bar{\tau} - s)\right] ds. \end{aligned}$$

Since, from (9), $E(0) = \int_0^{\bar{\tau}} e_0(\tau)\,d\tau$, and using the change of variable $\tau = \bar{\tau} - s$ in the last term of the equality, we have, for all $t \in [0, \bar{\tau}]$,

$$\begin{aligned} E(t) = &\exp\left(\int_0^t [\rho(P(u)) - \mu_E(E(u))]\,du\right) \int_0^{\bar{\tau}-t} e_0(\tau)\,d\tau \\ &+ \int_0^t \delta(P(s))N(s)\exp\left(\int_s^t [\rho(P(u)) - \mu_E(E(u))]\,du\right) ds. \end{aligned} \tag{15}$$

We still denote $E(t)$ the unique solution of (14b)–(14c), given by (15) on the interval $[0, \bar{\tau}]$. We define, for all $t \geq \bar{\tau}$,

$$V(t) = \int_{t-\bar{\tau}}^t \delta(P(s))N(s)\exp\left(\int_s^t [\rho(P(u)) - \mu_E(E(u))]\,du\right) ds. \tag{16}$$

From the properties of the different functions δ, ρ, μ_E, μ_P, which are supposed continuously differentiable, the function $(t, s) \mapsto \delta(P(s))N(s)\exp\left(\int_s^t [\rho(P(u)) - \mu_E(E(u))]\,du\right)$ is continuous with respect to t and s, and differentiable with respect to t, so $V(t)$ is differentiable for $t \geq \bar{\tau}$. Hence we obtain, for all $t \geq \bar{\tau}$,

$$\begin{aligned} \frac{dV}{dt}(t) = &[\rho(P(t)) - \mu_E(E(t))]V(t) + \delta(P(t))N(t) \\ &-\delta(P(t-\bar{\tau}))N(t-\bar{\tau})\exp\left(\int_{t-\bar{\tau}}^t [\rho(P(u)) - \mu_E(E(u))]\,du\right). \end{aligned}$$

And using (14c), for all $t \geq \bar{\tau}$,

$$\frac{d}{dt}(V-E)(t) = [\rho(P(t)) - \mu_E(E(t))](V-E)(t),$$

so, for all $t \geq \bar{\tau}$,

$$V(t) = E(t) + [V(\bar{\tau}) - E(\bar{\tau})] \exp\left(\int_{\bar{\tau}}^{t} [\rho(P(u)) - \mu_E(E(u))]\, du\right).$$

From (16),

$$V(\bar{\tau}) = \int_0^{\bar{\tau}} \delta(P(s))N(s) \exp\left(\int_s^{\bar{\tau}} [\rho(P(u)) - \mu_E(E(u))]\, du\right) ds,$$

and by (15)

$$E(\bar{\tau}) = \int_0^{\bar{\tau}} \delta(P(s))N(s) \exp\left(\int_s^{\bar{\tau}} [\rho(P(u)) - \mu_E(E(u))]\, du\right) ds = V(\bar{\tau}).$$

So, for all $t \geq \bar{\tau}$, $V(t) = E(t)$. We finally obtain an expression of $E(t)$, for all $t \geq 0$,

$$\begin{cases} E(0) = \displaystyle\int_0^{\bar{\tau}} e_0(\tau)\, d\tau, \\ E(t) = \exp\left(\displaystyle\int_0^t [\rho(P(u)) - \mu_E(E(u))]\, du\right) \displaystyle\int_0^{\bar{\tau}-t} e_0(\tau)\, d\tau \\ \qquad + \displaystyle\int_0^t \delta(P(s))N(s) \exp\left(\int_s^t [\rho(P(u)) - \mu_E(E(u))]\, du\right) ds, \quad \text{for} \quad t \in [0, \bar{\tau}], \\ E(t) = \displaystyle\int_{t-\bar{\tau}}^t \delta(P(s))N(s) \exp\left(\int_s^t [\rho(P(u)) - \mu_E(E(u))]\, du\right) ds, \quad \text{for} \quad t \geq \bar{\tau}. \end{cases} \tag{17}$$

We can note that this is not an explicit expression of $E(t)$, yet it defines $E(t)$ as the solution of a fixed point problem. This expression is useful to prove that

$$\lim_{t \to +\infty} E(t) = 0,$$

and we can finally prove the following result.

