1

An Automata based Microscopic Model inspired by the Clonal Expansion

F. Zanlungo[1,2] and G. Turchetti[1,2]

[1] Department of Physics, Via Irnerio 46, 40126 Bologna, Italy and Centro Interdipartimentale L. Galvani, Università di Bologna, Bologna, Italy
[2] Isituto Nazionale di Fisica Nucleare, sezione di Bologna

Summary. We present a simple model based on microscopic automata to describe the clonal expansion process. The model is based on a repertoire of antigens and T lymphocytes interacting via the APC cells which present the antigens peptides. Each cell is represented by an automaton moving randomly on a two dimensional lattice. We use this simplified model in order to introduce local and spatial considerations in the mathematical models of clonal expansion based on differential equations, and at the same time to attempt an analytical interpretation of the results of computer simulations. For this reason we derive also a mean field theory, whose results are in good agreement with the solutions of the of microscopic model, at least for situations that are not too far from equilibrium. This model may be used as the base of a more realistic one that could follow the clone expansion process on a simplified version of the lymphatic network.

Key words: Clonal expansion, automata, agent model

1.1 Introduction

In this work we try to create a bridge between two different ways to study the clonal expansion in the immune system (IS). One kind of approach consists in studying the concentrations of the different species of cells, whose behaviour and interaction is modelled through a system of differential equation (DE), the other one in studying the microscopic interactions between the single cells, that are usually modelled as Cellular Automata (CA).
It is our opinion that in immunology as in other fields of research the languages of microscopic (CA or agent) and macroscopic (DE) models could be integrated, in order both to use the analytical result to explain and partially predict the behaviour of the simulated models, and to utilise the simulations to enrich with microscopic details the assumptions of the the macroscopic models.
In this paper we present a simplified model of the clonal expansion, in which we stress our attention on the spatial interaction between T cells and Antigen

Presenting Cells (APC), while omitting the details of the T cell-antigen and APC-antigen interactions, and ignoring many other important agents of the IS. The aim of this paper is thus not to present a new model for the clonal expansion, but to start a project of work in which two different ways to model it could be combined.

1.2 Description of the model

1.2.1 Differential equations model

One of the major open question in immunology is the problem of understanding clonal expansion , i.e. how the T cells, that belong to a very large repertoire, are selected in response to a specific treat (the presence of an antigen) and proliferate to form a large clone, and how this proliferation is regulated.
De Boer and Perelson presented a model that justifies the maintenance of diversity in the periphery through the concept of competitive exclusion ([1]). This competition between T cells (between the different clones and inside the same clone) arose as competition for the peptides presented on the surface of APC. In fact these peptides can be freely available on the surface of an APC, or be captured in the receptor of a T cell bound to an APC; in the second occurrence they are no longer available to other T cells.
De Boer and Perelson imposed a quasi-steady-state condition for the number of complexes given the number of peptides, and obtained a system of differential equations for the different clones sizes, which corresponded to the well-known principle of competitive exclusion ,in biology (two different species cannot co-exist in equilibrium if they use just the same resource) and introduced also a capacity (equilibrium size for a single clone).
In this model the number of peptides is considered to be proportional to the antigen concentration, which is assumed as fixed. This assumption is well justified in case of self antigens, while for pathogens they assumed this fixed concentration to be the equilibrium value of a prey equation for the antigens, in which T cells had the role of predators. Using this assumptions, immune memory is attained through the persistence of antigen at a controlled concentration. (See [2] and appendix 1.5 for a treatment of prey-predator equations, and [3] for an application to the immune system).
This is one of the many models that describe the clonal expansion using a system of differential equations (see for example[4]) and has been further on studied and improved by the authors ([5]). Our interest in this version of the model is due to its simplicity and to the fact that its basic assumptions concern the microscopic spatial interactions between T cells and APC, averaged in the quasi-steady-state condition.
Since there are many experimental results concerning how these interactions happen [6, 7, 8, 9], we think that this model is well apt to a microscopic formulation, in which the different individual cells are represented as automata

