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ON THE INTERACTION BETWEEN THE IMMUNE SYSTEM AND AN EXPONENTIALLY REPLICATING PATHOGEN

Alberto d'Onofrio

Department of Experimental Oncology, European Institute of Oncology
Via Ripamonti 435, 20141 Milan, Italy

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Abstract. In this work, we generalize the Pugliese-Gandolfi Model [*A. Pugliese and A. Gandolfi, Math Biosc, 214,73 (2008)*] of interaction between an exponentially replicating pathogen and the immune system. After the generalization, we study the properties of boundedness and unboundedness of the solutions, and we also give a condition for the global eradication as well as for the onset of sustained oscillations. Then, we study the condition for the uniqueness of the arising limit cycle, with numerical applications to the Pugliese-Gandolfi model. By means of simulations, we also show some alternative ways to reaching the elimination of the pathogen and interesting effects linked to variations in aspecific immune response. After shortly studying some pathological cases of interest, we include in our model distributed and constant delays and we show that also delays may unstabilize the equilibria.

1. **Introduction.** Oscillations are among the core business of physiological processes, at all spatial and temporal scales [1]: from cell division cycle to circadian oscillations up to heart beats. Moreover, many interesting examples of oscillations are observed in cases of pathologies.

In particular, Mackey and coworkers were among the first to use the expression "dynamical diseases" [2, 3] to indicate some pathological states which, in terms of the behavior of their specific biological variables, are not characterized by steady states or by exponential explosions, but by persistent oscillations of various kinds (periodic, quasiperiodic or chaotic).

In particular, as stressed and documented by Stark et al. in their recent excellent review on oscillations in immune system[4], in the experimental and clinical immunology literature wide range of damped and also persistent oscillations is observed: from malaric periodic fevers [4] to tumor-immune system interactions [5, 6, 7, 8], passing through cyclic neutropenia[9, 4, 10] and, of course, to the response to antigens [4, 11, 12, 13, 14].

The possible causes of the arising of these oscillations have been theoretically investigated, and particular stress has been given to the possible role of delays [10, 15, 16, 17, 18], since it is well known that a key mechanism for the insurgence of oscillations in many biological interactions is the presence of delays [19, 20, 21], and, in particular, delays are a fundamental trigger of oscillations in the above mentioned 'dynamical diseases' [20, 3, 19].

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Also in tumour immuno-biology delays have been explored as sources of oscillations. For example, Galach [17] studied the role of delays in the tumour-stimulated proliferation of effectors by adding a constant lag in the corresponding term of the model by Kutsnetsov et al. [22], and Villasana and Radunskaja also proposed a delayed model of tumour-immune system interplay [15] huilt on the Kirschner-Panetta model [5].

However, among the causes of these phenomena there may also be the interactions, in absence of delays, of the involved populations: the immune system effectors and proteins and the nonself pathogens, for example the tumor cells. Indeed, delay independent oscillations have theoretically been shown in some papers with reference to tumor-immune system interaction [5, 23, 24] . In particular, it was studied in [24] whether the limit cycle arising from the instabilization of the equilibrium state between immune system and tumor cells is unique. However, as stressed above, cycles may arise in response of the immune system to more general pathogens, and the existence and uniqueness of limit cycle for a simple antibody-antigen model of immune system was studied in [25].

In [26] Pugliese and Gandolfi proposed an interesting bidimensional model of the interaction between an exponentially proliferating pathogen and the immune system, which takes into account both aspecific and specific immunity, and which was able to reproduce all the known basic phenomena of this interaction, including sustained oscillations. However, no further investigation was done on these periodic variations, neither on the conditions leading to the global eradication of the pathogen by the immune effectors.

However, the Pugliese-Gandolfi model is based on very strong assumptions on the specific effects, at the population scale, of the interactions between immune system and pathogen, assumptions translated in the use of specific functions modelling the action of the innate immune system, on the destroying activity of the effectors on the pathogen, and on the stimulation of the proliferation of immune cells by the presence of the pathogen. Is this acceptable given the general pourpose of the model and the wide extension of the diseases that may be modelled through this model ? Thus, in this work, following our previous studies on tumors, we will analytically study this topic within the mathematical framework of the approach, introduced by us in Refs. [23, 24], based on a *family* of finite-dimensional models, instead of the classic finite-dimensional approach based on *specific* models with constant parameters to be tuned. Indeed, the approach based on meta-models (where meta-model means a family of models) is a natural way to capture the common features of such a wide family of immune interactions, and it allows to get inferences that are independent on the specific choice of the functions representing the various interactions between the two populations. In this general framework we shall give conditions for the global eradicability of the pathogen. We shall then focus on the assessment of the uniqueness and, in some cases, global attractiveness of the limit cycle, which is a topic of interest not only from a mathematical but also from a biological point of view. Numerical simulations on the specific Pugliese-Gandolfi model will be performed to assess the influence of the various biologically relevant parameters on the amplitude of the oscillations. After briefly analysing some relevant cases of deranging of the standard behavior of the immune system, we finally also investigate the impact of delays by focusing in lags in the pathogen-stimulated proliferation of effectors. The effect of time delay in this process is taken into account to approximate missing dynamical components such as chemical

signals, B-lymphocytes maturation and activation of T-lymphocytes[27]. Immune system needs time, indeed, to identify the pathogens and to adequately react[28, 17]. It is also important to note that cell division length for IS effectors may range between 0.9 and 12 days [27, 15]. Here we shall focus on two particularly relevant patterns of delays: constant lags and exponentially distributed lags. In both cases we shall show that if the delay exceeds a threshold there is the onset of oscillations. To complement our analytical investigations, we also perform some numerical simulation on some specific instances of the proposed family of models.

2. **Modelling the interaction between a pathogen and the immune system.** In [26] Pugliese and Gandolfi proposed the following non-dimensional model for the dynamics of interaction between a pathogen and its host's immune response:

$$\begin{aligned} x' &= \alpha x - \frac{mx}{1+\beta_u x} - \frac{x}{1+\beta_s x} y \\ y' &= \frac{x}{1+\gamma x} y - y + \eta \end{aligned} \tag{1}$$

where $x(t)$ is the density of pathogen load, and $y(t)$ is the level of specific immunity, for example the density of specific T-cells or of antibodies. It is assumed that the pathogen is exponentially replicating with a net rate α (i.e. the net rate of positive growth for the pathogen population), which is related to the doubling time of the pathogen by the well known formula $\alpha = Ln(2)/T_d$. A characteristic feature of the system is that the immune predation is monotonically increasing with the pathogen burden up to a saturation level, namely a Holling type II functional response, for both the aspecific and the specific immune response. The a-specific response is modelled by: $mx/(1+\beta_u x)$ and the specific one by $yx/(1+\beta_s x)$. The replication of immune cells is stimulated by the presence of pathogen and up to a saturation again following a Holling type II function: $x/(1+\gamma x)$. The saturation level γ^{-1} is assumed to be in all cases greater than the death rate constant, which is equal to one, since the average life of the immune effectors is taken as time unit. Finally, there is a influx of effectors at a constant rate η. In [26], both the asymptotic and the transitory behavior of the system were studied, and it was analytically shown that periodic attractors may be possible.

