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Regulatory Process of T-cells in the Thymus

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Resumen

La dinámica del desarrollo celular en el timo es afectada por las interacciones de las diferentes subpoblaciones celulares. Experimentos recientes sugieren que las células maduras T, podrían afectar el crecimiento y diferenciación de los timocitos inmaduros. Aqui se presenta el análisis y modelamiento matemático sostenido con simulaciones computacionales que muestran el proceso de regulación celular. Nuestros resultados sugieren que cuando proporcionamos externamente células del tipo $CD4^+$ T, estas afectan positivamente a la célula simple y positiva $CD4^+CD8^-$ (subpoblación timocita), incrementando la diferenciación de las células doble positivo y reduciendo las células tipo $CD4^+CD8^-$.

Palabras Clave: *Timo, células T, células $CD4^+$, dinámica.*

Abstract

The dynamic of cell development in the thymus is affected by the interactions of the different subpopulations. Recent experiments suggest that mature T cells may affect the growth and differentiation of immature thymocytes. Here is presented mathematical analysis and modeling with computer simulations to present the process of regulation. Our results suggest that when we externally add $CD4^+$ T cells affect positively the single positive $CD4^+CD8^-$ thymocyte subpopulation, by increasing the differentiation of double positive cells ($CD4^+CD8^+$) and reducing $CD4^+CD8^-$ cells.

Keywords: *Thymus, T cells, $CD4^+$ cells, cell dynamic.*

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1. Introduction

The study of the immune system of the human body is critically important to better overall general health. That is why the thymus gland and its processes are a relevant subject of research. The thymus gland is located in the chest under the breastbone and its primary function is processing white blood cells, coming from the bone marrow, into T lymphocytes (T cells). T cells are distinguished by the presence on their surface of one or the other of designated glycoproteins $CD4$ and $CD8$. These thymic lymphocytes stimulate the production and growth of antibodies by other lymphocytes, they stimulate the growth and action of phagocytes that surround and engulf invading viruses and microbes, and they recognize and destroy abnormal and foreign tissue.
The generation of T cells in the thymus involves different cell populations from progenitors to mature cell types. The interactions of these cells affect the development of T cells and recent studies suggest feedback effects by which mature cells affect the generation of new T cells (*Fridkis-Hareli et al., 1993, 1994; Eren et al.,1989; Sharp et al., 1991, 1995*).
T cell development process begins with lymphohemopoietic cells coming from the bone marrow and settling in the thymic cortex. Settled cells are described as "double negative"(DN) because they lack the $CD4$ and $CD8$ glycoprotein of mature cells. When these cells divide eventually synthesize and express both the $CD4$ and $CD8$ markers, becoming "double positive"(DP) thymocytes. In this stage cells complete the rearrangement and expression of their specific antigen that will enable them to bind to the T cell receptor (TCR). Depending on the strength and context of the signal the cell receives from such binding, a developing DP thymocyte may be deleted or develop further into a "single positive"thymocyte of the type $CD4^+CD8^-$ or $CD4^-CD8^+$. These are the precursors of mature T cells of these two types. Positive and negative selection are the processes responsible for the death of most thymocytes and maturation of a few.
Previous investigations in this topic presented the dynamics of thymic cell development, without focusing in the feedback effects of the mature cells and biological experiments with mature cells. (*Feedback Regulation of T Cell Development in the Thymus, Mehr R., et al., 1996*) Then, the question is whether the presence of mature T cells affects thymocyte development the thymus. These question is supported with evidence from experiments were immature T cells (thymocytes) and mature T cells (splenocytes) were seeded onto fetal thymus explants. The results of these experiments demonstrated elevated levels of $CD4^+$ thymocytes from the progenitor cell origin were obtained when $CD4^+CD8^-$ splenocytes were seeded with DN thymocytes. Which gives the question of which cell compartment(s) are most likely affected by the presence of splenocytes, and how this interaction affects the parameters in the model (for example; growth or death rates).