in a computer simulation (we use the term automaton referring to the original definition by Von Neumann, and not just to cellular automata, i.e. we don't necessarily identify a biological cell with a site of a discrete grid, even if it is the case of the model that we are going to present). ,
These are the differential equations that describe our version of the De Boer-Perelson model

$$\dot{A}_i = aA_i - b{A_i}^2 - \sum_j c_{ij} A_i T_j \tag{1.1}$$

$$\dot{P}_i = dA_i - rP_i \tag{1.2}$$

$$\dot{N}_{APC} = 0 \tag{1.3}$$

$$\dot{T}_i^N = 2g{T_i}^A - h{T_i}^N - \sum_j k_{ij} {T_i}^N T_j - lf(\sum_j m_{ij} P_j) F {T_i}^N + oC_i + s \tag{1.4}$$

$$\dot{T}_i^A = qC_i - g{T_i}^A \tag{1.5}$$

$$\dot{C}_i^A = -qC_i - oC_i + lf(\sum_j m_{ij} P_j) F {T_i}^N \tag{1.6}$$

$$\dot{F} + \sum_i \dot{C}_i = 0 \tag{1.7}$$

Equation 1.1 tells us that the n_A species of antigens A_i follow a logistic prey equation in which the n_T T cell clones T_i have the role of predators. Equation 1.2 gives the probability to find a peptide of species i, P_i, (we assume for simplicity a one to one correspondence between peptides and antigens) in a site of an APC cell. This probability grows with the number of antigens and follows a decay rule (peptides remain on the APC's surface for a finite average time). With equation 1.3 we fix the number of APC cells.
Equation 1.4 and 1.5 concern the number of non-activated ${T_i}^N$ and activated ${T_i}^A$ T cells ($T_i \equiv {T_i}^N + {T_i}^A$). Non activated T cells are produced by duplication of activated ones with a rate g and die by apoptosis with rate h. The probability rate s represents an external source (thymus). F is the total number of free sites on the APC's surface, to which T cells can bind with a probability rate that depends on a function f of the probability to find a given species of peptides multiplied by its affinity m_{ij} to it. We call C_i a complex formed by a T cell T_i and a site of an APC. These complexes can unbind with probability rate q in case of successful activation (equation 1.5) and with probability rate o in case of unsuccessful activation (equation 1.4). The terms k_{ij} in equation 1.4 rule the fratricide competition between the T cells (see for example [10]).
The number of complexes and free sites is governed by equations 1.6, 1.7 coherently with the assumptions of equations 1.4, 1.5 and with the request that their sum has to be fixed as the total number of sites ($n_s N_{APC}$ if n_s is the number of sites on a single cell).

1.2.2 Microscopic model

In the differential equations based model we tried to write explicitely an equation for each agent of the process, and we defined a probability rate for each interaction between these agents, since we want these equations to be the mean field version of a microscopic model. Given the high number of equations and parameters we won't try an analytical treatment and we will rely on numerical integration for their solution. Our microscopic model is realized on two superposed 2D squared grids, one on which antigens move and one for APC and T cells. The physical region corresponding to each layer will be the same (creating a correspondence between sites "located in the same physical space") while the step of the grids and thus the number of sites could be different.
All the cells move by random walk obeying an exclusion principle (no more than a single cell on a given site of a layer), and the interaction between cells can happen by superposition when they are located on different layers, or by contact (i.e. if the are located on first neighbour sites) if they are on the same layer. We call these events that allow an interaction between the cells "encounters". An encounter between an antigen A_i and a T cell T_j leads to the elimination of the antigen with probability ${p^c}_{ij}$, while an encounter between an antigen and an APC leads with probability p^d to the presentation of a peptide on the "surface" (i.e. on one of the four sides) of the APC (in our convention we associate to the probabilistic rate x in the continuous macroscopic model the probability p^x in the discrete microscopic one). Encounters between a T cell T_i and an APC can form a complex, with a probability p^l multiplied by the affinity to the site $f(\sum_j m_{ij}P_j)$ (a function of the averaged affinity to the peptides). Encounters between the antigens lead to an over-population due "logistic" elimination of the antigen with probability p^b, while those between T cells in clones i and j lead to fratricide apoptosis with probability ${p^k}_{ij}$. These fratricide terms are in a certain sense "ad hoc" in our model (they are not present in the original formulation by De Boer and Perelson, even if they are present in other models, as in [10]), since we need them to avoid a filling of the grid. They should be chosen in such a way that they are not relevant under normal conditions (i.e. when the number of occupied sites is low with respect to the total number of sites). All the other processes are encounter independent and can happen with given probabilities at each time step.
It is quite clear that this model is too simple to describe all the complex processes that concern the clonal expansion in the immune system. A more complete formulation should use at least two different 2D grids to describe the site of infection and the lymph-nodes (connected in some way to allow the displacement of T and dendritic cells), while for a realistic description of immunological memory a differentiation between naive and memory T cells is necessary.