Here we generalize the above equations (1) by considering the following quite general family of models:

$$\begin{aligned} x' &= x\left(\alpha - M(x) - \phi(x)\pi(y)\right) \\ y' &= -y + P(x)y + \eta(x) \end{aligned} \tag{2}$$

where:

- $M(x)$ is the rate of pathogen-killing by the innate immune system. We assume that it is a decreasing function of the pathogen load: $M'(x) < 0$. For simplifying the following calculations we, without loss of information, deine the cojstant $m = M(0)$ and we assume that: $M(x) = \frac{m}{K_u(x)}$, with: $K_u'(x) > 0$ and (of course): $K_u(0) = 1$. We shall call m the baseline aspecific immune reaction;
- $K_u(x)$ for large x is an infinitum of order $q \geq 1$: $\Theta(x^q)$ (where $b = \theta(a^q)$ stands for infinitum of order q, i.e. for large a it is $b \approx a^q$). Biologically this means that for large burden of pathogens either the rate of their elimination by the aspecific immune effectors reach a saturation level ($q = 1$) as in model (1), or it reduces and goes to zero ($q > 1$);

- $\phi(x)$ is the rate at which the pathogens are killed by the specific immune system, and it is a decreasing function of x: $\phi'(x) < 0$. As in the Gandolfi-Pugliese model, the system is non-dimensionalized so that the baseline specific immune reaction is equal to the unity: $\phi(0) = 1$. Again for the sake of the notation simplicity we set: $\phi(x) = \frac{1}{K_s(x)}$, $K_s'(x) > 0$. The latter might be the case of some pathogens that are able to infect macrophages [29, 30, 31];
- $K_s(x)$ for large x is an infinitum of order $f > 0$: $\Theta(x^f)$;
- The predation function $\pi(y)$, unlike eq. 1 is, in general, not linear, although it is assumed increasing: $\pi'(y) > 0$. Moreover, for large y we assume that $\pi(y) = \theta(y^\nu)$ with $\nu > 0$, in order to model, in addition to the baseline linear case ($\nu = 1$), possible *i)* decreased predation efficiency due to competitive inter-effectors interactions ($\nu < 1$) or increased efficiency due to cooperative interactions ($\nu > 1$);
- The average lifespan of the immune effectors is the referencee time used in the non-dimensionalization;
- $P(x)$ is the pathogen-stimulated proliferation rate of the immune effectors, and it is increasing with the pathogen load: $P'(x) > 0$, is null in absence of the pathogen: $P(0) = 0$, and for large loads it is anycase greater than the death rate of the effectors: $P(+\infty) > 1$. The last assumption implies that a $x_m > 0$ exists where $P(x_m) = 1$;
- $\eta(x)$ is the influx rate of effectors, and we assume (see for the oncologic context [32, 23] and references therein) that it is a non-increasing function of x due to the possible negative feedback of the pathogens on the efficiency of the immunopoietic system: $\eta'(x) \leq 0$. However, we assume that this negative influence of pathogen is not able to totally suppress the production of effectors: $\eta(+\infty) = \eta_\infty > 0$. We denote the baseline influx $\eta(0)$ as η_0;

We stress here that the above family of models (2), although similar, is quite different from the tumor-related families of models of [23, 24]. In fact, the function $M(x)$ makes the dynamics of the pathogen density $x(t)$ quite different. For example, in absence of the specific response, i.e. setting $y = 0$ and $\eta(x) = 0$, one gets:

$$x' = x(\alpha - M(x)) \tag{3}$$

where the relative growth rate is now an increasing function, instead of being constant or decreasing. This also implies the possibility that the pathogen may be cleared, in some cases, by the aspecific immunity.

Moreover, here the interaction between the pathogens and the immunity effectors does not affect the death rate of $y(t)$, which deeply affects the dynamics of the systems introduced in [23, 24].

Significant biological differences also exist with respect to the model (1) proposed in [26]. In particular, here the immune system functional responses

$$U(x) := x\phi(x) \ , \ V(x) := xM(x)$$

that represent the killing ability of the, respectively, specific and a-specific immune response are not necessarily saturating : they may also be non-monotone. An example of non-monotone $V(x)$ is

$$V(x) = \frac{mx}{1 + \beta_{u1}x + \beta_{u1}x^2}.$$

Moreover, the predation function $\pi(y)$ is no longer linear, as in Ref. [23], but it is a generic growing function. The nonlinear behavior of $\pi(y)$ has been introduced

to model competitive (e.g. $\nu < 1$) [33, 34] and/or cooperative (e.g. $\nu > 1$) inter-effectors interactions [35, 36].

As far as the biological and mathematical soundness of the family (2), it holds that:

Proposition 1. *The model (2) is well posed since $\mathbb{R}^2_+$ is positively invariant and its solutions are defined for $t \in [0, +\infty)$. Moreover, the set $S = \mathbb{R}_+ \times [\eta_\infty, +\infty)$ is positively invariant and attractive.*

Proof. The positive invariance follows from the fact hat, as it is easy to verify, the normal of the vector field associated to (2) is directed inwardly, in both the x and y non-negative semi-axes. Moreover, since :

$$x' < \alpha x$$

and

$$y' < P(\infty)y - y + \eta(0) \tag{4}$$

thus both the state variables are exponentially bounded, and as such the orbits are defined for all $t > 0$. Finally, the positive invariance and attractiveness of the set S follows from the differential inequality:

$$y' > -y + \eta_\infty \Rightarrow minlim_{t\to+\infty} y(t) \geq \eta_\infty$$

□

3. **Nullclines and boundness (and unboundness) of orbits.** In order to determine the critical points, we need to assess the properties of the nullclines. Setting $x' = 0$, and defining:

$$\widetilde{y}_C(x) = K_s(x)(\alpha - \frac{m}{K_u(x)}) \tag{5}$$

it yields that the x-nullcline is given by:

$$\pi(y_C(x)) = \widetilde{y}_C(x) \Rightarrow y_c(x) = \pi^{-1}\left(\widetilde{y}_C(x)\right). \tag{6}$$

We start noticing that $y_c(x)$ is increasing since:

$$\pi'(y)y'_C(x) = K'_s(x)(\alpha - \frac{m}{K_u(x)}) + K'_s(x)\frac{mK'_u(x)}{K^2_u(x)} > 0.$$

Moreover, as the growth rate α increases the x nullcline shifts upward, whereas as n increases the x nullcline shifts downward. Indeed:

$$\partial_\alpha y_C(x;\alpha,m) = \frac{K_s(x)}{\pi'(y)} > 0, \partial_m y_C(x;\alpha,m) = -\frac{K_s(x)}{K_u(x)\pi'(y)} < 0,$$

which is biologically reasonable.

Moreover, remembering that $K_s(x) = \Theta(x^f)$, whereas $\pi(y) = \Theta(y^\nu)$, it follows that $y_C(x) = \Theta(x^{f/\nu})$.

Finally, there is another trivial nullcline, the positive semi-axe: $x = 0$, $y \geq 0$.

Setting $y' = 0$, one gets the y-nullcline:

$$y_i(x) = \frac{\eta(x)}{1 - P(x)} \tag{7}$$

which has a vertical asymptote at x_m, and to which we add the further constraint:

$$y'_i(x) > 0 \text{ if } 0 \leq x < x_m \tag{8}$$

Proposition 2. *If*

$$\alpha < \frac{\nu}{f}\left(P(\infty)-1\right) \tag{9}$$

then the orbits of (2) are bounded, whereas if $f \geq 1$ *and*

$$\alpha > \nu\left(P(\infty)-1\right) \tag{10}$$

the orbits are unbounded and:

$$lim_{t\to+\infty}\left(x(t),y(t)\right) = (+\infty,+\infty).$$

Proof. From the differential inequality (4) it follows that:

$$y(t) \leq \widehat{y}(t) = -\frac{\eta(0)}{P(\infty)-1} + \left(y(0)+\frac{\eta(0)}{P(\infty)-1}\right) Exp\left(\left(P(\infty)-1\right)t\right) \tag{11}$$

implying:

$$x' > \alpha x - V_S - U_S \pi\left(\widehat{y}(t)\right). \tag{12}$$

where:

$$V_S = Supremum_{x\geq 0} V(x), U_S = Supremum_{x\geq 0} U(x).$$

Thus, since for large t it is:

$$\pi\left(\widehat{y}(t)\right) = \Theta\left(Exp\left(\nu\left(P(\infty)-1\right)t\right)\right),$$

it follows that if (10) holds then the orbits are unbounded.

