A STOCHASTIC MODEL FOR THE CLONAL EXPANSION AND HOMEOSTASIS OF CYTOTOXIC T LYMPHOCYTES

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Received July 26, 2003

ABSTRACT. We propose a stochastic model to explain the long time scale dynamics of Cytotoxic Antigen- experienced (AE) (memory) T Cell Clones. The clonal expansion is the result of a complex dynamical process in which the main actors are the acute and chronic antigenic stimuli, and the local environment created by the Cytokinic network in peripheral tissues. To describe the homeostasis, we introduce a size-landscape with several minima corresponding to the local quasi-equilibrium states of the clonal size. These states are reached under: i. the impulse of external acute stimuli which cause rapid jumps; ii. the chronic stress causing a slow diffusion; iii. physiological regulatory mechanisms. The chronic stress has a relevant role on long time scale dynamics via cross-reactivity and induced cytokinic activity during heterologous pathogen infection.

1 Introduction In this paper we propose a model which describes the dynamics of an antigen-experienced (AE) T cell clone under the effects of two main driving forces. The first is the acute antigenic stimulus (acute infection) which impinges upon the specific T cell receptor of a clone. The second is the chronic immune stress, consisting of endogenous stimuli (self-antigens) and exogenous stimuli (non self antigens), which in turn affect the dynamics of the AE T cell clone in two distinct ways: the T cell receptor cross-reactivity and the inflammatory cytokine response. We define the chronic immune stress as the effects of the ceaseless and lifelong stimulation of the immune system due to the continuous challenge with antigenic stimuli. As a consequence of the nature of the chronic immune stress, the dynamical time scale taken into consideration will be long (months, years)with respect to the duration of an acute stimulus and its related response (days, weeks). In this respect, many authors suggest[1] that the dynamics of AE T cells can be affected by i) MHC-mediated[1, 4, 5, 3, 2] and ii) by MHC non-mediated mechanisms[6, 10, 7, 8, 9, 1] . Concerning the former mechanisms, based on MHC mediated stimuli, we recall that the dynamical expansion of a AE T cell clone is affected not only by specific peptides causing high affinity TCR-MHC peptide binding but also by cross reactive antigens that repeatedly stimulate the AE T cell clone previously created in response to a preliminary specific antigen. Moreover, it has recently been shown that AE T cell clone may not only expand but also contract in response to a new antigen. This phenomenon, known as "heterologous immunity" has relevant consequences for our model, as in the case of the quoted "attrition", namely the possible clonal size contraction after an heterologous antigenic stimulus [12, 13] .

The latter mechanism (MHC non-mediated stimuli) is based on inflammatory cytokines (such as IL-15 and its cognate network INFa,b,g, IL-12,IL-18) produced during most immune challenges. These macromolecules can elicit the so called "bystander effect", i.e. the

2000 *Mathematics Subject Classification.* 65C20,92B05,68U20.

Key words and phrases. Aging, Clonal Expansion, Eterologous Infections, Stochastic Processes, Immune System Models.

proliferation of AE T cells even in the absence of a specific antigenic stimulus. In this case, in fact, during an antigen specific T cell expansion, other T cell clones, sharing with this clone the same local environment (lymphoid tissues) will expand because of the presence of the local cytokinic field[7, 1, 10, 11] . The secretion of inflammatory cytokines is the hallmark of the natural immunity, whose main actors are macrophages and natural killer cells when they are aspecifically challenged by every sort of antigenic stimuli. Inasmuch the constitutive cytokine production plays as the principal factor in the homeostasis (proliferation, survival) of AE T cells, the bursts of cytokine secretion being due to specific (antigen specific T cells) and aspecific (macrophage and natural killer cells) immune response. Such cytokines contribute to generate a fluctuating "cytokine noise" which is expected to affect the long time scale dynamics of AE T cell clones[9] . Different lymphoid, as well as non lymphoid tissues, determine different combinations of the intensity of antigenic stimuli, different availability of antigen presenting cells and the local production and clearance of inflammatory cytokines, giving rise to the particular intensity and temporal evolution of the clone in each peripheral district. [9, 11, 15, 16] Studies in the mouse show that T cells with immediate effector function [14, 4] preferentially localize in non lymphoid tissue .

This picture, emerging from the literature, will be expressed in mathematical terms in the model, which describes an AE T cell clone which can be directly stimulated through its T cell receptor by specific antigen (acute antigenic stimulus), as well as by cross-reacting antigens and pro-inflammatory cytokines (chronic immune stress). The effect of different peripheral tissues on the AE T clones will be discussed in more detail in Appendix A.

2 Description of the model We assume that an acute antigenic stimulus is due to a pathogen against which the AE T cell clone is specific, and that a chronic immune stress is a combination of both, the effects of various pathogens against which the T cell clone has different degrees of cross-reactivity, and the modulatory effects of the cytokine network. The model refers to a single AE T cell clone located in an inflammed tissue whose size (number of cells) is denoted by $c(t)$. We introduce the variable $x(t) = \log_{10} c(t)$ and its time variation, we denote by $v(t)$. The body of the model is a description of the homeostatic process as a mechanism of relaxation to a given equilibrium size after the action of an external antigenic stimulation. Changes of the size of the clone are due to both the acute antigenic stimulus, represented by an impulsive function, and the chronic immune stress described as a stochastic process (eq. 1).

$$(1) \qquad \begin{aligned} \frac{dx}{dt} &= v \\ \frac{dv}{dt} &= F(t) + f_0 + \gamma\xi(t) - \beta v - \frac{dV(x)}{dx} \end{aligned}$$

The acute antigenic challenge is represented by the impulsive fuction $F(t)$ acting for a time interval T with constant mean load of intensity (eq. 2).

$$(2) \qquad F(t) = \begin{cases} F_0\omega^2 & \text{for} \quad 0 \le t \le T \\ 0 & \text{for} \quad t > T \end{cases}$$

where ω^{-1} is a constant with the dimension of a time.