Proposition 4 *The solution* $(N(t), E(t), P(t))$ *of system* (14)*, with any non-negative initial condition* (N_0, E_0, P_0) *given by* (9)*, converges to the steady state* $(H/\mu_N, 0, 0)$.

Proof First, from (14d), $\lim_{t\to+\infty} P(t) = 0$. Second, from (14a), since $\lim_{t\to+\infty} P(t) = 0$ and $\delta(0) = 0$, then $\lim_{t\to+\infty} N(t) = H/\mu_N$. Finally, we prove that $\lim_{t\to+\infty} E(t) = 0$.

By (17), for all $t \geq \bar{\tau}$,

$$E(t) = \int_0^{\bar{\tau}} \delta(P(t-s))N(t-s)\exp\left(\int_0^s [\rho(P(t-u)) - \mu_E(E(t-u))]\,du\right) ds,$$

therefore, for all $t \geq \bar{\tau}$,

$$|E(t)| \leq \int_0^{\bar{\tau}} \delta(P(t-s))N(t-s)\exp(\bar{\rho}s)\,ds.$$

We assumed $\delta(0) = 0$, this yields

$$\lim_{t\to+\infty} \int_0^{\bar{\tau}} \delta(P(t-s))N(t-s)\exp(\bar{\rho}s)\,ds = 0,$$

which proves that

$$\lim_{t\to+\infty} E(t) = 0.$$

We proved that for $N_0 \geq 0$, $E_0 \geq 0$ and $P_0 \geq 0$, the solution $(N(t), E(t), P(t))$ of system (14) tends to the steady state $(H/\mu_N, 0, 0)$. □

When we focused on local asymptotic stability of the steady states in Sect. 5, we studied system (8) endowed with general initial conditions defined on the interval $[0, \bar{\tau}]$. In this general situation, we obtained two steady states whose local asymptotic stability is dependent on the sign of $\rho(0) - \mu_E(0)$. Above, we focused on the global asymptotic stability of system (7)–(8), that is we endowed system (8) with the particular initial conditions described in (7). This particular case really corresponds to the biological situation of the immune response we want to model. Consequently, the convergence result obtained in Proposition 4 does not depend on the sign of $\rho(0) - \mu_E(0)$, which may appear, at first, confusing, but only describes a situation in which there is no other steady state than the trivial one $(H/\mu_N, 0, 0)$, due to the particular initial conditions considered in the analysis.

Biologically, we have an acute infection which does not destabilize the system on a long period of time, but only represents a perturbation, ended when pathogen is eliminated and when other populations come back to a state corresponding to a healthy organism. Hence, system (7)–(8) is able to correctly reproduce qualitatively a primary CD8 T cell response to a non-proliferative infection. In the next section, we

illustrate how this model can reproduce quantitatively the T CD8 kinetics, coherent with experimental data found in the literature.

7 Simulations

We present here the results of simulations for the model presented in Sect. 6. We use parameters able to reproduce data found in the literature (Murali-Krishna et al. 1998). The goal of this approach is to study how the model can reproduce a CD8 T cell immune response, characterized by its kinetics with the expansion and contraction phases, generation of memory cells and elimination of pathogen. The parameters used in simulations are described as follows.

The delay $\bar{\tau}$ is discrete. Only the flow H of naive cells produced from hematopoietic stem cells, death rate of naive cells μ_N and death rate of memory cells μ_M are constant. The death rate of effector cells μ_E, their proliferation rate ρ, the rate δ of differentiation of naive cells into effector cells, and the pathogen death rate μ_P are taken as Hill functions, that is, bounded, positive functions, according to their dependencies on the different populations, and can be denoted

$$\delta(P) = \delta_1 \frac{P^{\delta_2}}{P^{\delta_2} + \delta_3}, \quad \rho(P) = \rho_0 + \rho_1 \frac{P^{\rho_2}}{P^{\rho_2} + \rho_3},$$
$$\mu_E(E) = \mu_E^0 + \mu_{E1} \frac{E^{\mu_{E2}}}{E^{\mu_{E2}} + \mu_{E3}}, \quad \text{and} \quad \mu_P(E) = \mu_P^0 + \mu_{P1} \frac{P^{\mu_{P2}}}{P^{\mu_{P2}} + \mu_{P3}},$$

where the values of parameters H, μ_N, μ_M, constants ρ_0, μ_E^0, μ_P^0, δ_i, ρ_i, μ_{Ei}, μ_{Pi} (i = 1, 2, 3), and discrete delay $\bar{\tau}$ are given in Table 1. These values have been determined to fit correctly the data from Murali-Krishna et al. (1998), but there was no systematic investigation of parameters to determine the best values able to fit the data.