To show that the constraint (9) implies the boundeness of the orbits, we shall construct a bounded set. Let us start from an initial point (x_0, y_0) lying on the lower border of S, i.e. with $y_0 = \eta_\infty$. Moreover, let us suppose that x_0 is sufficiently larger than x_m to be such that $P(x_0) = P(\infty) - \epsilon$ with $P(\infty) >> \epsilon$. Thus, the orbits will "start" and remain in the region $y < y_P(x)$ AND $x > c_m$ where $x' > 0$ and $y' > 0$. Thus, the initial branch of the orbit will lie there on a curve $Y(x)$ defined by:

$$\frac{dY}{dx} = \frac{(P(x)-1)Y(x)+\eta(x)}{x\left(\alpha - M(x)m - \phi(x)\pi(Y(x))\right)}$$

which, thanks to the fact that $x' > 0$ is such that:

$$\frac{dY}{dx} > \frac{\left(P(\infty)-1-\epsilon\right)Y(x)}{\alpha x} \Rightarrow Y(x) \geq \eta_\infty \left(\frac{x}{x_0}\right)^{\frac{P(\infty)-1-\epsilon}{\alpha}}.$$

Now, remembering that the x-nullcline is: $y_c(x)\ \theta(x^{f/\nu})$ if follows that if:

$$\frac{P(\infty)-1-\epsilon}{\alpha} > \frac{f}{\nu}$$

i.e. if (9) holds then the curve $(x, Y(x))$ crosses the x nullcline, let us say a to a point (x_A, y_A). Since now the orbit is in a region where $y' > 0$ and $x' < 0$, this orbit either crosses $y_i(x)$ or is such that

$$lim_{t\to+\infty}\left(x(t),y(t)\right) = (x_w,+\infty)$$

with $x_w > x_m$. However, in the second case it would be $x(t) \to 0^+$, contradicting the fact that $x_w > x_m$. thus, the orbit crosses the y nullcline at $(x_A, y_i(x_A))$ and enter in a region where $y' < 0$. As a consequence, we showed that the orbits are bounded. □

Remark 1. From (9) it follows that if $f << 1$, i.e. if the killing rate is $U(x) \approx kx$, then the orbits are bounded up to very high values of α, and similarly if $P(\infty) >> 1$. This means that a mass action law for killing of pathogens is enough to at least impede the exponential expansion of the pathogens, and the control is also reached if the replication rate of effectors is sufficiently stimulated.

Moreover, it holds that:

Proposition 3. *In the case $f \neq 1$, if the initial condition on the pathogen burden $X(0)$ is sufficiently large and:*

$$\alpha > \frac{\nu}{f}\left(P(\infty) - 1\right) \tag{13}$$

then the orbits are unbounded.

Proof. Proceeding as in the first part of the preceeding proof, we observe that for large times and large initial conditions since it has to be;

$$(C - \epsilon)x^f < K_s(x) < (C + \epsilon)x^f, 0 < M(x) < \epsilon_1$$

as a consequence it follows that:

$$x' > (\alpha - \epsilon_1)x - Ax^{1-f}\pi\left(Exp\left((P(\infty) - 1)t\right)\right). \tag{14}$$

So that $x(t) > w(t)$, where $w(t)$ is the solution of the differential equation:

$$w' = \alpha w - Aw^{1-f}\pi\left(Exp\left((P(\infty) - 1)t\right)\right)\ , w(0) = x(0)$$

which can be solved by transforming it the following linear ODE:

$$z' = f\alpha z - fA\pi\left(Exp\left((P(\infty) - 1)t\right)\right)\ , z(0) = x^f(0).$$

where: $z = w^f$.

Thus, being $\pi(u)$ an infinite of order $\nu > 0$, we have that if (13) holds then $z(t)$ is divergent, implying the unboundedness of $x(t)$ and in turn also of $y(t)$, i.e. the orbits are unbounded. □

Remark 2. Constraints (10) and (13) show that if the replication rate is sufficiently large, then there is the possibility of unbounded orbits.

4. **Attractors.** Since also $x = 0$ is a x-nullcline, we observe that all model belonging to our family have a 'pathogen-free' equilibrium point:

$$PFE = (0, \eta(0))$$

to which is associated the following linearized equation for x:

$$x_l' = \left(\alpha - m - \phi(0)\pi\left(\eta(0)\right)\right)x_l$$

implying that:

Proposition 4. *If:*

$$\alpha < m + \phi(0)\pi\left(\eta(0)\right), \tag{15}$$

i.e. if

$$y_i(0) > y_c(0) \tag{16}$$

then PFE is LAS. If

$$\alpha > m + \phi(0)\pi\left(\eta(0)\right), \tag{17}$$

i.e. if

$$y_i(0) < y_c(0) \tag{18}$$

then PFE is unstable.

and this corollary:

Corollary 1. *If $m > \alpha$ then the pathogen is locally eradicated.*

Remark 3. The above proposition provides the conditions needed in order that the immune system may neutralize small amounts of pathogens, which are summarized in constraint (15) whose biological meaning is straightforward: the sum of baseline killing rate of the aspecific and of the specific immunity weighted by $\pi(\eta(0))$ (i.e. by the 'killing efficiency' of the baseline amount of effectors) must be greater than the replication rate of the pathogen. The above corollary simply says that if the innate immunity itself is greater at the baseline than the replication rate of the pathogen, then the aspecific immune effectors are able to clear out small amounts of pathogens.

In absence of the specific immunity the dynamics of the pathogens density x would be ruled by equation (3), so that the behavior would be the following: *i)*if $\alpha > M(x)$ then $x(t) \to +\infty$ (i.e. the innate defense would only be active in decelerating the replication of the pathogen); *ii)* If $m > \alpha$ thus it exists a x_{inn} such that $M(x_{inn}) = \alpha$ and the behavior is dichotomic. If $0 < x(0) < x_{inn}$ then $x(t) \to 0$, if $x(0) > x_{inn}$ then the pathogen density diverges.

Coming back to the general case where both the immune reaction are present, depending on the specific functions $P(x)$, $K_u(x)$ etc... the model may have 0, 1, 2 or more other critical point. Of course, if $y_i(x) > y_c(x)$ there is only an equilibrium: the PFE, and:

Proposition 5. *If $y_i(x) > y_c(x)$ and (9) hold then PFE is globally asymptotically stable in $\mathbb{R}^2_+$*

Proof. If $y_i(x) > y_c(x)$ and (9) hold then the set

$$Q = \left\{(x, y) \in \mathbb{R}^2_+ | y > y_c(x)\right\}$$

is positively invariant and attractive. Thus, defining the following LaSalle-Liapounov function $L = x$ and applying the LaSalle's invariance principle, it easily follows our claim. □

Remark 4. Note that, although the pathogen free equilibrium does not depend on the parameter m, its stability may be influenced by the aspecific immune reaction that may induce not only local, but also global eradication of the pathogen since $\partial_m y_c(x; m) < 0$. The contribute of aspecific immunity in eradicating pathogens is illustrated in fig. 1 where we plotted the nullclines $y_i(x)$ and three instances of $y_c(x; \alpha, m)$, as well as an example of global eradication of the pathogen.