The chronic immune stress is composed by: 1. a constant mean intensity f_0 and a fluctuating term $\gamma\xi(t)$, where $\xi(t)$ is a white noise. The chronic immune stress is responsible for the long time scale expansion or contraction of the clone. From a probabilistic point of view, the latter phenomenon is quite unlikely because the average fluctuating chronic stress is positive therefore the clone predominantly expands. However the impulse of the chronic stimulus on short time intervals can be negative so inducing a temporary clonal reduction: from a biological viewpoint this is a description of the attrition phenomenon [13, 12] (see discussion).

Finally the term $-\beta v$ represents the effect of immune microenvironmental mechanisms counteracting the rate of variation of the clone size for restoring the equilibrium and to maintain the homeostasis of the system. Biologically this term account for both, the apoptotic down regulation as well as for the cellular proliferation induced by cytokines. The constant β fixes the time scale to restore the clone equilibrium size. In our model its value has been fixed as $\beta = .02\,\text{days}^{-1}$ corresponding to a time scale of 50 days, which is the typical time scale found during experiments on T cell expansion and down regulation in mice after secondary influenza challenge[16]

The homeostasis of T cell clone is a tissue dependent mechanism [7, 8, 3, 14, 10, 4] in which its size expands or reduces depending on a "bidirectional cross-talk" between the T cell clone and the immunological microenvironment. The size-landscape function V, which can be seen as a sort of mean field approximation of this interaction, regulates the dynamics that underlies the clonal relaxation to an homeostatic quasi-steady state after an immune response [3] For simplicity we assume the function $V(x)$ to be periodic and simmetric with period $2\pi/\omega$, with an excursion between the minima and maxima equal to ω^2. The shape of the function is not relevant once the period is fixed. We have examined explicitly two different cases, the saw-tooth and sinusoidal functions, as shown in figure 1.

$$V(x) = \begin{cases} \omega^2 \left|(x+1) \bmod 2 - 1\right| & 0 < x < L \quad \text{saw-tooth} \\ \omega^2 \dfrac{1-\cos(\pi x)}{2} & 0 < x < L \quad \text{cosine} \\ +\infty & x < 0 \quad x > L \end{cases} \tag{3}$$

In order to describe the homeostasis we only require the size-landscape function $V(x)$ to have a sequence of minima, and the term $-\beta v$. The equilibrium size of x follows the linear law $x = n$ which implies an exponential increase in the clone size c, as suggested by experimental results [16] . The way $x(t)$ rises during an acute antigenic impulse depends on the shape of the landscape function, but the average growth is linear in t, as suggested by experimental results. Different shapes of the size-landscape-function would correspond to different peripheral tissues. We introduce an effective size-landscape function V_{eff} by adding to $V(x)$ the linear term $-f_0 x$ which corresponds to the average antigenic stress f_0. As shown in fig.1 this term "bends" the graph of the function, giving a preferred direction to the clone size evolution when the white noise is switched on. The equilibrium sizes are metastable: even in the case of the absence of an acute antigenic stimulus, the effects of small fluctuations due to random immune stress can cumulate, thus allowing the clone to reach a new equilibrium state.

According to the hypothesis that the "immunological space" (total number of T lymphocytes in the whole body) is finite, and that the total T cell pool in a single organ cannot exceed a fraction of the total mass of the organ itself, we introduce a size limit of the clone L by using an infinite barrier in the size-landscape function corresponding to that size. The estimate of the number of T cell repertoire and of the tissue mass can be used to guess this limit size. Most of the experimental studies performed with mice revealed that the spleen

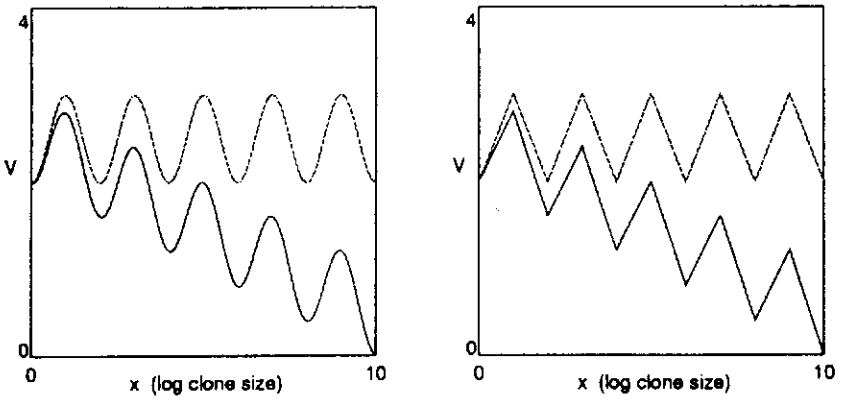

Figure 1: Sinusoidal (left panel) and saw-tooth (right panel) size-landscapes (upper curves) and effective landscape (lower curves). The effective landscape function $V_{\text{eff}}(x) = V(x) - f_0 x$ includes the contribution of the mean antigenic stress.

is a site accounting for at least 50% of the total virus-specific CD8+ memory T cells during an acute infection. Splenic T cells fill up to 70-80% of the splenic volume[16] .

All the parameters , except for the amplitudes of the acute stimulus F_0 and those related to the chronic stress, γ and f_0, correspond to a time scale. The first parameter β^{-1} corresponds to the time needed to reach the equilibrium when the chronic antigenic stress is switched off. The second T is the duration of the acute antigenic insult. The third parameter $2\pi/\omega$ is the period of the oscillations of the clone size around an equilibrium value after an antigenic impulse. The value of T is rather well known (1 to 3 weeks), and the value of β can be determined from recent experiments on mice[16] concerning the diaspora of virus-specific CD8+ T cell in peripheral tissues after a secondary antigenic challenge. The values we choose in our numerical example concerning an acute stimulus without chronic stress are $T = 24$ days, $F = 2$ and $\beta = \omega = 0.1\,\text{days}^{-1}$ corresponding to an oscillation period of about 40 days and a decay period $2/\beta$ of 20 days, as suggested by experimental data [16] . For the general case, in which the chronic stress is present, slightly different values have been chosen: $T = 9$ days, $\beta = .02$ days^{-1} whereas we still have $\omega = .1$. The small oscillations period is approximately $2\sqrt{2}/\omega \sim 30$ days.