We use experimental data given by Murali-Krishna et al. (1998), displayed in Fig. 4. BALB/c mice were infected with lymphocytic choriomeningitis virus. CD8 T cells specific for lymphocytic choriomeningitis virus are counted at days indicated on Fig. 4, in the spleen of mice. The authors obtain an expansion phase between days 1 and 8 post-infection, from about 4×10^2 cells to $2.8 \pm 1.0 \times 10^7$ cells at the peak of response. Then, between days 9 and 20 post-infection, a contraction phase occurs, during which CD8 T cell population switches from the peak to 1.0×10^6 cells. After these phases, CD8 T cell population keeps on dying out, but a part of the population is relatively maintained on a long term. Indeed, about 5.0×10^5 cells remain at day 400, which is a similar range to cell count at day 30. Hence, from day 30 post-infection, authors consider that remaining CD8 T cells are memory cells.

Kinetics, experimentally obtained by Murali-Krishna et al. (1998), are reproduced with the model presented and mathematically studied in Sect. 6. Results obtained from the model are given in Fig. 4. The total CD8 T cell count, that is $N(t) + E(t) + M(t)$ with notations of the model, is represented by the continuous line on Fig. 4. The same characteristic phases and ranges in time and CD8 T cell counts are obtained. An expansion occurs, with the same ranges than Murali-Krishna et al. (1998) from $10^3 - 10^4$ to $10^7 - 10^8$ CD8 T cells. A contraction follows, during which effector cells

Table 1 Parameter values for simulations (see Figs. 4, 5, 6) of the model described by system (14)

Biological parameter	Associated parameters	Value
Flow of naive cells produced from hematopoietic stem cells (number of cells per day)	H	10
Death rate of naive cells (day^{-1})	μ_N	0.1
Death rate of memory cells (day^{-1})	μ_M	10^{-5}
Differentiation rate of naive cells in effector cells (day^{-1})	δ_1	0.9
	δ_2	2
	δ_3	10^3
Proliferation rate of effector cells (day^{-1})	ρ_0	0.2
	ρ_1	2.1
	ρ_2	2
	ρ_3	10^2
Death rate of effector cells (day^{-1})	μ_E^0	0.2
	μ_{E1}	0.9
	μ_{E2}	1
	μ_{E3}	10^7
Death rate of pathogen (day^{-1})	μ_P^0	0.1
	μ_{P1}	0.7
	μ_{P2}	2.5
	μ_{P3}	10^4
Discrete delay (days)	$\bar{\tau}$	3.5

die by apoptosis and decrease from 10^7 to $10^5 - 10^6$ CD8 T cells. Expansion occurs between days 1 and 7 post-infection and contraction follows. During the response against infection, most of the population is made of effector cells, because of their great differentiation and proliferation rates from a relative small pool of naive cells. Hence during the expansion and contraction phases of the response, total cell count is mainly due to contribution of effector cells (represented by the continuous curve on Fig. 5). After this complete response, as can be observed on Fig. 5, a pool of $10^5 - 10^6$ cells is maintained on a long period of time, up to 400 days post-infection, such as in experimental data of Murali-Krishna et al. (1998). In the model, we observe that generation of memory cells has provided a pool of cells which contributes to the total T cell count mostly after a long time, as this population is still maintained 400 days post-infection, while effector cell population decreases. Moreover, memory cell count becomes greater than effector cell count after 30 days post-infection. This result, dealing with the remaining of memory cells on a long time, is in agreement with Murali-Krishna et al. (1998) explanation. Indeed, they observed that after expansion and contraction phases, that is after day 30 in their data, a memory phase starts, where CD8 T cells still present correspond to a pool of memory cells.