Let us consider a generic non-eradicative equilibrium $EQ = (x_e, y_e)$ with $x_e > 0$ (and $y_e > 0$). We start noticing that, since $y_c'(x) > 0$ and $y_i'(x_m) = +\infty$, it follows that if $y_i(0) > y_c(0)$ (i.e. if PFE is LAS) then there is an odd number of those nontrivial equilibria: 0,2,.... Moreover, by rewriting (2) in the following convenient form:

$$\begin{aligned} x' &= U(x)\left(\widetilde{y}_C(x) - \pi(y)\right) \\ y' &= \frac{\eta(x)}{y_i(x)}\left(y_i(x) - y\right) \end{aligned} \tag{19}$$

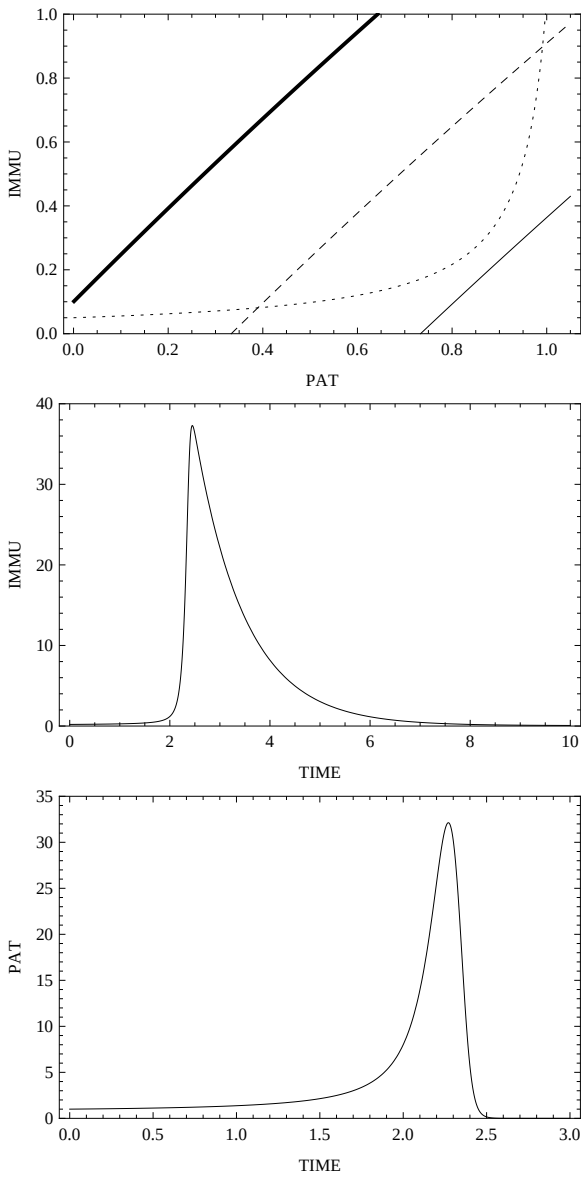

FIGURE 1. Contribute of aspecific immunity in eradicating pathogens. In upper panel, plot of the nullclines $y_i(x)$ (dotted lines) and of three instances of $y_c(x;\alpha,m)$ for $\alpha = 15$ and *i* $m = 14.9$ (thick solid line): unstable PFE; *ii)* $m = 15.5$ (dashed line): LAS PFE and *iii)* $m = 16.1$ (solid line): GAS PFE. In central and lower panels, respectively, plots of $x(t)$ and $y(t)$ for $m = 16.1$ and corresponding to the following 'non-small' initial condition: $(1, 0.3)$. Values of the other parameters: $\eta = 0.05$,$\gamma = 0.05$, $\beta_u = 0.1$ and $\beta_s = 0.001$.

after some algebra, one can show that the linearization of (2) has an associated characteristic polynomial $\lambda^2 + a_1\lambda + a_0$ where:

$$a_1 = \frac{\eta(x_e)}{y_i(x_e)} - \pi'(y_e)U(x_e)y'_C(x_e)$$

$$a_o = \frac{\eta(x_e)}{y_i(x_e)}\pi'(y_e)U(x_e)\left(y'_i(x_e) - y'_C(x_e)\right).$$

Thus:

Proposition 6. *If:*

$$y'_i(x_e) > y'_C(x_e) \ \textit{AND}\ y'_C(x_e) < \frac{\eta(x_e)}{\pi'(y_e)y_i(x_e)U(x_e)} \tag{20}$$

then EQ is locally asymptotically stable. IF

$$y'_i(x_e) < y'_C(x_e) \ \textit{OR}\ y'_C(x_e) > \frac{\eta(x_e)}{\pi'(y_e)y_i(x_e)U(x_e)} \tag{21}$$

then EQ is unstable.

Remark 5. The LAS condition (20) simply means that, in order that EQ may be LAS, the slope of the tangent line at $x = x_e$ of the y-nullcline must be greater than the slope of the tangent of the x-nullcline, and that this second slope must not be 'excessively large'.

Remark 6. Note that, in the case $\pi(y) = y$, if α is sufficiently large, then there is a unique equilibrium point $x_e \approx x_m$ that is unstable. In fact, the derivative of the x nullcline is increasing with α: $\partial_\alpha y'_C(x;\alpha) = k_s(x) > 0$.

Moreover, in case of unique non-trivial equilibrium point the Poincare' Bendixon thricotomy easily yields that:

Proposition 7. *Let us suppose that EQ is the unique non-trivial equilibrium and that (9) holds. Thus, if EQ is unstable with $a_0 > 0$ then EQ is surrounded by at least a locally stable limit cycle.*

Proof. Note preliminarily that we supposed that there are only two equilibria: PFE and EQ, i.e. for $x \in (0, xe) \cup (xe, x_m)$ there is no other intersection between the two null-clines. PFE is unstable and its stable manifold, as it is immediate to verify, is the y-axis. Furthermore, since $a1 < 0$ and $a0 > 0$ there are no homoclinic orbits. Thus, thanks to the Poincare'-Bendixon trichotomy it follows the existence of at least a LAS limit cycle. □

Remark 7. Note that if $a_0 < 0$ then the eigenvalues at $x = x_e$ are real and of opposite signs, so that there can be either a homoclinic orbit or a heteroclinic orbit linking EQ to PFE.

Finally, note that since, by applying the implicit functions theorem, one easily gets:

$$\frac{\partial x_e}{\partial m} = -\frac{1}{\varphi(x)K_u(x)}\frac{1}{y'_i(x_e) - y'_i(x_e)}\ ,\ \frac{\partial y_e}{\partial m} = y'_i(x_e)\frac{\partial x_e}{\partial m}$$

it follows that increasing the baseline aspecific immune reaction m decreases both the coordinates of of a LAS equilibrium, as well as those of an unstable equilibrium point around which a periodic orbit cycles. On the contrary, there is an increase of the coordinates of an unstable equilibrium.

5. **Uniqueness of the limit cycle and its dependance on the parameters.** Given a simple nonlinear ODE bi-dimensional model, however simple it may be, the determination of the number n of its limit cycles and, in particular, determining if $n = 1$ is a non trivial task. However, some significant theoretical results have been obtained in recent years by reduction of prey-predator models to equivalent Lienard's equations [37, 38]. Since we are dealing with a family of models, we limit to find Lienard' equations starting from the family 2 and then we state some generic propositions in which we simply translate theorems of the theory of limit cycles in terms of our biomedical problem. We shall also provide some numerical examples on the model 1, by showing that it may admit a unique and GAS limit cycle.

In the case of non-null immune influx ($\eta(x) > 0$) and $\pi(y) = y$, after defining the variable

$$u = ln(x) \tag{22}$$

and the functions:

$$\widehat{\phi}(u) = \phi\left(E^u\right) \ ,\widehat{y}_C(u) = y_C\left(E^u\right) \ ,\widehat{B}(u) = B\left(E^u\right) \ ,\widehat{\eta}(u) = \eta\left(E^u\right) \tag{23}$$

we may rewrite model 2 in the form:

$$\begin{aligned} u' &= \widehat{\phi}(u)(\widehat{y}_C(u) - y) \\ y' &= \widehat{B}(u)y + \widehat{\eta}(u) \end{aligned} \tag{24}$$

If we define:

$$d\tau = \widehat{\phi}(u(t))dt \ , \ A(u) = \frac{\widehat{B}(u)}{\widehat{\phi}(u)} \ , \ R(u) = \frac{\widehat{\eta}(u)}{\widehat{\phi}(u)}, \tag{25}$$

and eliminate the y variable, one obtains the following equivalent Lienard's equation:

$$u''(\tau) - \big((\widehat{y}'_C(u) + A(u)\big)u'(\tau) + A(u)\widehat{y}_C(u) + R(u) = 0, \tag{26}$$

i.e. a Lienard's equation with Lienard's functions:

$$f_L(u) = -\big((\widehat{y}'_C(u) + A(u)\big) \ , \ g_L(u) = A(u)\widehat{y}_C(u) + R(u). \tag{27}$$

Thus, one may apply various theorems for the uniqueness of limit cycle of (26). Among them, the application of Zhang's theorem [39] leads to the following proposition:

Proposition 8. *For model (24) with $\eta(x) > 0 >$ and $\pi(y) = y$, if there hold the conditions listed in prop (6) and if in $\mathbb{R}_+ - \{log(x_e)\}$ it is:*

$$\frac{d}{du}\Big(\frac{\widehat{y}'_C(u) + A(u)}{A(u)\widehat{y}'_C(u) + R(u)}\Big) < 0, \tag{28}$$

then there is a unique GAS limit cycle.