3 Analytical solutions The system of equations (1) can be analytically solved in particular conditions.
Strong damping approximation: a high value of β means that the IS strongly counteracts any acute stimulus $F(t)$, which changes the homeostatic metastable equilibrium of the clone. From a mathematical point of view this hypothesis is represented by the condition $\beta \gg \omega$. In this hypothesis the term dv/dt is negligible, and the equation reduces to

$$\beta \frac{dx}{dt} = -V'(x) + F(t) \tag{4}$$

If $V(x)$ is zero the clone expands linearly and cosequently the clone size $c(t)$ expands exponentially; when the acute stimulus is over the clone reaches the constant value $x(T) =$

Table 1: Comparison of the thresholds T_n of the impuse duration which allow to jump after the maximum $x = 2n - 1$ for both the cosine and saw-tooth size-landscapes. The parameters are $\omega = 0.1$, $\beta = 0.5, F_0 = 2$ so that $\beta_* = \sqrt{2}\,\pi\omega = 0.44$

Landscape	T_1	T_2	T_3	T_4
cosine	64	143	223	302
saw-tooth	52	116	180	244
approx (5)	50	116.6	183.3	250

$F_0 T/\beta$, namely the increase is proportional to the antigenic impulse $F_0 T$. The case of the saw-tooth function can be easily solved, and the result is an expansion of the clone up to the equibrium value, which still depends on the antigenic impulse. The condition for a jump to the interval $[2n - 1,\ 2n + 1]$ between two subsequent maxima is given by

$$1 + (n-1)\left(1 + \frac{F_0 - 1}{F_0 + 1}\right) \leq T\,\frac{\omega^2}{\beta}\,(F_0 - 1) < 1 + n\left(1 + \frac{F_0 - 1}{F_0 + 1}\right) \tag{5}$$

and the time needed to reach the maximum $x = 2n - 1$ at rest is

$$T_n = \frac{\beta}{\omega^2}\left(\frac{n}{F_0 - 1} + \frac{n-1}{F_0 + 1}\right) \tag{6}$$

Consequently the duration T of the impulse which allows the solution to overcome the maximum $x = 2n - 1$ without overcoming the next one $x = 2n + 1$ is $T_{2n-1} < T < T_{2n+1}$. In this case the solution remains trapped in the corresponding interval, and evolves asymptotically to the minimum $x = 2n$. We have checked that the above conditions are approximately verified also for the cosine size-landscape (2a) when the strong damping condition $\beta \gg \beta_*$ is fulfilled. In this case $\beta_* = \sqrt{2}\,\pi\omega$ is the critical damping value above which no oscillations occur. In Table 1 we compare the jump time T_n evaluated by accurate numerical integration of the exact equation with the corresponding estimate (5).
As it can be seen from figure 1, the clone may expand beyond the equilibrium size and then relax, decreasing monotonically to this value. Considering $\beta \ll \omega$, which corresponds to a weak damping condition, we obtain that under the acute antigenic stimulation the clone reaches a new homeostatic metastable equilibrium size on a time scale much longer with respect to the previous case, and exhibits a sequence of oscillations (see figure 2).

The rise of $x(t)$ during the acute stimulus is monotonic and can be approximated by a linear function after an averaging process. This corresponds to an exponential growth of the clonal size $c(t)$. In the strong damping case the rise is piecewise linear and close to linear when $T\omega^2 F_0/\beta \gg 1$.

3.1 The Role of size-landscape function: tissue specificity It has been recently shown that the peripheral cytotoxic T cell clone may have different expansions for the same antigenic insult, depending on the organ in which it is located (spleen, bone marrow, liver, etc.) [16, 15] and that oscillations are observed before the relaxation to an equilibrium size. Here we show that by moderate changes of the shape of the landscape, one can reproduce this periphery-dependent behaviour for the same antigenic impulse (intensity F_0 times duration T). It is very plausible that the parameters of V (time scale and equilibrium sizes) might vary in the different organs. To this end we keep the heights of the maxima unchanged and vary the periodicity from 2 to $2/(1+\epsilon)$ namely $V = \frac{1}{2}\,\omega^2\,[1 - \cos\pi\,(1+\epsilon)\,x]$.

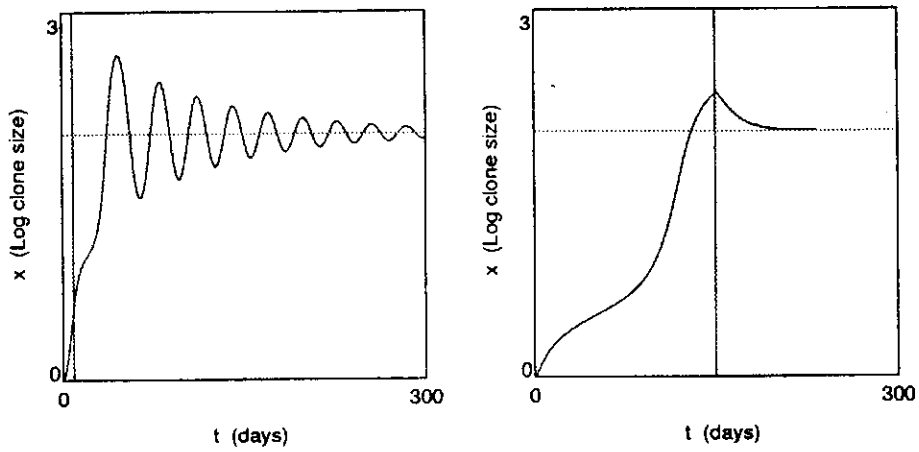

Figure 2: Left panel: weak damping case ($\omega = 0.1, \beta = 0.02$, $F_0 = 2\omega^2$, $T = 9$) T cell clonal expansion in the case of a sinusoidal size-landscape function $V(x)$. Right panel: overdamping case ($\omega = 0.1, \beta = 0.8$, $F_0 = 2\omega^2$, $T = 150$). The jump to the first minimum occurs for $T \simeq 100$, whereas for the saw-tooth size-landscape the jump threshold is $T = \beta/(F_0 - \omega^2) \sim 80$