1.2.3 Mean field equations

All the probability rates in a macroscopic model have to be chosen on the base of macroscopic observations, in such a way that the behaviour of the solutions will correspond to the behaviour of the biological species under some given assumptions.
According to the spirit of this work, the probabilities of the microscopic model should be given on the base of microscopic observations, as reported for example in [6, 7, 8, 9]. The time step step should be chosen smaller than the shorter characteristic time of the processes involved, and all these characteristic times should be expressed as probabilities. An average process would be necessary to describe 3 dimensional cells with a complex shape as 2D squared objects, and probably also minor changes on the geometry (allowing for example APC and T cells to have different size) could be necessary.
Given the preliminary stage of this work and its general purposes, and considering also our limits in the interpretation of experimental data given our scientific formation, we just do very simple considerations that allow us to have some qualitative result, without any claim to quantitative or predictive results.
We can obtain the mean field equations for the microscopic model in the following way. Let us assume for example that the average time for antigen duplication is one day. If we choose a time step of 15 minutes, the probability for antigen duplication is fixed to $p^a = 0.01$. Defining N_A as the number of sites of the antigen's grid and assuming random distribution for all the cells, the probability for an antigen to have an encounter with another antigen on one of its 4 sides is A/N_A, and thus the time evolution of the number of antigens in absence of T cells is given by

$$A(t + \Delta t) = A(t) + p^a A(t) - p^b A(t)^2/N_A \tag{1.8}$$

The value of p^b can be fixed given the wanted maximum density of antigens (the capacity),

$$A_{max}/N_A = p^a/p^b \tag{1.9}$$

and in the continuous limit we obtain equation 1.1 through the identifications $a = p^a/\Delta t$, $b = p^b/(N_A \Delta t)$.
The discrete version of equation 1.2 is, referring to Π_i as the number of peptides of species i on a single side of an APC,

$$\sum_{APC} \Pi_i(t + \Delta t) = \sum_{APC} \Pi_i(t) + p^d A_i(t) N_{APC}/N_T - p^r \sum_{APC} \Pi_i(t) \tag{1.10}$$

or, averaging over all the sides

$$P_i(t + \Delta t) = P_i(t) + p^d A_i(t)/(4N_T) - p^r P_i(t) \tag{1.11}$$

where N_T is the number of sites of the APC-T cell grid. The continuous version of 1.11 is equation 1.2, through the identification $d = p^d/(4\Delta t N_T)$, $r = p^r/\Delta t$. Equation 1.2 has solution

$$P_i(t) = e^{-rt}\Big[\int A_i(t')e^{rt'}d + const\Big] \tag{1.12}$$

that reduces to

$$P_i(t) = \frac{A_i d}{r}\Big[P_i(0) - \frac{A_i d}{r}\Big]e^{-rt} \tag{1.13}$$

in case of constant A_i concentration. We use $p^d = 1$ (the APC always recognises the antigen) and $p^r = 0.02$, corresponding to a permanence of the peptide on the antigen surface for an average time of 12 hours.
We compare in figure 1.1 the numerical integration of equation 1.1 (for a single species) with the corresponding results given by the microscopic model, and in figure 1.2 we present the same comparison for the analytical result of equation 1.11. (We have used for these simulations $N_T = 9\text{x}10^4$, $N_A = 3.6\text{x}10^5$ and $p^b = 0.05$ which corresponds, according to equation 1.9, to a capacity of an antigen every 5 sites).