Finally, the model allows to describe not only kinetics for total cell count, but also kinetics per population, naive, effector and memory (see Fig. 5). Effector and

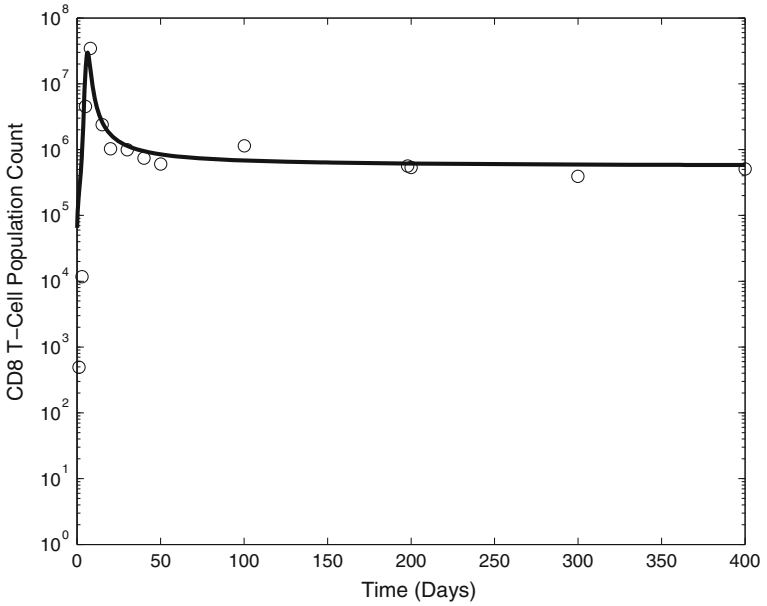

Fig. 4 CD8 T cell immune response on 400 days postinfection. Experimental data, represented by *circles*, correspond to a response to lymphocytic choriomeningitis virus infection, in BALB/c mice (Murali-Krishna et al. 1998). The *straight line* corresponds to simulation of the kinetics of the total CD8 T cell population, $N(t) + E(t) + M(t)$, described by system (14)

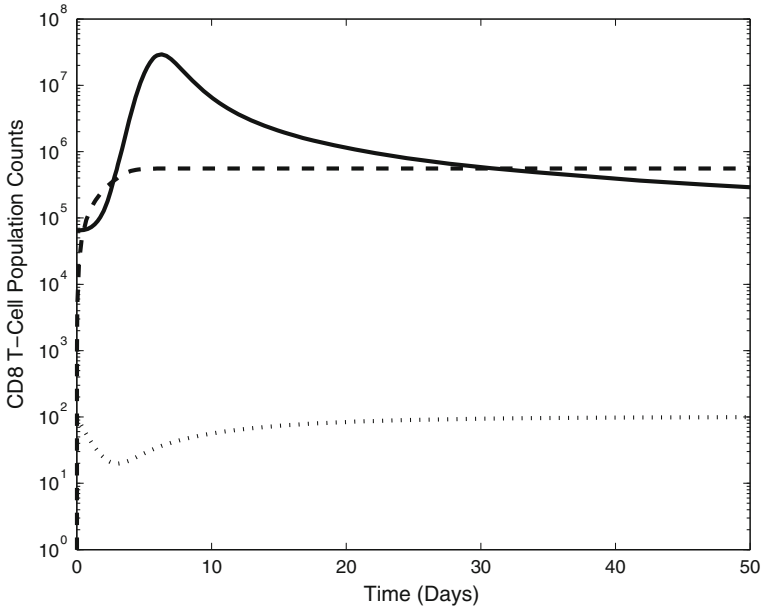

Fig. 5 Simulation of the model described by system (14) on the first 50 days postinfection (zoom of Fig. 4 on the first 50 days). The *dotted line* corresponds to naive cell population kinetic, the *straight line* to effector cell population kinetic and the *dashed line* to memory cell population kinetic