2. The Model

The model present the dynamics of thymocyte differentiation. The thymic population is divided in subpopulations defined by the expression of $CD4$ and $CD8$ cell surface glycopro-

teins named double negative (DN), double positive (DP), single positive $CD4^+$ and $CD8^+$.

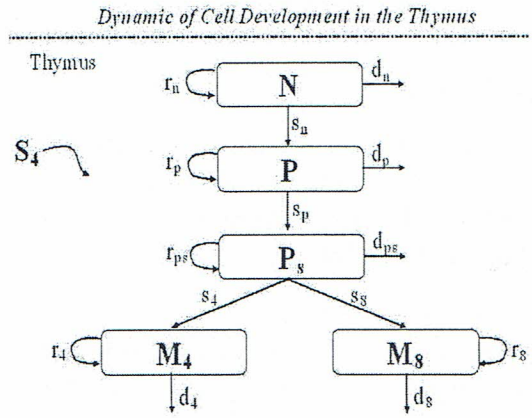

Figura 1: Scheme representing T-cell development, interactions between thymic subpopulations.

2.1. Model Description

The model representation with parameters present the subpopulations with the following variables, N: double negative cells (DN), P: double positive cells (DP) that are not sensitive to deletion, P_s: double positive cells (DP) that are sensitive to deletion, M_4: maturing single positive $CD4^+CD^-$ cells, M_8: maturing single positive $CD4^-CD8^+$ cells. The division in two compartments of the double positive cells is based on the hypothesis that mature DP cells are less resistant to deletion by negative selection. Also, there is a maximum number of cells possible in the thymus, which is based on data indicating that the total number of thymic cells is autonomously controlled. Since there is lack of information on this control, all the compartments (except DN) are similarly affected by competition. Only the DN compartment has a separate upper bound. The parameters s_i represent maturation rates, or rate of passage from one compartment to the next. The parameters r_i represent cell division rates, and d_i represent rates of cell death.
It is important to clarify that for this model there is no input of cells except during the initial seeding, and there is no output of mature T cells from the thymus since it is not connected to a blood circulation.

3. Mathematical Model

Our goal is to arrive at a model that would enable us to study mature T cells effects on thymic differentiation in a cell population model.

3.1. The Standard Model

This mathematical model is based on a formulation given first for [1] when there is no consideration of effect due to external influence and on a second work [2] where is taken in account the mature T cells effects. For the earliest work, the Thymic subpopulations of cells are represented for the following variables:

N	:	double negative (DN) cells
P	:	early double positive (DP) cells not sensitive to deletion
P_s	:	double positive (DP) cells sensitive to deletion
M_4	:	maturing single positive $CD4^+CD^-$ cells
M_8	:	maturing single positive $CD4^-CD^+$ cells
S_4	:	externally added $CD4^+$ spleen cells

An upper bound, K, for the total number of cells in the thymus is assumed to exist. This is based on data indicating that the total number of thymocites is autonomously controlled (Metcalf, 1963). The number of DN cells (N) is subject to a separate upper bound $K_n \ll K$. The equation for the time evolution of thymic subpopulations in this model are:

$$\begin{aligned}
\dot{N} &= \left(1 - \tfrac{N}{K_n}\right) r_n N - (d_n + s_n)N \\
\dot{P} &= s_n N + \left(1 - \tfrac{T}{K}\right) r_p P - (d_p + s_p)N \\
\dot{P}_s &= s_p P + \left(1 - \tfrac{T}{K}\right) r_{ps} P_s - (d_{ps} + s_4 + s_8)P_s \\
\dot{M}_4 &= s_4 P_s + \left(1 - \tfrac{T}{K}\right) r_4 M_4 - d_4 M_4 \\
\dot{M}_8 &= s_8 P_s + \left(1 - \tfrac{T}{K}\right) r_8 M_8 - d_8 M_8
\end{aligned}$$

where

$$T \equiv N + P + P_s + M_4 + M_8$$

For $i = n, p, ps, 4, 8$, the parameters are given by :

r_i	:	cell division rate
s_i	:	maturation rates or rates of differentiation (passage) from one compartment to the next
d_i	:	cell death rates

In this model, there is no input of cells except during the initial seeding and there is no output of mature T cells from the thymus.