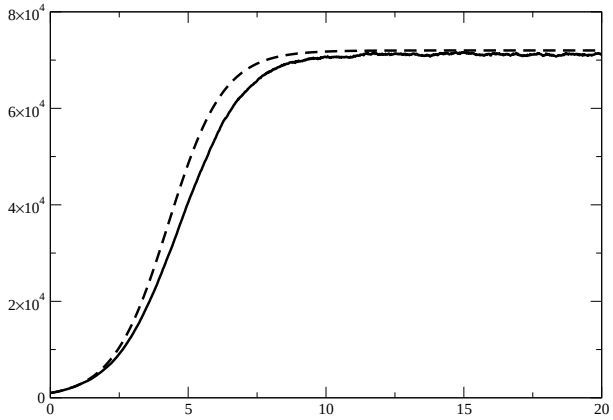

Fig. 1.1. Comparison between the free growth of the antigen number $A(t)$ as obtained from the microscopic model (continuous line) and the mean field equations. The time unit is one day, as in all the figures to follow.

While there is an almost perfect correspondence between the curves in figure 1.2, there is a slight difference between those in figure 1.1. This effect is due to the fact that while the behaviour described by equation 1.2 depends on the interaction between cells located on different layers, and thus is not actually based on microscopic spatial interactions, the behaviour described by equation 1.1 relies on and influences the spatial distribution of antigens. For this reason the mean field equation describes well the microscopic model in the initial configuration, when a uniform distribution is imposed, and at the equilibrium, while the discrepancy is stronger during the expansion.

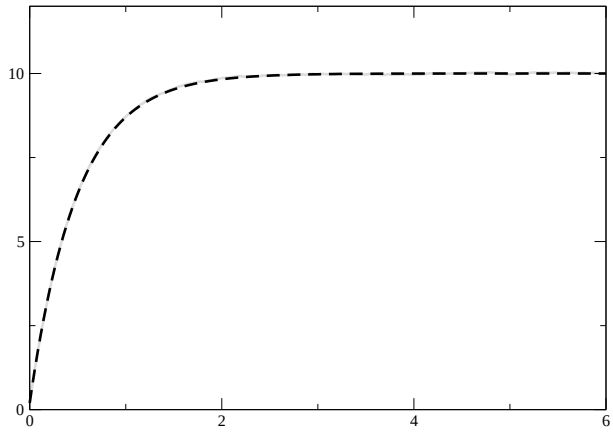

Fig. 1.2. Average number of peptides as obtained by the microscopic model (continuous line in grey) and the mean field equations. The two lines are almost indistinguishable.

The local effects are obviously stronger when we consider the spatial T-APC interaction. Let us fix $N_{APC} = 2\text{x}10^3$ on the $N_T = 9\text{x}10^4$ grid, use the sigmoid function

$$f(\sum_j m_{ij}P_j) = \frac{1 - e^{\sum_j m_{ij}P_j}}{1 + e^{\sum_j m_{ij}P_j}} \tag{1.14}$$

to obtain the affinity of a T cell to a site on the APC surface, $p^g = 0.05$ (an activated T cell needs 5 hours to split referring to the time step of 15 minutes), $p^h = 0.001$ (a life span of 10 days for the T cells), $p^l = 0.25$ (an hour to form a complex in case of maximum affinity), $p^q = 0.2$, $p^o = 0.04$. (These are the probabilities to unbind with and without activation in case of maximum affinity. The dependence of these microscopic probabilities on the affinity has been chosen "ad hoc" is such a way that the first one grows and the second one decreases with affinity).
If now we consider a single clone T with maximum affinity to a single species of antigen A ($m \equiv m_{11} = 1$) and fix A to its maximum capacity ($c \equiv c_{11} = 0$, i.e. antigens are not removed), we can obtain in the usual way the discrete mean field equations for T, F and C whose continuous limit leads to equations 1.4-1.6, redefining the parameters on the base of the microscopic probabilities. Figure 1.3 refers to the growth of the clone, and compares the integration of the mean field equation with the results given by the microscopic model (the fratricide term value is fixed to $p^k = 0.1$). In this case the discrepancy is

stronger, and also a qualitative one. The growth in the microscopic model is lower at the beginning, while the equilibrium value is higher. Two different effects are present, both due to the presence of zones around the APC in which T cells reproduce: the fratricide effect is enforced because of the higher density in these zones, but also the probability to meet an APC and thus to be activated is enhanced. Since these effects depend strongly on the density of cells, is possible to obtain the parameters of equations 1.1-1.7 by a process of best-fitting only on regions in which the values of A and T are almost constant (this means that those equations are able to describe properly the behaviour of the microscopic system only if we introduce a dependence of the parameters on A and T).