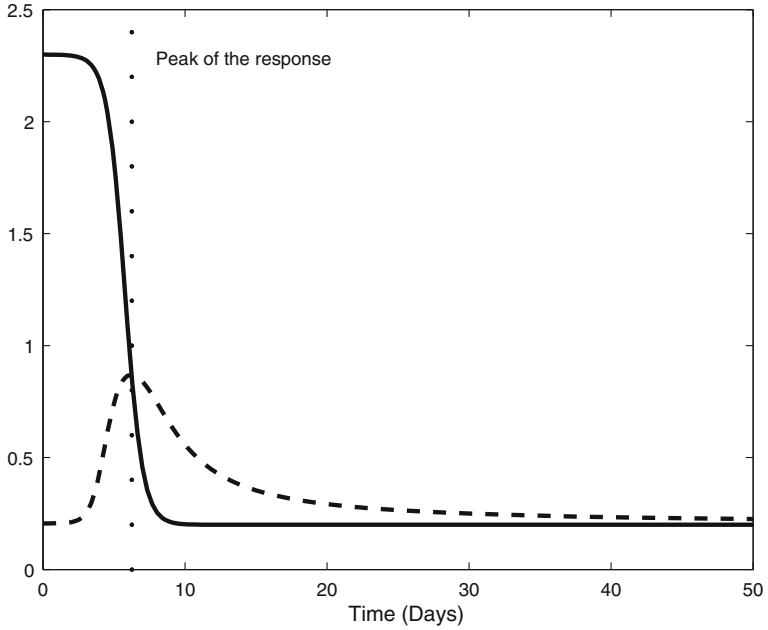

Fig. 6 Proliferation rate of effector cells (*straight line*) and death rate of effector cells (*dashed line*) (day^{-1}) during the first 50 days of the immune response, illustrated on Fig. 4. *Dotted line* points out the switch between the two phases of the response, expansion when proliferation rate of effector cells is greater than their death rate, and contraction when death rate of effector cells is greater than their proliferation rate

memory counts are the main contributions to the total CD8 T cell population. Naive cell population, which is at an equilibrium between production from hematopoietic stem cells and natural death when infection occurs, undergoes a slight decrease during expansion, because of the great differentiation of these cells into effector cells. Then naive population almost returns to its steady state during contraction. Pathogen is eliminated during the time of expansion and contraction (not shown here), this was expected since replication of the virus was not taken into account in the model. Information about the different rates describing evolution of death, differentiation and proliferation of cells during the phases of expansion and contraction of the immune response was also obtained. For example, on Fig. 6, proliferation rate of effector cells, which is assumed to be dependent on pathogen, is at maximum during the first 4 days of the response, that is during the expansion phase, before the effector cell count reaches a maximum on days 6–7 postinfection. Then proliferation rate strongly decreases during the contraction phase until days 8–9 postinfection, after that effector cell count decreases less fast and proliferation rate is maintained at its minimum level. The death rate of effector cells stays low during the beginning of the response, and increases between days 4–6 postinfection when effector cell proliferation is maximum. When effector cell population begins to die strongly, during the contraction phase, its death rate decreases, and is maintained at its minimum level after days 14–15 postinfection. It can be noticed that proliferation rate is greater than death rate during the first 6 days of the response,

and when the maximum of effector cell count is reached death rate becomes greater than proliferation rate, due to the feedback loops included in the model.

8 Discussion

We developed a model of T CD8 immune response to study kinetics of the different populations of CD8 T cells, naive, effector and memory cells, focusing in particular on the importance of generation of memory cells from effector cells (see Fig. 1). This model is based on the model of Antia et al. (2005), with an age-structured system to take into account effector cell dynamics. Contrary to the model of Antia et al. (2005), which is linear, in this model we introduced feedback loops to describe interactions between the different CD8 populations and the pathogen. Hence most of the rates in our model (differentiation, proliferation and death), are nonlinear. We took into account the fact that the response is partly independent of pathogen, which means expansion, with proliferation of effector cells and their regulation, is not completely determined by the amount of pathogen. Antia et al. (2005) modeled generation of memory cells with a fixed age considering that below this age, only naive and effector cells are present in the pool of immune cells, and beyond this fixed age, all cells remaining in the system are memory cells. In our model, we also consider that differentiation of effector cells into memory cells is dependent on effector cell age, but differentiation of an effector cell into memory cell is progressive and the two populations can be produced at the same time, which seems more realistic. For example, recent studies deal with memory precursors, which seem to be present with specific markers at the beginning of the response, eventually as a special effector cell subset, so differentiation of effector cells in memory cells seems to be very progressive and does not begin only after a fixed time (Appay and Rowland-Jones 2004; Jenkins et al. 2008; Sprent and Surh 2001).