3.2. The Model with Effects

By [2] the model verify the experimental results only if it was assumed that the effect of mature $CD4^+$ T cells on thymocite development is exerted through two separate processes: *negative* regulation of the proliferation of early thymocite (DN and DP); and *positive* regulation of the DP cells differentiating into the $CD4^+$ lineage. These both effects has to be assumed in the next model and they are given by the introduction of the *modifying* fucntions :

$$F_{rp} = 1 + H_{rp}\frac{S_4 + M_4}{S_4 + M_4 + K_4}$$

which multiplying by r_p is used to study effects on proliferation of DP thymocites. And, to study the effects on death of $CD4^+SP$, d_4 is multiplied by:

$$F_{d4} = 1 + H_{d4}\frac{S_4 + M_4}{S_4 + M_4 + K_4}$$

where for $i = rp, d4$, H_i are the parameters that change the direction and magnitude of the effects and K_4 is the half-maximum 'constant'depending on the population size.
To explore these effects , we examined the following model

$$\begin{aligned}
\dot{N} &= \left(1 - \tfrac{N}{K_n}\right) r_n N - (d_n + s_n)N \\
\dot{P} &= s_n N + \left(1 - \tfrac{T}{K}\right) F_{rp} r_p P - (d_p + s_p)N \\
\dot{P}_s &= s_p P + \left(1 - \tfrac{T}{K}\right) r_{ps} P_s - (d_{ps} + s_4 + s_8)P_s \\
\dot{M}_4 &= s_4 P_s + \left(1 - \tfrac{T}{K}\right) r_4 M_4 - F_{d4} d_4 M_4 \\
\dot{M}_8 &= s_8 P_s + \left(1 - \tfrac{T}{K}\right) r_8 M_8 - d_8 M_8 \\
\dot{S}_4 &= \left(1 - \tfrac{T}{K}\right) r_s S_4 - d_s S_4
\end{aligned}$$

where

$$T \equiv N + P + P_s + M_4 + M_8 + S_4$$

with
S_4 : externally added $CD4^+$spleen cells and the same variables, constants and parameters described above.

3.2.1. Steady State Analysis

We are interesting to find non trivial steady states in the positive orthant by the nature of the problem. We are going to use the *quasi steady-state* approximation.
To obtain $\left(1-\frac{T}{K}\right)$ as an explicit function of N and P we have to solve a sixth order algebraic equation. Getting conditions to reduce our sixth-equation system of differential equations to the first two and then finding the steady state of the last system, we can construct a quasi steady-state for our original system under appropiate conditions and variation of the parameters. This last is possible because of the conservation of behavior of the steady-states of our system to study with the quasi steady-state to be constructed in the way above mentioned.

Lemma 3.1 *Assuming the following conditions:*

•

$$\dot{P}_s, \dot{M}_4, \dot{M}_8, \dot{S}_4 \approx 0$$

• *relaxation of the logistic limits*

$$\left(1-\frac{T}{K}\right) \approx 1$$

$$\left(1-\frac{K_4}{S_4+M_4+K_4}\right) \approx 1$$

the system (2) *will be written in a simpler form in order to approximate its steady state.*

Proof
Under the given conditions , the system (2) takes the following form

$$\begin{aligned}
\dot{N} &= \left(1-\tfrac{N}{K_n}\right) r_n N - (d_n + s_n)N \\
\dot{P} &= s_n N + F_{rp} r_p P - (d_p + s_p)N \\
\dot{P}_s &= s_p P + r_{ps} P_s - (d_{ps} + s_4 + s_8)P_s \\
\dot{M}_4 &= s_4 P_s + r_4 M_4 - F_{d4} d_4 M_4 \\
\dot{M}_8 &= s_8 P_s + r_8 M_8 - d_8 M_8 \\
\dot{S}_4 &= r_s S_4 - d_s S_4
\end{aligned}$$

We can check that we linearize the right-hand side of the last four equations, then the steady state of the subpopulations P_s, M_4, M_8, S_4 will be written in terms of N and P.