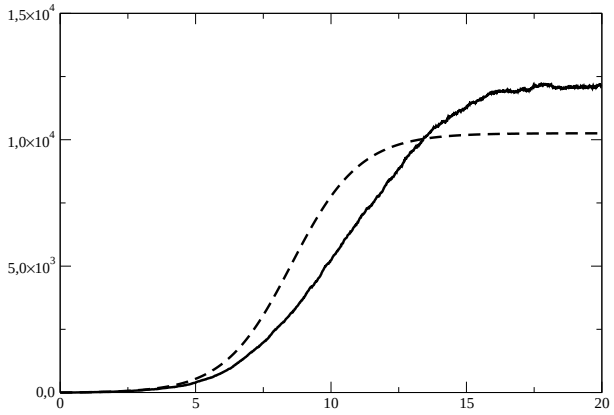

Fig. 1.3. T clone expansion in response to a fixed number of antigens in the microscopic (continuous line) and mean field models.

1.3 Results of the simulations

1.3.1 Acute antigenic impulse

In order to complete the model we have to fix the value of the parameters $c_{ij} \equiv c\, m_{ij}$ (we are assuming that the ability of a T cell in removing an antigen is proportional to its affinity to it). We have used $c = 0.2$ in order to obtain a realistic time scale for the response of the immune system to the infection. In figure 1.4 we plot the evolution of the clone size T and antigen A population, comparing the results of the microscopic model with the solutions of the mean field equations. In agreement with the previous discussion the results are very similar at equilibrium values, while the agreement is only qualitative

during the transient part. Damped oscillations are present in both models, and both the period and the height of peaks and valleys are of the same order of magnitude (the damping rate and the period of oscillations are higher in the microscopic model).

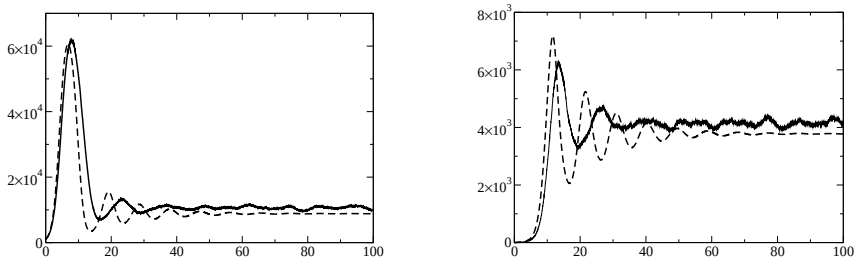

Fig. 1.4. Evolution of the system under an acute antigenic stimulus. The evolution of the antigen number in the microscopic (continuous line) and mean field model is shown at left, while the size of the T cell clone is reported on the figure at right (the continuous line corresponds to the microscopic model)

This behaviour corresponds to that of a prey-predator system (see appendix 1.5 and [2]). To an equilibrium value with $A \neq 0$, $B \neq 0$ corresponds a "memory" effect due to the permanence of the antigen. In this situation the response to a secondary stimulus is obviously quicker (figure 1.5).

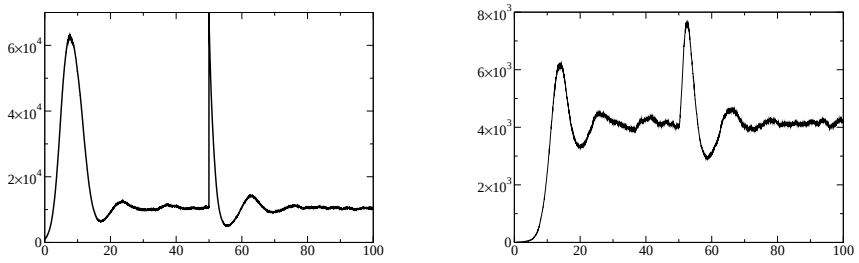

Fig. 1.5. Left: evolution of the antigen population after a secondary impulse occurring 50 days after the primary; microscopic model. Right: corresponding evolution of the T cells clone.