Let us consider the particular case of model 1. Applying proposition (8), and setting back $E^u = x$, and, finally, applying formula (28) one obtains a very complex rational function of x (and of all the parameters), whose denominator is positive. Thus the sign of the constraint is determined by the numerator which is in the following form:

$$S(x; \alpha, \eta, \gamma, \beta_u, \beta_s, m) = x\sum_{i=0}^{8} c_i(\alpha, \eta, \gamma, \beta_u, \beta_s, m)x^i,$$

where the coefficients c_i are second order polynomials in α.

Using the set of parameters:

$$\alpha = 1.9, \eta = 0.05, \gamma = 0.05, \beta_u = 0.1, \beta_s = 10^{-10}, m = 1.5 \tag{29}$$

that were used in [26] and that leads the onset of at least a limit cycle (see figure 2-left), it yields:

$$S(x) = x(-0.1525 + 0.26975x - 0.105731x^2 - 0.0294475x^3$$
$$-0.00148431x^4 - 9.3564310^{-13}x^5 - 1.5430410^{-14}x^6$$
$$-3.429510^{-24}x^7 - 1.8862310^{-34}x^8).$$

The function $S(x)$ is negative, as illustrated in figure 2-right, and as one can see from the following inequality:

$$S(x) < xS_3(x) = x(-0.1525 + 0.26975x - 0.105731x^2 - 0.0294475x^3)$$

since, as it is easy to verify with standard methods, $S_3(x) < 0$.

Similar results can be obtained by using greater values of β_s, up to $\beta_s = \beta_u = 0.1$. The effect of the increase of β_s (for this and other value of α) is of increasing the amplitude (both for pathogen and for the immune system).

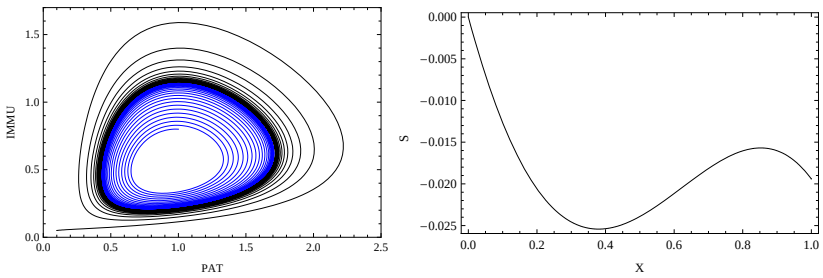

FIGURE 2. Left: replica of the Figure 3 of [26]: orbits starting by two randomly chosen points, one internal to the limit cycle the other external, and both converging to the unique limit cycle. Right: plot of the associated $S(x)$. The values of the parameters are as in (29)

.

Increasing α alone up to $\alpha = 3.0$, we see that the constraint (28) is again fulfilled, since:

$$S(x) = x(-0.3175 + 0.25325x - 0.129244x^2 - 0.0325825x^3 - 0.00156269x^4$$
$$-1.26174 * 10^{-12}x^5 - 2.27963 * 10^{-14}x^6 - 5.415 * 10^{-24}x^7 - 3.135 * 10^{-34}x^8)$$

and it is:

$$S(x) < x(-0.3175 + 0.25325x - 0.129244x^2) < 0.$$

More in general, assuming that all the other parameters are constant, we verified that for a wide range of α it is $S(x;\alpha) < 0$, i.e. there is a unique limit cycle. Moreover, in order to perform some semi-analytical computations, we note that the coefficients $c_i(\alpha)$ $i = 0, \ldots, 8$ are such that : $c_2 < 0$, $c_7 < 0$, $c_8 < 0$ and c_3, c_4, c_5, c_6 are negative up to huge values of α (e.g. $c_3 > 3.1610^9$), whereas $c_0 < 0$ for $\alpha > 0.883$. On the contrary, $c_1 < 0$ only for big values of α, namely for: $\alpha > 19.88$. So, we may conclude that for a wide range of α it is:

$$S(x, \alpha) < xT_2(x; \alpha)$$

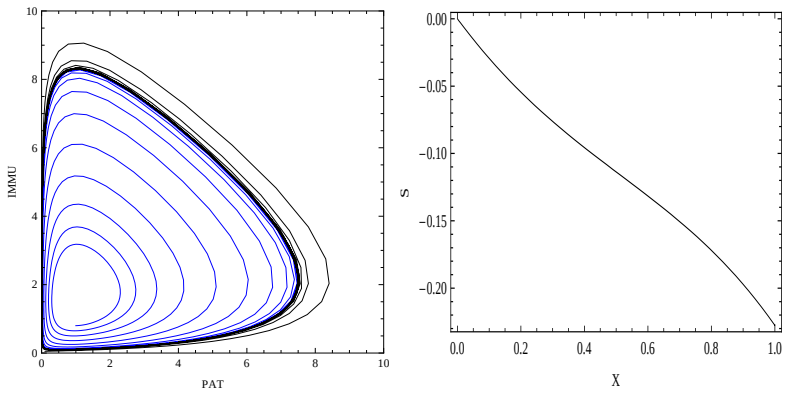

FIGURE 3. Simulation for the case where $\alpha = 3$ and the other values are as in (29). Left: orbits starting by two randomly chosen points, one internal to the limit cycle the other external, and both converging to the unique limit cycle. Right: plot of the associated $S(x)$

where (neglecting the coefficients smaller than 10^{-10}):

$$T_2(x;\alpha) \approx 0.1325 - 0.15\alpha + x(0.29825 - 0.015\alpha) + x^2(-0.0651187 - 0.021375\alpha)$$

whose maximum is:

$$T_2^{MAX}(\alpha) = -\frac{0.147368(\alpha - 1.99639)(\alpha + 4.9083)}{\alpha + 3.04649}$$

As a consequence, we may say that for α greater than 1.99639 up to huge values of α there is a unique limit cycle. Numerically, it is easy to show that, in reality, $S(x,\alpha)$ is negative also for $1.9 < \alpha < 1.9936$. Moreover, since for $\alpha < P(\infty) - 1 = 19$ the orbits are bounded, implying that for $1.9 < \alpha < 19$ the unique limit cycle is also globally attractive in $\mathbb{R}^2_+$.

The simulations we performed to assess the influence of α on limit cycles were of some interest since they revealed a paradoxical effect. Indeed, by using $\alpha = 3$ and, for the other parameters the values of (29), we obtained a limit cycle that, for the variable $x(t)$, has maximum value $x_{max} = 7$ about (fig. 4-left), whereas by increasing α the maximum burden of the pathogen fastly increased, for example for $\alpha = 18$ it is $x_{max} = 850$ about (fig. 4-right). This makes sense, since a faster replicating pathogen intuitively should reach higher levels. However, if we we plot the logarithm in base 10 of x as in the figure 5, we note that the increase of α has also the effect of decreasing the minimum of x. Moreover, the decrease of x_{min} is so sharp that it decrease from 10^{-2} down to 10^{-30}, which is well under the limit of validity of this deterministic model. The effect on the minima of $y(t)$ is, instead, very small, whereas the maxima of $y(t)$ increase from 8 to 120 about. Thus, by taking into account that for small values of x a stochastic model should be used, the biological interpretation of this behavior is that, for this constellation of parameters values, it is very likely that the increase of the replication rate of the pathogen stimulates an immune reaction such that the pathogen burden reduces to zero. Note that the immune system effectors $y(t)$ at least for large t are bounded by η_∞.