In figure 3 (left panel) we show an example choosing a set of parameters such that the time scales are similar to the ones observed in the data of ref.[16] . We notice that the clone may expand beyond the equilibrium size and then relax by some oscillations of decreasing amplitude.
In figure 3 (right panel) we show the effect of a second antigenic insult with two different values of the parameters (ω, ϵ), which can be associated to two different organs where it takes place. The jump to a higher clonal size does not only depend on its duration and intensity, but also on the delay between two subsequent stimuli (phase dependence), see figure 4. ¿From a biological viewpoint this is a relevant prediction of our model: depending on the time the second stimulus occurs, the final (equilibrium) clonal size is enhanced if the clone is expanding (during the previous relaxation stage), whereas it is depressed if the clone is contracting. This behaviour of the clone after the second acute challenge, which depends on the previous stimulus and the consequent oscillatory behavior in the peripheral systems, is relevant and could be helpful in understanding the recalls in vaccination protocols.

3.2 Chronic stress, some analytical results The full model includes a second mechanism of clonal expansion: the chronic antigenic load, which is described by the stochastic forcing term $f_0 + \gamma\xi(t)$. The two terms, the average forcing term f_0 , and the function $V(x)$ considered together give the effective clone size-landscape function $V_{\text{eff}} = V - xf_0$ which regulates the peripheral-dependent homeostasis. On the basis of the analytical treatment of stochastic dynamical systems, we can state that the probability density $\rho(x, v, t)$ to obtain a certain solution $x(t)$ of the clone size at time t and rate of change $v(t)$ satisfies the Kramer-Fokker-Planck equation

$$\frac{\partial \rho}{\partial t} + \frac{\partial (v\rho)}{\partial x} - \frac{\partial}{\partial v}\left[(\beta v + V_{\text{eff}})\,\rho\right] = \frac{\gamma^2}{2}\frac{\partial^2 \rho}{\partial v^2} \tag{7}$$

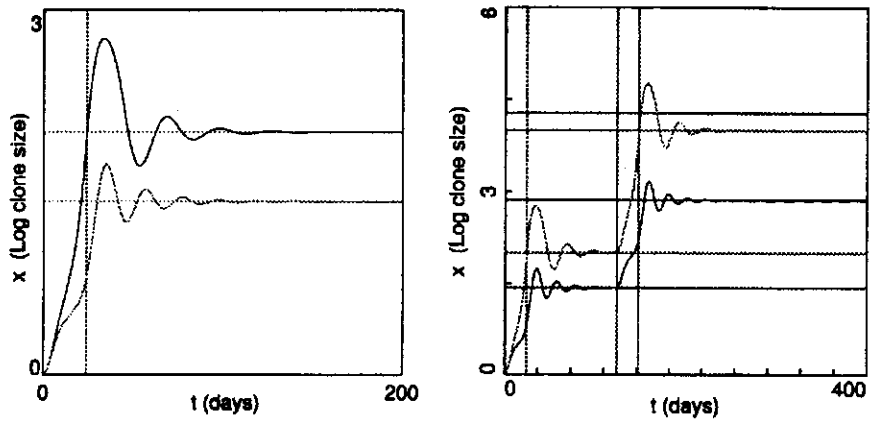

Figure 3: Left panel: comparison of the clonal expansion for two different sinusoidal landscapes defined by $V = \frac{1}{2}\omega^2 [1 - \cos \pi (1 - \epsilon) x]$ with a moderate damping. The chosen parameters are $\omega = 0.1$, $\beta = 0.1$, $F_0 = 2$, $T = 24$ and $\epsilon = 0$ for the first landscape (grey curve) and $\epsilon = 0.4$ for the second landscape (black curve). Right panel: same comparison as in the left panel for a clone undergoing a second antigenic impulse of the same duration and a delay of 100 days.

The equilibrium configuration, reached as $t \to \infty$, is described by:

$$\rho_{\,\mathrm{eq}}\,(s,v) = \frac{1}{Z} \exp\left(-\frac{v^2}{2\,k_B\,T}\right) \exp\left(-\frac{V_{\,\mathrm{eff}}}{k_B T}\right) \qquad k_B\,T = \frac{\gamma^2}{2\beta} \tag{8}$$

where Z is a normalization constant. To reach this configuration the system undergoes a relaxation process where a diffusion with respect to the deterministic trajectory occurs with a diffusion coefficient $D = \frac{1}{2}\gamma^2 = \beta k_B\,T$.

The chronic antigenic stress gives a finite probability for the expansion of the T cell clone to an equilibrium size $x = 2, 4, \ldots, 2N$ over a long time period. This is even possible if no restimulation by the original specific peptide occurs, so that the fluctuating heterologous chronic antigenic stress is the unique factor continuously stimulating the IS. As a consequence, the number of T cells constituting the clone depends not only on the acute antigenic challenge but also on the chronic stress impinging upon the IS during the period of its persistence in the periphery. We can estimate the transition time necessary to move among the equilibrium states, evaluating the time needed to complete this transition, as the inverse of the probability, according to Kramer's formula

$$\tau = C \exp\left(\frac{V_{\,\mathrm{eff}}\,(x_2) - V_{\,\mathrm{eff}}\,(x_1)}{k_B T}\right) = C \exp\left(\frac{2\beta(\omega^2 - f_0)}{\gamma^2}\right) \tag{9}$$

where

$$C = \frac{\omega_{2n}}{2\pi\omega_{2n+1}} \left[-\frac{\beta}{2} + \sqrt{\frac{\beta^2}{2} + \omega_{2n+1}^2}\right] \tag{10}$$