1.3.2 The clonal repertoire model

We finally consider the effects of both fratricide and spatial competition terms between different clones in presence of a differentiated antigen repertoire. By using a fratricide term in which the decrease is proportional to the overall size of the clones, $\Delta_- T_i = -kT_i \sum_j T_j$, we obtain a mutual exclusion principle. In fact, if we summarise with $\Delta_+ T_i$ the growth terms, the relative variation of the clone size is

$$\frac{\dot{T}_i}{T_i} = \frac{\Delta_+ T_i}{T_i} - \frac{\Delta_- T_i}{T_i}$$

Since $\Delta_- T_i/T_i$ is the same for all the clones, supposing that there is a unique antigen with the highest affinity to the clone T_j (namely $\Delta_+ T_j/T_j > \Delta_+ T_i/T_i \ \forall i \neq j$), if the clone j reaches an equilibrium $\Delta_+ T_j = \Delta_- T_j$ then any other clone extinguishes since $\Delta_+ T_i - \Delta_- T_i < 0$. (These are the basics of competitive exclusion, see [2]).
To show that in our model there is competition for peptides presented at the APC surface (the mechanism investigated in [1]), we can use a "pure fratricide" term $-kT_i^2$. This is actually a "non-competitive" one since it favours the small clones. In fact, studying the expansion of 3 clones under the stimulus of a single antigen, using an affinity matrix $m_{i,1}$ such that $m_{i,1} \ll m_{1,1} = 1$ if $i \neq 1$ we have (figure 1.3.2) an equilibrium with $T_2 \neq 0$ (figure 1.6)

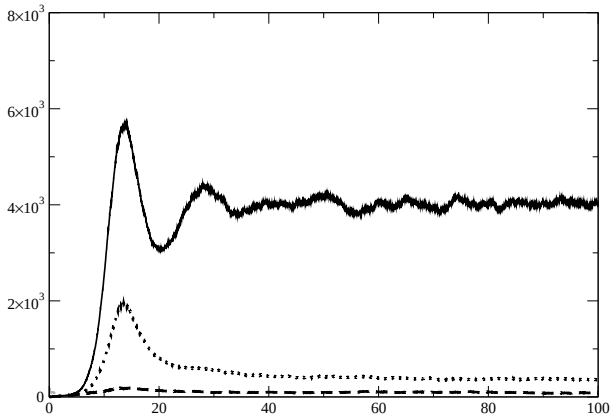

Fig. 1.6. Time evolution of the size of three clones one of which (continuous line) has higher affinity to a given antigen

Nevertheless even in this situation the competition for the peptides on the APC surface leads to a control in the overall number of T cells, at least when the number of clones is large. To study this effect we introduce an antigen with constant concentration, to which 10 clones have maximal affinity. Once

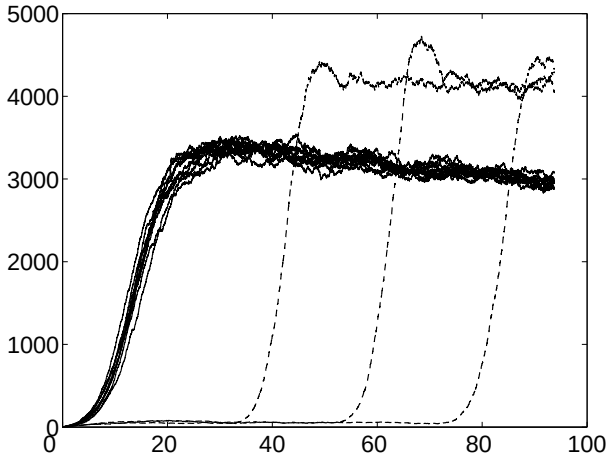

Fig. 1.7. Time evolution of the size of 10 clones (continuous curves) stimulated by a single antigen and shrinkage due to the expansion of three new clones (not continuous)

these clones have reached their equilibrium size, we introduce three different additional antigens at which three new clones are highly affine. The results of figure 1.7 show that the size of the "old" clones shrinks as a a reaction to the growth of the new ones.