We reduced this model to a system with a delay differential equation, and studied basic properties of the solutions. The analysis of existence and local asymptotic stability of steady states, for the system (8), with general initial conditions on $[0, \bar{\tau}]$, brought existence of two steady states. One which leads to extinction of effector cells always exists and can be locally stable or unstable. It corresponds to the complete resolution of an infection, with on a mid-term, two populations remaining in the organism, memory cells and naive cells, and asymptotically, only one population, the naive ones. The second steady state, with a positive state for effector cells, exists only when, in the absence of pathogen, proliferation rate of effector cells is greater than their natural apoptosis rate. In this case, this steady state is also locally stable. Finally, results of local asymptotic stability being independent of the delay, an analysis of global asymptotic stability was performed, with the system simplified by considering a discrete delay instead of a distributed delay (see Figs. 2–3). This analysis showed that the system (8) with particular initial conditions (7) on $[0, \bar{\tau}]$, equivalent to the initial structured system, always converges towards the healthy steady state. Let us briefly comment on the existence and stability of the positive steady state of system (8). This steady state exists (and is stable) under particular conditions, namely that proliferation of effector cells in the absence of pathogen is larger than their death by apoptosis.

Moreover, in a "classical" situation, represented by initial conditions (7), this steady state does not appear. The biological relevance of such a steady state, with persistence of effector cells but not of memory cells, could be investigated. Apparently, such a steady state is not observed during a primary infection, although one could think about a chronic infection, rather than an acute one. However, even during chronic infections the scenario expansion/contraction with generation of memory cells is preserved and we found no clue regarding a sustained effector population (Althaus et al. 2007). Consequently, this steady state may be relevant during a secondary immune response, when the immune system starts to react with levels of effector and memory cells low but non-zero. This should be further investigated.

This model allows to provide simulations we have confronted with experimental data (Murali-Krishna et al. 1998), to verify whether kinetics of the different populations, with expansion and contraction phases and elimination of pathogen, may be correctly reproduced by the model. The order ranges for total T CD8 population count and durations of the different phases of the response obtained are coherent, and simulations also show generation of memory cells and a progressive convergence to the expected steady state. We obtained, as in experimental data, a CD8 T cell immune response characterized by expansion and contraction phases on the first 30 days after infection. This response begins with an expansion phase during approximately 7 days. This period is characterized by a great increase of CD8 T cell population, multiplied by 10^3, because of differentiation of naive into effector, and of effector cell proliferation. After 8 days post-infection, we observe a contraction phase with a decrease of the effector cell population, which begins to die while memory cell population is still maintained, in agreement with the biological experiments (Murali-Krishna et al. 1998). Hence, the model proposes kinetics for the response of total CD8 T cell population, but also details about kinetics of the different populations, in particular proportions of effector and memory cells in the organism according to the time after infection.

In this study, the viral replication was not considered, whereas it actually occurs in the experimental setting. In order to obtain a more realistic behavior of the model, one should therefore add the ability for the virus to replicate in the model. It brings a more complex mathematical analysis but it also adds other biological questions, such as analyzing if virus replication is dependent on the amount of effector cells, or not. But before performing other mathematical studies, we have to complete the present analysis of the model by an experimental work aimed at generating experimental data and fitting the model to these data. Indeed, what is presented here is almost exclusively based on the mathematical study of the model. Numerical simulations performed in Sect. 7 aimed at demonstrating the ability of the model to describe a "typical" CD8 immune response, without considering a systematic investigation of parameters. Although our present study brings relevant information on the biological problem, particularly regarding the role of feedback responses, we will pursue the confrontation with experimental data. Such an investigation will be the subject of a forthcoming paper and will consider different types of nonlinearities, not only Hill functions as presented in this work. We plan to compare systematically fit errors between the different choices of nonlinearities to determine their shapes and ranges of parameter values, needed to correctly reproduce the data. With the same method, we will also have to validate kinetics of the three sub-populations of T cells. This

last point requires to generate data which distinguish the different subtypes of T cells (whether naive, effector/activated, or memory), sampled during the total duration of a response, which involves massive experimental work. Experimental measurements of the different rates used in the model (differentiation, proliferation, apoptosis) should also bring valuable information on the relevance of linear models (De Boer et al. 2001; Rouzine et al. 2005; Kim et al 2007; Antia et al. 2003, 2005) versus nonlinear models, like the present one, for the description of the CD8 T cell response.

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