and $\omega_{2n} = V''(x_{2n}) \quad \omega_{2n+1} = V''(x_{2n+1})$

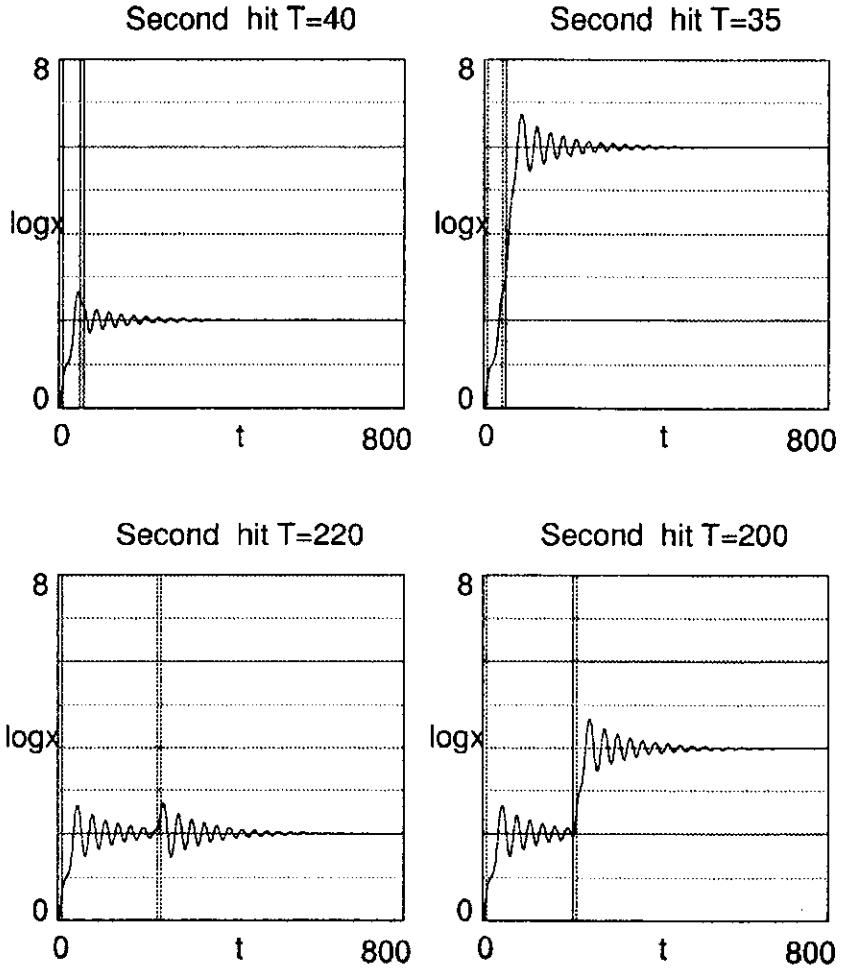

Figure 4: Phase dependence on the clone expansion in presence of a second acute stimulus. The right panels show that the second stimulus does not cause the jump to the next equilibrium size because it acts in counterphase, namely when the clone size is decreasing. The right panels show that the clone jumps to the next equilibrium size when the second impulse is in phase namely when the clone is expanding. The top panels refer to a secondary impulse starting at $T = 35$ and $T = 40$ days respectively, the bottom panels to $T = 220$ and $T = 200$. The duration of the primary and secondary impulses is $t = 9$ days.

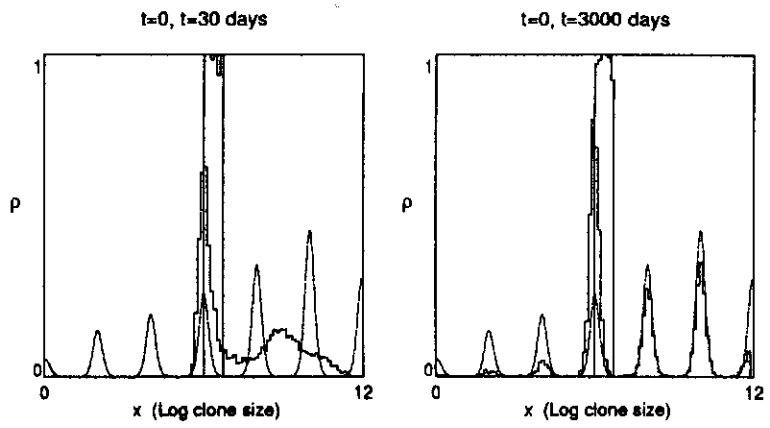

Figure 5: Clonal expansion due to an acute antigenic insult of intensity $F_0 = 2$, and duration $T = 9$ days and chronic stress with average value $f_0 = 0.02$ and fluctuation amplitude $\gamma = 0.0075$. The remaining parameters are $\omega = 0.1, \beta = .02$. The pictures represent the probability density function ρ, at a given time t, of having a clone size $c = 10^x$. Initially the clone size is uniformly distributed around the value $x_0 = 6$ with a spread $\Delta x = 0.35$. Under the stochastic stimulation the probability distribution of the clone size evolves and asymptotically peaks around the equilibrium values. The histogram corresponds to the results of the simulation, whereas the continuous curve is the analytical solution of the asymptotic equilibrium solution of the Fokker-Planck equation. There is a small but finite probability that the clone size decreases with respect to the initial value. The histogram in the left panel refers to the distribution at time $t = 30$, whereas the histogram in the right panel corresponds to time $t = 3000$. The central rectangle in both panels represents the initial distribution and is the same for both panels