Lemma 3.2 *Under the conditions given by* Lemma 1 *and the following conditions over the parameters*

$$\begin{aligned} d_{ps} + s_4 + s_8 &> r_{ps} \\ (1 + H_{d4})d_4 &> r_4 \\ d_8 &> r_8 \\ d_s &> r_s \\ r_n &> d_n + s_n \\ d_p + s_p &> (1 + H_{rp})r_p \end{aligned}$$

we get a quasi steady-state for the system (2).

Proof

By the *Lemma 1*, we get the steady state of the system (3), solving first the steady state of the first two differential equation and then expressing the other four variables in terms of this steady state.

Solving the following algebraic equations

$$\begin{aligned} s_r P + r_{ps} P_s - (d_{ps} + s_4 + s_8)P_s &= 0 \\ s_4 P_s + r_4 M_4 - F_{d4} d_4 M_4 &= 0 \\ s_8 P_s + r_8 M_8 - (d_8 M_8 &= 0 \\ r_s S_4 - d_s S_4 &= 0 \end{aligned}$$

we get

$$\begin{aligned} P_s &= \frac{s_p}{d_{ps}+s_4+s_8-r_{ps}} P \\ M_4 &= \frac{s_4}{(1+H_{d4})d_4-r_4} P_s \\ M_8 &= \frac{s_8}{d_8-r_8} P_s \\ S_4 &= 0 \end{aligned}$$

with

$$\begin{aligned} d_{ps} + s_4 + s_8 &> r_{ps} \\ (1 + H_{d4})d_4 &> r_4 \\ d_8 &> r_8 \end{aligned}$$

Now finding the steady state (N, P) of the first two differential equations we get

$$\begin{aligned} N &= \frac{K_n}{r_n}(r_n - d_n - s_n) \\ P &= \frac{s_n K_n (r_n - d_n - s_n)}{r_n (d_p + s_p - (1+H_{rp}) r_p)} \end{aligned}$$

Then we get the quasi steady-state for the system (2) given by

$$\begin{aligned} N &= \frac{K_n}{r_n}(r_n - d_n - s_n) \\ P &= \frac{s_n K_n (r_n - d_n - s_n)}{r_n (d_p + s_p - (1+H_{rp}) r_p)} \\ P_s &= \frac{s_p s_n K_n (r_n - d_n - s_n)}{r_n (d_p + s_p - (1+H_{rp}) r_p)(d_{ps} + s_4 + s_8 - r_{ps})} \\ M_4 &= \frac{s_4 s_p s_n K_n (r_n - d_n - s_n)}{r_n ((1+H_{d4}) d_4 - r_4)(d_p + s_p - (1+H_{rp}) r_p)(d_{ps} + s_4 + s_8 - r_{ps})} \\ M_8 &= \frac{s_8 s_p s_n K_n (r_n - d_n - s_n)}{r_n (d_8 - r_8)(1+H_{d4}) d_4 - r_4)(d_p + s_p - (1+H_{rp}) r_p)(d_{ps} + s_4 + s_8 - r_{ps})} \\ S_4 &= 0 \end{aligned}$$

Proposition 3.3 *Under the hypothesis of* Lemma 1 *and* Lemma 2, *the quasi steady state of the system* (2) *is stable.*

Proof
The eigenvalues of the Jacobian matrix (a diagonal matrix) for the system (3) evaluated in the quasi-steady state are

$$\begin{aligned} \lambda_1 = -(r_n - d_n - s_n) &< 0 \\ \lambda_2 = (1 + H_{rp}) r_p - (d_p + s_p) &< 0 \\ \lambda_3 + r_{ps} - (d_{ps} + s_4 + s_8) &< 0 \\ \lambda_4 = r_4 - (1 + H_{d4}) d_4 &< 0 \\ \lambda_5 = r_8 - d_8 &< 0 \\ \lambda_6 = r_s - d_s &< 0 \end{aligned}$$

then the quasi steady state given by *Lemma 2* is stable under the conditions that satisfy the parameters.