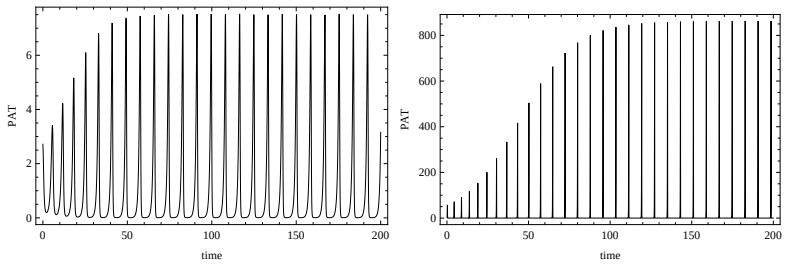

FIGURE 4. Plot of $x(t)$ for two values of α, the other parameters being as in (29). Left subfigure: $\alpha = 3$, right: $\alpha = 18$.

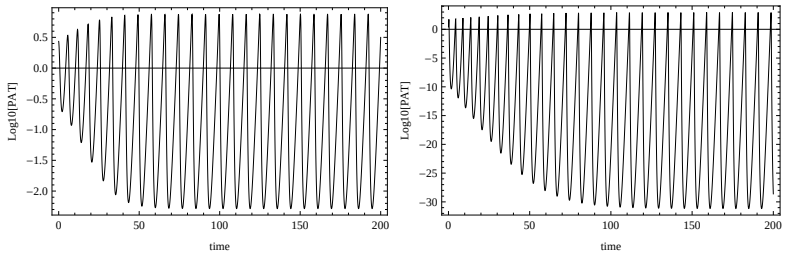

FIGURE 5. Plot of $Log_{10}(x(t))$ for two values of α, the other parameters being as in (29). Left subfigure: $\alpha = 3$, right: $\alpha = 18$.

The influence of β_s is even more intriguing. Increasing it means decreasing the saturation threshold of $U(x)$, and decreasing globally $U(x)$, thus one would expect a great increase of the maximum x. However, one can also note (see figure 6): *i)* a strong decrease of x_{min}, which goes well under the limits of validity of the deterministic modelling, so that the probability of stochastic extinction is very likely big; *ii)* a fast increase of the length of the period of the limit cycle, which has the effect of reducing the average value of $x(t)$.

A decrease of the parameter γ implies an increase of the pathogen-stimulated replication rate of the effectors such that the maximima of x sensibly reduces.

The variations of m, i.e. of the killing rate by the aspecific immunity, are the most surprising. Indeed, the period increases and also the variance of the spikes reduces, so that also in this case there is a reduction of the average value of $x(t)$, however increasing m the increase of x_{max} is nonmonotone. For example for $\alpha = 18$ and the other parameters (except m) as in (29) we obtained that $x_{max}|_{m=1} = x_{max}|_{m=16} \approx 600$ and around $m = 5$ it is $x_{max}| \approx 1500$.

Finally, by setting $\alpha = 100$, $\gamma = 5 * 10^{-4}$ and the other values as in (29), we performed some simulations on the following quadratic family of rates $\pi(y)$:

$$\pi(y) = y + ry^2.$$

The family includes for $r = 0$ the linear rate $\pi(y) = y$ that is such that $x_{max} \approx 480$ and $y_{max} \approx 650$, whereas in case of quadratic $\pi(y)$ with $r = 0.1$ we observe a reduction in the maximum x: $x_{max} \approx 300$. Moreover, there is a remarkable decrease of the time course of y: $y_{max} \approx 85$.

6. **Pathological cases.** Our family of models refers to the case of physiologic behavior of the host organisms. However, it is also useful to consider dys-functionalities,

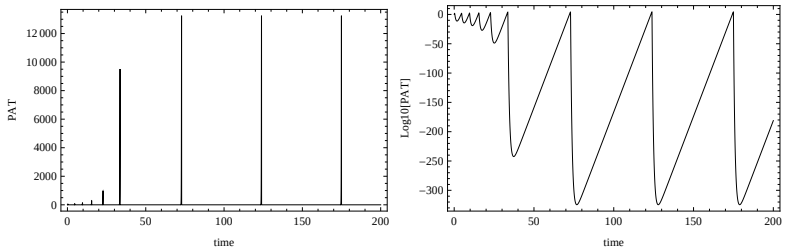

FIGURE 6. Simulations of $x(t)$ (left) and of $Log_{10}(x(t))$ (right) for two values of $\alpha = 18$, $\beta_s = 0.001$ and the other parameters being as in (29).

and for this reason we shall briefly study two main cases: *i)* insufficient pathogen-driven replication of immune particles: $P(\infty) < 1$; *ii)* absence of influx: $\eta(x) = 0$.

In the case *i)*, in model (2) we have only to take into account that $P(x) < 1$, which, however, implies that:

$$-y + \eta_\infty < y' < -y\,(1 - P(\infty)) + \eta(0)$$

i.e. that variable y is bounded: $y \in (y_m, y_M)$. Moreover, since thus:

$$x' > x\,(\alpha - M(x) - \phi(x)\pi(y_M))$$

it follows that for sufficiently large $x(0)$ there is unbounded proliferation of the pathogen: $x(t) \to +\infty$.

In case *ii)* our model reads:

$$\begin{aligned} x' &= x\,(\alpha - M(x) - \phi(x)\pi(y)) \\ y' &= P(x)y - y \end{aligned} \tag{30}$$

Concerning the equilibria the point $(x_m, y_C(x_m))$, which is as well unstable since the characteristic polynomial of the associated Jacobi's matrix reads as follows:

$$\lambda^2 - U(x_m)y_C'(x_m)\lambda + U(x_m)\pi'\left(y_c(x_m)\right)y_c(x_m)P'(x_m).$$

A second equilibrium is $(0, 0)$, which is unstable provided that $M(0) < \alpha$. If $M(0) > \alpha$ then $(0, 0)$ is LAS, and there also is a third equilibrium point: $\left(M^{-1}(\alpha), 0\right)$, which, as it is easy to verify, is unstable.

Finally, in case of coexistence of both these two abnormalities, biologically it is a very simple matter to infer the behavior: since there is both absence of influx and insufficient stimulation of the immune proliferation, as a consequence there will be the extinction of the immune response. This phenomenon is correctly reproduced by our model since :

$$y' = (P(x) - 1)y < (P(\infty) - 1)\,y \Rightarrow y(t) \to 0$$

and

$$x' \to x\,(\alpha x - M(x))\,.$$

7. **Effects of delays.** In this section we shall briefly assess the effects of delays concerning the stimulation of immune system replication in perturbing an equilibrium state, mainly focusing to the case where, in absence of lags, the equilibrium is

locally asymptotically stable:

$$x' = x\left(\alpha - M(x) - \phi(x)\pi(y)\right) \tag{31}$$

$$y' = P(z(t))y - y + \eta(x) \tag{32}$$

$$z(t) = \int_{-\infty}^{t} x(w)K(t-w)dw \tag{33}$$

$K(t)$ is the distributed delay kernel acting on the variable $x(t)$ [19]. In order to obtain simple but biologically robust results, we adopted as kernel the so called Erlang distribution:

$$Erl(\tau; n, a) = \frac{a^n}{(n-1)!}\tau^{n-1}Exp(-a\tau).$$

These distributions are such that the average delay is $T = n/a$ and the standard deviation is $\sigma = \sqrt{n}/a$. Note that if $a = nq$, q being constant, it is: $T = 1/q$ and $\sigma = 1/(q\sqrt{n})$

Moreover, as it is well known, this family has the noteworthy property that it allows to reduce the delayed models to finite dimensional models [19], by adding the following linear differential equations involving n auxiliary state variables:

$$z_i' = a(z_{i-1} - z_i),\ i = 1, \ldots, n \tag{34}$$

with

$$z_0(t) = x(t)$$

and

$$z(t) = z_n(t).$$

Finally, the Erlang family of distributions is such that it includes for $n = 1$ the exponential distribution, and also the Dirac's delta distribution as limit case for $n \to +\infty$:

$$lim_{n\to+\infty} Erl\left(\tau; n, nq\right) = \delta\left(\tau - \frac{1}{q}\right)$$

i.e. the case of constant delay.