4 Numerical results The effect of the average chronic stress in equation (1) is a change of the shape of the size-landscape function from V to $V_{\text{eff}} = V - f_0 x$. The bending of this function , which increases with the average stress intensity, enhances the probability of having large clones, by reducing the activation threshold. The fluctuating part of the chronic stress is responsible for jumps in the size-landscape function even in the absence of acute stimuli. This diffusion process leads to an asymptotic stationary state where the probability of having a clone of size $c = 10^x$ is peaked at the minima of $V_{\text{eff}}(x)$. The height of the peaks increases with f_0. In figure 5 we consider a case in which both the acute stimulus and the chronic stress are present. In the absence of chronic stress the clone would reach a new equilibrium size, after an acute stimulus, and perform damped oscillations around it, according to figure 2. Due to the presence of chronic stress, a population of clones will evolve independently and we can only estimate the probability of reaching a given size at time t. As shown in the left panel of figure 4, short after (3 weeks) an acute stimulus (duration T=9 days) we observe that the probability density of the clone size is spread out with a peak at the initial value ($x = 6$), and a second broader peak between the next two equilibrium sizes. After a long time ($t = 3$ years) the asymptotic stationary distribution is approached, as shown by the comparison with the analytical solution (8). A longer time or a smaller damping ($\beta = 0.01$) are required to reach more closely the equilibrium distribution, being the relaxation time proportional to β/ω^2 according to a strong damping estimate. Taking into account that the simulation of the initial probability distribution is uniformly

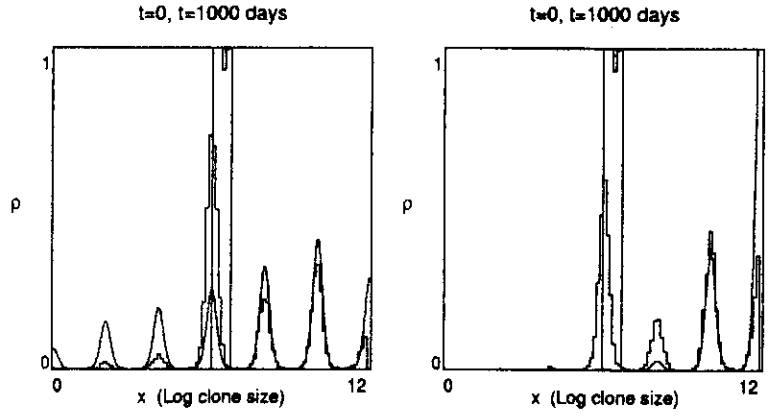

Figure 6: Clonal expansion due to an acute antigenic stimulus with the same parameters as in figure 5. Left panel: probability density ρ of the clone size at time $t = 1000$ for an average value of the chronic stress $f_0 = 0.02$ and a fluctuation amplitude $\gamma = 0.008$. Right panel: probability density of the clone size at time $t = 1000$ for an average value of the chronic stress $f_0 = 0.2$ and a fluctuation amplitude $\gamma = 0.008$. This figure shows that by increasing the average value of the chronic stress the expansion probability is strongly enhanced and that the probabily of reducing the clone size becomes very small.

distributed around a value $x(0) = 6$ with a small spread $\Delta x = 0.35$, the possibility of having smaller clone sizes with respect to its initial value $x(0)$ is due to the fact that the impulse of the chronic stress in a small time interval can be positive or negative ($f_0\Delta t+\gamma\xi\sqrt{\Delta t}$ where ξ is here a random number uniformly distributed in [-1,1] with variance 1). From a biological view point this negative contribution causing a temporary reduction of the clone size, is acceptable since it has been experimentally observed that under heterologous stimulations the clone size can decrease (attrition) [13, 12]. ¿From a probabilistic point of view this mechanisms would imply that during the homeostasis of a peripheral T cell clone, the size does not grow monotonically but, due to further different environmental stimulation, it can even reduce, in order to make possible the expansion of other specific T cell clone. A finite probability to have a clone with a size several times the initial configuration, is observed within a long time period. This phenomenon is enhanced by increasing the amplitude of the chronic fluctuations.

In figure 6 we analyze the effect of a change in the average value of chronic stress f_0. The right panel shows the results of the probability density of the clone size for a value of f_0 ten times bigger with respect to the left panel. With this larger value of f_0 the probability of having clones smaller than the initial value is practically zero, which means that the attrition phenomenon is negligible. On the contrary, for the smaller value of f_0 the effect of the attrition is visible.

5 Discussion and Conclusion In this work we proposed a model to describe a possible mechanism to generate and to maintain a long lived AE T cell clone in the peripheral infected tissues. Even though the TCR engagement with MHC complex is fundamental for CD8+ T cell activation and differentiation, the signals and mechanisms regulating the peripheral homeostasis of AE T CD8+ pools in long time scale are still unclear [15] . According