4. Simulation Results

We used the following values of parameters given by [1].

Parameter	Value
K	2×10^5
r_n	1,5
d_n	0
s_n	1
r_p	1,25
d_p	,6
s_p	,5
r_{ps}	0
d_{ps}	,8
K_n	7×10^4
s_4	,3
s_8	1
r_4	0
r_8	0
d_4	,6
d_8	,6
r_s	0
d_s	,12

Fig (1) and fig (2) shows the results of the model with out externally added $CD4^+$ spleen cells. These results demonstrate that our simple model, eqn $(1-5)$, which does not include any effects of mature T cells on the developing thymocytes, is insufficient for explaining the differences between thymocyte differentiation in the presence and absence of mature T cells. We conclude from these results that the differences in the experiments are due to some type of interaction, direct or indirect between the mature splenocytes and DN cells, and not only due to competition between the various sub populations. We also study the effect of some parameters on the dynamics of T cells. Figure (3) and (4) shows the results of the model with externally added $CD4^+$ spleen cells. Figure (3) shows the dynamics of all the populations on one graph verses time and figure (4) shows the subplots of all the populations against time. Now we will see the effect of the critical parameters H_{d4} and H_{rp}. In fig (3) and fig (4), H_d4 and H_rp are both negatives with $H_{d4} = -1$ and $H_{rp} = -0{,}5$. It is clear that the dynamics of fig (3) is same as of in fig (1) for first five equations. Now if we change H_{d4} from -1 to $+1$ while keeping Hrp same, then as shown in fig (5) that M_4 changes its dynamics. First M_4 increases and then decreases before going towards steady state. Also S_4 changes by increasing before going to steady state. But if we change Hrp to 0,5 instead of $-0,5$ the there is no change at all, you can see it in fig (6). If both H_{d4} and H_{rp} are taken to be positive,

then fig (7) and fig (5) have same dynamics.

5. Conclusions

So we notice that the parameter H_{d4} is critical. If we change Hd4 from negative to positive, we will have different dynamics. Which confirm from the lab results. It proves the system is more sensitive for the parameter H_{d4} as compare with H_{rp}. To consider the variation of the other rates of division cell and/or death , it means the addition of modifying functions, is not biological realistic. Mathematically, there are more ways to approximate the steady-state of this sixth differential equations system, they refine this approximation.

Referencias

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Appendix A: Matlab file used to solve the System

function thymus

$K = 2 * 10^5; rn = 1{,}5; dn = 0; sn = 1; rp = 1{,}25; dp = {,}6; sp = {,}5; rps = 0;$

$Kn = 7 * 10^4; dps = {,}8; s4 = {,}3; s8 = 1; r4 = 0; d4 = {,}6; r8 = 0; d8 = {,}6;$

$Hd4 = -1; Hrp = -{,}5; te = 0 : {,}1 : 60; rs = 0; ds = {,}12;$

$[T1, Y1]$=ode15s(@antibiotic1,te,[7*$10^4 10^3 10^3\ 10^3 10^3\ 10^3$], [], $K, rn, dn, sn, rs, ds,$
$rp, dp, sp, rps, Kn, dps, s4, s8, r4, r8, d4, d8, Hd4, Hrp$);

Total= $Y1(:,1) + Y1(:,2) + Y1(:,3) + Y1(:,4) + Y1(:,5) + Y1(:,6)$;

figure(1),clf for $k = 1 : 6$

subplot (6,1,k), plot (T1,Y1(:,k))

xlabel('time')

end figure(2) plot(T1,Total,'–r','lineWidth',2);

hold on

plot(T1,Y1(:,1),'k')

hold on

plot(T1,Y1(:,2),'r')

hold on

plot(T1,Y1(:,3),'b','lineWidth',2) hold on plot(T1,Y1(:,4),'g')

hold on

plot(T1,Y1(:,5),'–b') hold on plot(T1,Y1(:,6),'m')

hold on

legend('T','Ñ','P','Ps','M4','M8','S4')

function

[$Ydot$] =antibiotic1(t,Y,K,rn,dn,sn,rp,dp,rs,ds,sp,rps,Kn,dps,s4,s8,
r4,r8,d4,d8,Hd4,Hrp)