1.4 Conclusions

Analytical models and simulations are usually treated as completely distinct fields of research, even when they face the same problem. In this paper we have presented a microscopic dynamical model inspired by the clonal expansion in the immune system, together with a system of differential equations that could be interpreted as its mean field theory. We have shown how the mean field equation can be used to interpret the results of simulations, while the microscopic model can be used to add a local and spatial character to a macroscopic system based on differential equations.
We don't claim that the results of our model are biologically relevant, but we present it as a starting point for a more complex model and as a solution for a compromise between pure analytical and pure simulated models that could be used in different fields of research.

1.5 Appendix

The dynamics of the model can be described by a simplified system of differential equations for A and T. We assume that the antigen-APC-T average

interaction consists of a growth term for the T clone proportional to A. The equations become

$$\dot{A} = A(a(1 - cA) - bT) \qquad \dot{T} = T(-d + eA - fT) \tag{1.15}$$

These Lotka-Volterra equations with a logistic term have been extensively investigated and if $e > cd$ they exhibit a critical stable point

$$T_c = \frac{a(e - cd)}{eb + caf} \qquad A_c = \frac{af + db}{eb + caf} \tag{1.16}$$

Every solution in the positive sector $T > 0$ $A > 0$ is attracted by this point which is topologically a focus. Convergence rate to equilibrium and the oscillations period are determined by the eigenvalues of the Jacobian matrix. From its trace and determinant

$$\text{Tr } J = -a\frac{acf + bcd + ef - cdf}{eb + acf} < 0 \qquad \det J = \frac{a(e - cd)(bd + af)}{eb + acf} > 0 \tag{1.17}$$

we obtain the eigenvalues $\lambda_\pm = \frac{1}{2}[\,\text{Tr}\, J \pm \sqrt{\text{Tr}\, J^2 - 4\det J}\,]$ which are real negatives or complex with negative real part. We have oscillations if is $\Delta = trJ^2 - 4detJ = -\omega^2 < 0$ and their period is $2\pi/\omega$.

References

1. R. De Boer, A. S. Perelson *T cells repertoires and competitive exclusion* J. Theor. Biol. **169**, 375-390 (1994).
2. J. Hofbauer, K. Sigmund *Evolutionary games and population dynamics* Cambridge University Press (1998)
3. M. Novak, R. May, K. Sigmund *Immune responses against multiple epitopes* J. Theor. Biol. **175**, 325-350 (1994).
4. R. Antia, V. Ganusov, R. Ahmed *The role of models in understanding CD8$^+$ T-cell memory* Nature Reviews Immunology, published online 20 January 2005
5. R. De Boer, A. S. Perelson *Competitive control of the self renewing T cell repertoire* International Immunology, Vol. 9, No. 5, pp. 779, 1997 Oxford University Press
6. A. Lanzavecchia, F. Sallustio *Lead and follow: the dance of the dentritic cell and T cell* Nature Immunology Vol. 5 No.12, 1201-1202 (2004)
7. S. Hugues, L. Fetler, L. Bonifaz, J. Helft, F. Amblard, S. Amigorena *Distinct T cell dynamics in lymph nodes during the induction of tolerance and immunity* Nature Immunology Vol. 5 No.12, 1235-1242 (2004)
8. R. Lindquist, G. Shakhar, D. Dudziak, H. Wardemann, T. Eisenreich, M. Dustin, M. Nussenzweig *Visualizing dendritic cell networks in vivo* Nature Immunology Vol. 5 No.12, 1243-1247 (2004)
9. R. Germain, M. Jenkins *In vivo antigen presentation* Current opinion in Immunology **16** 120-125
10. R. Callard, J. Stark, A. Yates *Fratricide: a mechanism for T memory-cell homeostasis* TRENDS in Immunology Vol.24 No.7, 370-375 (2003)