We want to recall here that a delay in the dynamics of immune effectors accounts for a complex chain of events[40]. For instance, in the case of a humoral immune response, the pathogen must be processed by antigen presenting cells, T-lymphocites must be activated and proliferate to become mature T-helper cells, specific B-lymphocite must proliferate and differentiate in mature antibody producing cells[41].

As usual when adding an Erlang distributed lag in a ODE model, system (31) is such that if $EQ = (x_e, y_e)$ is an equilibrium of (2), then $EQ_{del} = (x_e, y_e, x_e, \ldots, x_e)$ is an equilibrium for (31)-(32)-(34). The equilibrium is independent of the delay parameter a, however its stability depends on a, which, thus, we shall assume as bifurcation parameter.

For $n = 1$, i.e. for exponentially distributed delays, by applying the Routh-Hurwitz criterion, it is possible to obtain a second order polynomial in a determining the local stability properties of the equilibrium, and the possibility of Hopf bifurcation. Although in the general case the symbolic expression we obtained is very complex, in the case $\eta(x) = constant = \eta$ it is considerably simpler and more

understandable. In fact, in such a case, one may rewrite the above delayed system as follows:

$$\begin{aligned} x' &= U(x)\left(\widetilde{y}_C(x) - \pi(y)\right) \\ y' &= \frac{\eta}{y_i(z)}\left(y_i(z) - y\right) \\ z' &= a(x - z) \end{aligned} \tag{35}$$

whose characteristic polynomial at an equilibrium point $EQ_{del} = (x_e, y_e, x_e)$ is :

$$\lambda^3 + c_2\lambda^2 + c_1\lambda + c_0$$

where:

$$c_0 = a\frac{\eta U\left(x_e\right)\pi'(y_e)}{y_i\left(x_e\right)}\left(y_i'\left(x_e\right) - y_C'\left(x_e\right)\right)$$

$$c_1 = a\frac{\eta - U\left(x_e\right)y_i\left(x_e\right)\pi'(y_e)y_C'\left(x_e\right)}{y_i\left(x_e\right)} - \frac{\eta U\left(x_e\right)p'(y)y_C'\left(x_e\right)}{y_i\left(x_e\right)}$$

$$c_2 = a - U\left(x_e\right)\pi'(y_e)y_C'\left(x_e\right) + \frac{\eta}{y_i\left(x_e\right)}$$

The Routh-Hurwitz condition $c_1c_2 - c_0 > 0$ (to be complemented by $c_2 > 0$) reads as follows:

$$RH(a) = B_2a^2 + B_1a + B_0 > 0$$

where:

$$B_0 = \frac{\eta U\left(x_e\right)p'\left(y_e\right)y_C'\left(x_e\right)\left(U\left(x_e\right)y_i\left(x_e\right)p'\left(y_e\right)y_C'\left(x_e\right) - \eta\right)}{y_i\left(x_e\right)^2}$$

$$B_1 = \frac{N_1}{y_i\left(x_e\right)^2}$$

$$\begin{aligned} N_1 &= \eta^2 - U\left(x_e\right)y_i\left(x_e\right)\pi'\left(y_e\right)\left(2y_C'\left(x_e\right) + y_i'\left(x_e\right)\right)\eta \\ &= +U\left(x_e\right)^2y_i\left(x_e\right)^2p'\left(y_e\right)^2y_C'\left(x_e\right)^2, \end{aligned}$$

$$B_2 = \frac{\eta}{y_i\left(x_e\right)} - U\left(x_e\right)\pi'\left(y_e\right)y_C'\left(x_e\right)$$

We have:

Proposition 9. *Let EQ_{del} be an equilibrium point corresponding, in the unlagged model (2) to an equilibrium EQ. Thus:*

1. *If EQ is unstable with $y_i'(x_e) < y_c'(x_e)$ then EQ_{del} is unstable*
2. *If EQ is LAS, then it exists an $a_1 > 0$ such that EQ_{del} is LAS for $a \in (a_1, +\infty)$ whereas it become unstable for $0 < a < a_1$, i.e. for sufficiently large delays the equilibrium is unstabilized*
3. *If EQ is unstable with $y_C'(x_e) > \frac{\eta(x_e)}{\pi'(y_e)y_i(x_e)U(x_e)}$ and*

$$a_* = U\left(x_e\right)\pi'(y_e)y_C'\left(x_e\right) - \frac{\eta}{y_i\left(x_e\right)} < a_1 \tag{36}$$

then EQ_{del} is LAS for $a \in (a_*, a_1)$, i.e. for small average delays the equilibrium remains unstable, for sufficiently large average delays it is stabilized, and for larger delays it is again unstable.

Proof. **Case 1**: if $y_i'(x_e) < y_c'(x_e)$ then $c_0 < 0$, implying that the characteristic polynomial has at least a real positive root.

Now, note that $B_0 \times B_2 < 0$, so that the equation $RH(a)$ has in all cases a positive solution a_1. Thus, in **Case 2** being ${}_c2 > 0$ $B_0 < 0$ and $B_2 > 0$, then the Routh-Hurwitz condition $RH(a) > 0$ is fulfilled in $a \in (a_1, +\infty)$. On the contrary in **Case 3** being $B_0 > 0$, $B_2 > 0$ but c_2 being of variable sign, the LAS condition $RH(a) > 0$ AND $c_2 > 0$ is fulfilled in $a \in (a_*, a_1)$. □

As far as the behavior at the Hopf point $a = a_1$, the existence of a Hopf bifurcation requires verifying that

$$Re\,(\lambda'(a)) \neq 0$$

at $(a, \lambda) = (a_H, \sqrt{C_0})$. We computed this expression, and it resulted very complex. Thus we report here the results of our numerical simulations. We used the following set of values for the parameters:

$$\alpha = 1.6, \eta = 0.05, \gamma = 0.05, \beta_u = 0.1, \beta_s = 10^{-2}, m = 1.5.$$

We obtained that in all cases, including the exponential, a Hopf bifurcation arises with the onset of a limit cycle, as in figure 7. However, at least for the values we used, we noticed that the oscillations arise for values of a that are roughly in the interval $(n, n+1)$, which means that the average delay T_H triggering the onset of the limit cycle is around $T_H \approx 1$, i.e. T_H is of the same order of the lifespan of immune system effectors, which might not be a realistic value. However, for another constellation of parametric values more realistic T_H might occurr.

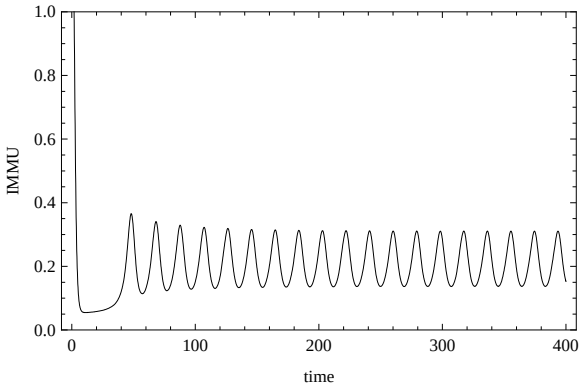

FIGURE 7. Erlangian distributed delay of order $n = 4$ for $a = 1/4$. Behavior of $y(t)$.