Ydot=Y;

T=Ydot(1)+Ydot(2)+Ydot(3)+Ydot(4)+Ydot(5)+Ydot(6);

K4=.05*T;

Frp=1+Hrp*(Y(4)+Y(6))/(Y(4)+Y(6)+K4);

Fd4=1+Hd4*(Y(4)+Y(6))/(Y(4)+Y(6)+K4);

Ydot(1)=(1-Y(1)/Kn)*rn*Y(1)-(dn+sn)*Y(1);

Ydot(2)=sn*Y(1)+(1-T/K)*Frp*rp*Y(2)-(dp+sp)*Y(2);

Ydot(3)=sp*Y(2)+(1-T/K)*rps*Y(3)-(dps+s4+s8)*Y(3);

Ydot(4)=s4*Y(3)+(1-T/K)*r4*Y(4)-Fd4*d4*Y(4);

Ydot(5)=s8*Y(3)+(1-T/K)*r8*Y(5)-d8*Y(5);

```
Ydot(6)=(1-T/K)*rs*Y(6)-ds*Y(6);
```

Appendix B: Simulations

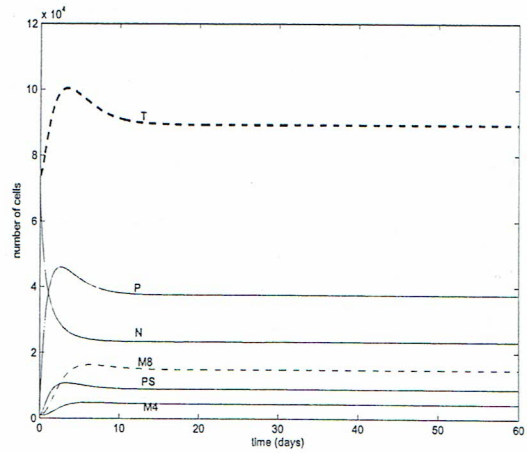

Figura 2: Computer simulation for the system without external added $CD4^+$

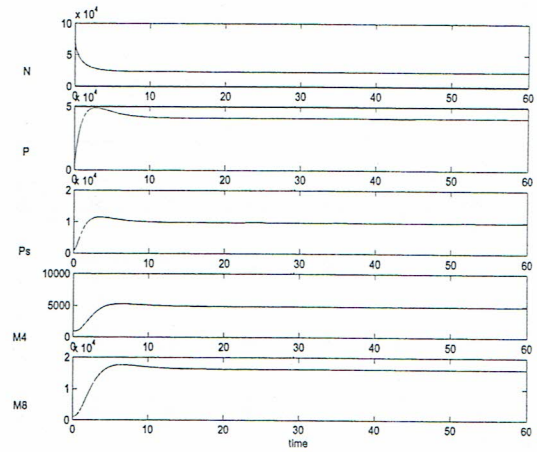