to recent experimental studies [4, 11, 9] we introduced the acute stimulus, of a given duration and intensity, in order to boost the IS to generate AE T cells. The recruitment and maintainance of T cells in the periphery [16] is not only due to acute challenges but also to chronic non specific stimulation. The role of the chronic stress is to maintain without any specific antigenic challenge the cycling of CD8+ T cells in the peripheral system by enhancing the clone size, because of the cross reactive interaction and/or of to cytokine-induced stimulations. The chronic stress can also play a relevant role in the reduction of the clone size caused by the attrition mechanism, i.e. the decline of the memory T cells clone size specific for a first antigen during successive infections by different pathogens[12, 13] . A combination of all the signals involved in the T cell differentiation, and the intermittent contact with some infection-induced cytokines (IL2, IL15, IL6) and cross reactive antigens can be fundamental for the memory activity even if no specific antigen is present. This model confirms the two envisaged different roles played by the cytokines produced during infections for promoting long term memory T cells. The first related to the survival of pre-existing AE T cells because of the fluctuating interactions with the cytokines released during undercurrent infections; the second related to their adjuvant function for the homeostasis of new AE T cells in peripheral tissues. In this minimal model we have introduced the size-landscape function in order to to describe the role of the infected organs in the differentiation and proliferation of AE T cell pool. The minima of this function correspond to the dynamical equilibrium (balance of cell proliferation and apoptosis rates), which depend on the specific immunological microenvironment of the infected organ [17, 18] . As recently suggested [14] , AE T cell pools dynamics can be described with a preliminary expansion phase in the lymph-node, and with a subsequent recruitment to the site of the antigenic exposure. It has been observed that, after the homing from lymph-node in the peripheral infected tissue, there is a regulation of microenvironment[3, 14]. In our model the dynamics of T cells clones is governed by the environment, described by the size-landscape function V, which fixes the size, and by the counteracting term $-\beta v$, both acting as a spring-dashpot system, which describes the homeostatic process. The average of the chronic stress bends the landscape making the activation threshold progressively decreasing, and thus favouring the expansion of the clone size with respect to its contraction, which is nevertheless still allowed. To conclude we confirm the hypothesis that the antigenic specificity might not be the main determinant of the kinetic behaviour of AE T cells clones [19, 5, 10, 9] ; the clone can survive in the peripheral tissues where infectious stimuli are present even without a specific challenge (MHC restricted mechanism), the chronic stress being a possible mechanism to explain its expansion and its survival over long time scale. The model is adequate to describe the sensitivity of the clone homeostasis to the local environment as suggested by recent experiments [16] . Some mathematical models[21, 20] highlight the role of cross reactive antigens during clonotipic immune response, but the study of the effect of the chronic stress over long time scale has not been fully addressed[22] . from both a biological and a mathematical point of view. These preliminary results on the long time evolution of T cell clones could be of potential relevance for vaccination strategies and protocols. It is well known that a percentage of people acquires a poor memory boost after vaccination; our model shows that a specific AE T clone can shrink (with a low probability), after an acute stimulus as a consequence of the chronic stress and subsequent attrition. The effect on long time scale dynamics of the chronic stimulation is to strongly influence the maintainance of the T cell clones. This would suggest that during vaccine recalls the size of the long lived AE T cell pool could not change at all, or worst, could even decrease in some individuals, with a negative effect on the maintainance of the clone. In future works it would be very interesting to introduce in the model a population of regulatory T cells. The presence of the antigenic stress could act as a mechanism for the maintainance of regulatory cells over

a certain given threshold strongly influencing the specific T cell clone dynamics.

Aknowledgements This work was supported by grants from M.I.U.R. Project COFIN 2001 "A multidisciplinary approach to the regu;ation of the immune system"

A Biological Mechanism for the homeostasis of T cells in peripheral infected tissues In this model the presence of the term $-\beta v$, which modulates the variation of the T cell number, and the presence of the landscape-function as a collection of a finite number of metastable states, which allows the oscillations around the homeostatic T cells size, suggest a potential general mechanism accounting for the maintainance of the homeostatic level of T cells in the infected tissues. The control of the lymphocyte number is not entirely a function of hematopoietic cells but a dynamical interaction between activated T cells and peripheral epithelia through microenvironment cytokine concentration. For instance, the Fas-Fas Ligand interaction is an important peripheral mechanism which regulates apoptosis of activated T cells [17, 18, 19] via different mechanisms such as AICD (Activation Induced Cell Death). It has been reported that in several tissues the epitelial cells (keratinocytes in the skin, epithelium of small intestines, liver cells) do express Fas-Ligand and that its expression is strongly modulated via cytokines. Unlike Fas, the expession of Fas-Ligand appears to be more restricted to activated T cells, NK cells of immune privileged organs and epitelial infected cells. Although the peripheral lymphocyte deletions depends on lymphocytes Fas-Ligand concentration, experimental evidence suggests a remarkable contribution to T cell apoptosis (AICD) by Fas-Ligand expression due to Fas-Ligand expressing cells present in non-lymphoid tissues after the lymphocyte expansion. Changes in the concentration of microenvironmental cytokines due to the fluctuations of the antigenic stress lead to a modification of the Fas-Ligand expression in the peripheral microenvironment. This variation affects the balance between Fas and Fas Ligand concentrations and therefore the regulation of the apoptotic infiltrated T cells. The homeostasis of the T cell clone will be finally the result of a countinuous cross-talk between specific infiltrated T cell and the epitelial tissues expressing the Fas-Ligand. In our model the damping term acts as a general mechanism which prevents uncontrolled proliferation as well as the extreme rapid depletion of cytotoxic T cells during the oscillatory phase around the homeostatic level of the clone size (see fig.3). If the rate of the pro-inflammatory cytokines increases, the expression of Fas-Ligand on the epitelial cells also increases and those T cells expressing Fas are more prone to apoptosis. As a final result the total number of T cells surviving in the periphery are diminished. When the rate of cutokines decreases because of a temporary reduction of the antigenic chronic stimuli, the expression of Fas-Ligand on the epitelial cells could decrease giving rise to a moderate re-expansion of the T cell number.

B Strong damping approximation Such approximation for the proposed deterministic model

$$\begin{aligned} \frac{dx}{dt} &= v \\ \frac{dv}{dt} &= -\beta v + F(t) - V'(x) \end{aligned} \tag{11}$$

consists in neglecting the term dv/dt when β^{-1} is small with respect to a typical time scale of the system without damping. We have chosen both $F(t)$ and $V(x)$ proportional to ω^2, where ω^{-1} defines such a scale. Indeed if $F = 0$ the period of oscillations around the minima scales as ω^{-1}. If $V = 0$ such a scale is given by the pulse duration T, as one can check directly by solving the linear equation, and we choose T of order ω^{-1}.

When $\beta \gg \omega$ the equations (A1) are approxmated by the first order equation

$$\beta \frac{dx}{dt} = F(t) - V'(x) \tag{12}$$

Taking into account (2)and (3), introducing the rescaled time $t' = t\omega^2/\beta$ and pulse duration $T' = T\omega^2\beta$ equation (A2) reads

$$\begin{aligned} \frac{dx}{dt'} &= \begin{cases} \sigma(x) + F_0 & t' < T' \\ \sigma(x) & t' > T' \end{cases} \\ \sigma(x) &= \begin{cases} -1 & 2n-2 < x < 2n-1 \\ 1 & 2n-1 < x < 2n \end{cases} \end{aligned} \tag{13}$$

Solving with initial condition $x(0) = 0$ we obtain

$$\begin{aligned} x(t') &= (F_0 - 1)\, t' && 0 < t \le t'_1 = \frac{1}{F_0 - 1} && x(t'_1) = 1 \\ x(t') &= 1 + (F_0 - 1)\,(t' - t'_1) && t'_1 < t \le t'_2 = t'_1 + \frac{1}{F_0 + 1} && x(t'_2) = 2 \\ x(t') &= 2 + (F_0 + 1)\,(t' - t'_2) && t'_2 < t \le t'_3 = t'_2 + \frac{1}{F_0 - 1} && x(t'_3) = 3 \end{aligned} \tag{14}$$

and so on until some n such that $t'_{2n-1} < T' < t'_{2n+1}$. Then if $T' < t'_{2n}$ the point reaches $x = 2n$ and stops; in the last interval $[x(T'),\, 2n]$ the points slows down changing its speed to 1. Instead if $T' > t'_{2n}$ the point overcomes the equilibrium point and after reaching $x(T') > 2$ comes back, with speed 1, to the equilibrium point. In figure (7) we show the piecewise linear trajectories which can be followed to reach a minimum of $V(x)$.

The times at which the maxima $2n-1$ and the minima $x = 2n$ of $V(x)$ are reached are given by the recurrence

$$t'_{2n-1} = t'_{2n-2} + \frac{1}{F_0 - 1} \qquad t'_{2n} = t'_{2n-1} + \frac{1}{F_0 + 1} \tag{15}$$

whose solution reads

$$t'_{2n} = \frac{n}{F_0 - 1} + \frac{n}{F_0 + 1} \qquad t'_{2n+1} = \frac{n+1}{F_0 - 1} + \frac{n}{F_0 + 1} \tag{16}$$

so that the condition for the trajectory to end in the interval $[2n-1,\, 2n+1]$ stopping at the minimum $x = 2n$ is $t'_{2n-1} < T' < t'_{2n+1}$ from which equation (4) follows.

C Weak damping approximation When $\beta \ll \omega$ an analytical study of the the problem is still possible in the weak damping approximation, which is basically a first order perturbative expansion in β. However since we are left with a system of two differential equations, the calculations are more involved. We show only how to construct the solution in the interval $0 < x < 1$ starting with initial conditions $x(0) = v(0) = 0$. The equation to solve reads

$$\begin{aligned} \frac{dx}{dt} &= v \\ \frac{dv}{dt} &= -\beta v + \omega^2(F_0 - 1) \qquad 0 < x < 1 \end{aligned} \tag{17}$$

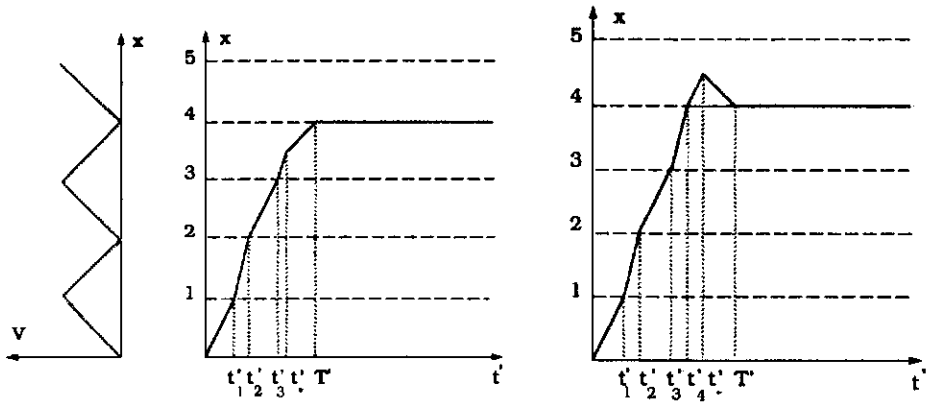

Figure 7: Sawtooth landscape and time evolution $x(t)$. Left panel : $t'_3 < T' < t'_4$ the second minimum is not overcome. Left panel : $t'_4 < T' < t'_5$ the second minimum is overcome and it is reached after an expansion up to a larger value. In the figure we have chosen $\omega = 1$.

In order to compute the solution we introduce the function H which satisfies the following equation

$$H = \frac{v^2}{2} - \omega^2(F_0 - 1)\,x \qquad \frac{dH}{dt} = -\beta v^2 \tag{18}$$

The unperturbed ($\beta = 0$) reads $x(t) = \frac{1}{2}\omega^2\,(F_0 - 1)\,t^2$ and the time to reach $x = 1$ is $t_1 = \omega^{-1}\sqrt{2/(F_0 - 1)}$. The first order is computed according to

$$H(x,v) - H(0,0) \equiv \frac{v^2}{2} - \omega^2(F_0 - 1)x = -\beta \int_0^t \omega^4\,(F_0 - 1)^2\,t'^2\,dt' = -\beta\,\omega^4(F_0 - 1)^2\frac{t^3}{3} \tag{19}$$

where t is expressed in terms of x using the unperturbed solution. As a consequence

$$v = \omega\sqrt{2(F_0 - 1)}\,x^{1/2}\left(1 - \frac{4}{3}\,\frac{\beta\,x^{1/2}}{\omega\sqrt{2(F_0 - 1}}\right)^{1/2} \simeq \omega\sqrt{2(F_0 - 1)}\,x^{1/2} - \frac{2}{3}\beta\,x \tag{20}$$

Solving $dx/dt = v(x)$ by separation of variables we find at the first order in β the solution $x(t)$ and the time t_1 at which the first maximun is reached (provided that $T_1 < T$)

$$x = \frac{\omega^2}{2}\,(F_0 - 1)\left(t^2 - \frac{3}{4}\beta t^3\right) \qquad t_1 = \frac{1}{\omega}\sqrt{\frac{2}{F_0 - 1}} + \frac{3}{4}\,\frac{\beta}{\omega(F_0 - 1)} \tag{21}$$

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