Figura 3: Computer simulation for the system without external added $CD4^+$

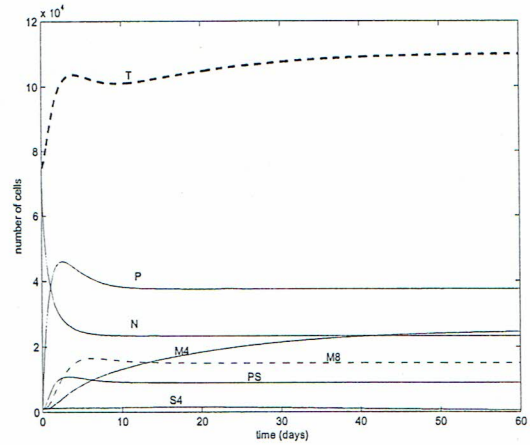

Figura 4: Computer simulation for the system with external added $CD4^+$

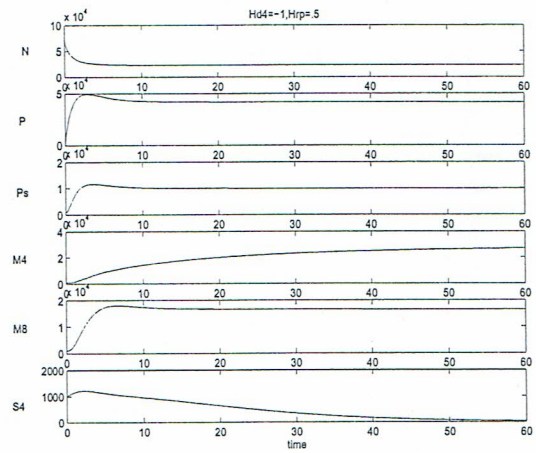

Figura 5: Computer simulation for the system with external added $CD4^+$

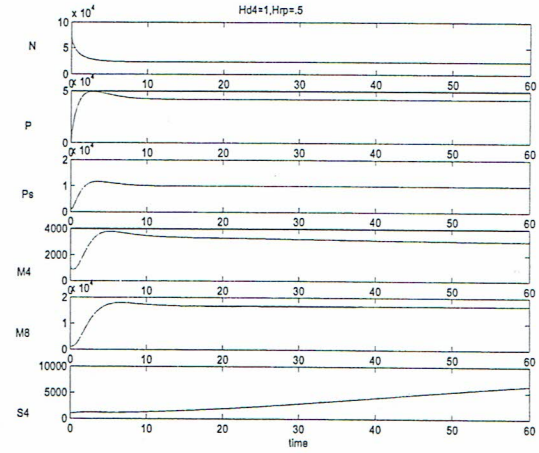

Figura 6: Computer simulation for the system with external added $CD4^+$

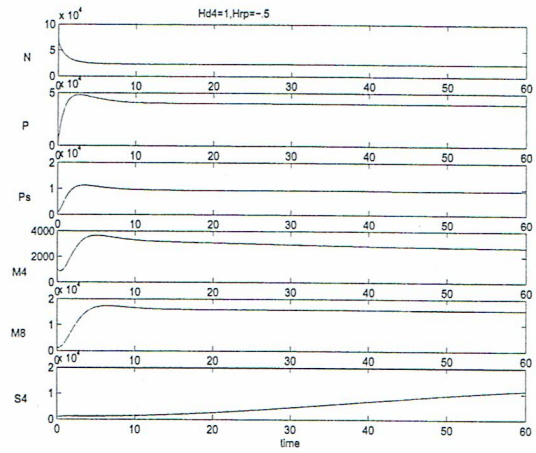

Figura 7: Computer simulation for the system with external added $CD4^+$

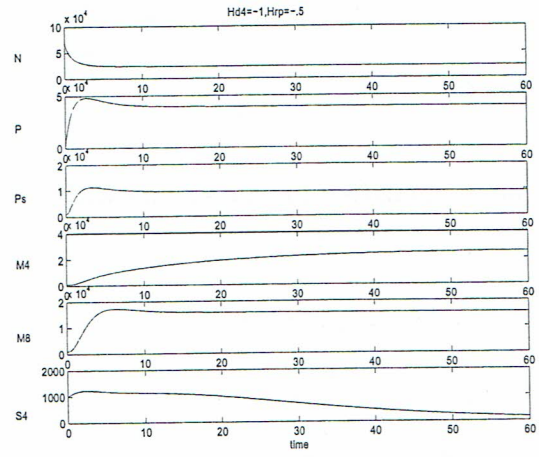

Figura 8: Computer simulation for the system with external added $CD4^+$