In the case of presence of a constant lag, denoted as T,the model (2) reads:

$$x'(t) = U(x(t))\,(y_C(x(t)) - \pi(y(t))) \tag{37}$$

$$y'(t) = \frac{\eta}{y_i(x(t-T))}\,(y_i(x(t-T)) - y) \tag{38}$$

The linearization at an equilibrium point $EQ_{del} = (x_e, y_e)$ is thus:

$$x_1'(t) = U(x_e)\pi'(y_e)\,(y_C'(x_e)x_1(t) - y_1(t)) \tag{39}$$

$$y_1'(t) = \frac{\eta}{y_i(x_e)}\,(y_i'(x_e)x_1(t-T) - y_1(t)) \tag{40}$$

whose characteristic equation is:

$$s^2 + Vs - AC = -E^{-sT}AJ \tag{41}$$

where, for the sake of the notation simplicity we set:

$$V = \frac{\eta}{y_i(x_e)} - U(x_e)\pi'(y_e)y'_C(x_e)$$
$$A = \frac{\eta}{y_i(x_e)}U(x_e)\pi'(y_e)$$
$$J = y'_i(x_e), C = y'_C(x_e)$$

We remember here that the condition for LAS of an equilibrium of the un-lagged system are $V > 0$ and $J > C$. Searching for Hopf point by setting $s = i\omega$ yields:

$$cos\,(T\omega) = \frac{\omega^2}{AJ} + \frac{C}{J} \tag{42}$$

$$sin\,(T\omega) = \frac{V}{AJ}\omega \tag{43}$$

By applying the fundamental trigonometric equality, we get the bi-quadratic equation:

$$\omega^4 + (2AC + V^2)\omega^2 + A^2\left(C^2 - J^2\right) = 0 \tag{44}$$

Since (x_e, y_e) is LAS it is $C < J$, thus eq. (44) has this unique solution:

$$\omega_H = \pm\sqrt{\frac{-(2AC + V^2) + \sqrt{(2AC + V^2)^2 + 4A^2\left(J^2 - C^2\right)}}{2}} \tag{45}$$

As a consequence, from (42) and (43), and taking in account that the r.h.s of (42) is positive, we gets:

$$T_k = \frac{1}{\omega_H}\left(ArcTan\left(\frac{V\omega_H}{\omega_H^2 + AC}\right) + 2k\pi\right) \tag{46}$$

Now since:

$$\frac{ds}{dT}|_{s=i\omega} = \frac{iAJ\omega E^{-i\omega T}}{V + i2\omega - TAJE^{-i\omega T}} = AJ\omega\frac{(2\omega + iV)E^{-i\omega T} - iTAJ}{|V + i2\omega - TAJE^{-i\omega T}|^2} \tag{47}$$

Thus the real part of ds/dT at the hopf points is proportional to

$$2\omega_H^2 cos(\omega_H T) + V\omega_H sin(\omega_H T) = 2\omega_H^2\left(\frac{\omega^2}{AJ} + \frac{C}{J}\right) + \frac{V^2}{AJ}\omega_H^2 > 0 \tag{48}$$

As a consequence, it holds that:

Proposition 10. *It exists a*

$$T_0 = \frac{1}{\omega_H}ArcTan\left(\frac{V\omega_H}{\omega_H^2 + AC}\right)$$

with

,

such that: if $0 < T < T_0$ *then* EQ_{del} *is LAS, if* $T_0 < T < T_1$ *then* EQ_{del} *is unstable and at* $T = T_0$ *there is a Hopf bifurcation with the arising of a limit cycle whose period is:*

$$T_{LC} = \frac{2\pi}{\omega_H}$$

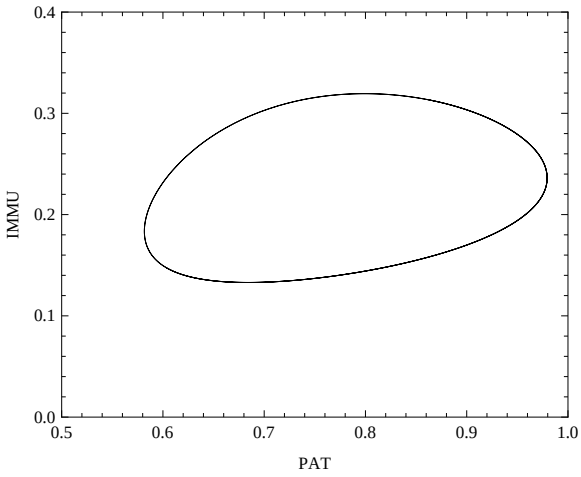

FIGURE 8. Constant delay $T = 1$: arising limit cycle.

8. **Concluding remarks.** We would like to stress that some results we present here seems to well correlate with some known biological knowledge, but other inferences seem new, so that they might lead to further theoretical and experimental research of some biological interest.

For example, we showed that the effect of the aspecific immunity alone is local. This well correlates with the common knowledge that the aspecific immunity, in absence of specific immunity (as in the Severe Combined Immunodeficiency [41]), fails when the host organism is initially attacked by a moderate or large amount of pathogens ($x(0)$ sufficiently big), or by fastly replicating pathogens ($\alpha > m$). Increasing the baseline aspecific reaction, however, has to be considered positive since it can switch form instability to local stability the pathogen-free equilibrium, thus protecting against contatcs with small loads of pathogen. However, our results analytically confirm, at least in principle, that the aspecific immunity can induce global eradication of the pathogen only if in conjunction with the specific immunity.

Moreover, the model shows that the dynamical effect of the increase of the baseline aspecific immune reaction m in case of oscillating immune-pathogen patterns is even more nonlinear, since it induces a nonmonotone response in the maxima of the oscillations of the pathogen. This new theoretical finding, of course, should have a confirm by means of available clinical data or by means of experiments based on animal models.

The strong dependence of the minimum of the pathogen load during oscillations on its replication rate very likely may lead to stochastic extinction of the population. Thus, our model suggests that quickly replicating pathogens may more easily induce acute than chronical diseases. The latter, in fact, would be characterized by constant or periodic attractors with 'not so small' values for x_{min}. This finding well correlates with the literature hypothesis that slowly replicating viruses induce weaker immune responses than those that are more rapidly replicating [40]. Of course, we must stress that since also the maximum burden of the pathogen fastly increases when α is increased, this means that particularly high proliferation rates of the pathogen may correspond to 'fulminant' diseases, as observed in HBV and HCV[40].

Our simulations also showed that a global reduction of the killing rate $U(x)$ obtained by increasing β_s has the effect of improving the response, since also in this case x_{min} dramatically decreases, in other term decreasing this killing rate seems to make the pathogen eradication more likely through stochastic effects. In order to better understand this point we think that experimental and theoretical investigation should be done on how modelling the interactions pathogens-effectors when a very limited number of pathogens are present. For example, as follow up of this work we are planning to change this model by allowing, for low levels of pathogens, a different probability of killing them.

We devoted some space to the assessment of the uniqueness and GAS of oscillations in pathogen-immune system interaction. This point is not only mathematically of some importance, but it also has some biological relevance. In fact, for the general family (2), in proposition 2 we showed that the orbits may be bounded. As a consequence, as illustrated in proposition (7), if the equilibrium is unstable there is at least one stable limit cycle. We may think to the following three main configurations:

- There is an unique limit cycle, which must be GAS;
- There is one stable limit cycle L_o and one or two unstable LCs, which, because of their instability, are not physically observable: in the "real world" all the orbits will tend to L_o. Roughly speaking, it is "as if" the system had an unique GAS LC;
- There may be two or more locally stable cycles (birhytmicity), or even more than two.

In the third case, the periodic behavior of pathogen and immune system effectors interaction and, in particular, the period of the oscillations depend not only on the kinetic features (such as the parameter α and the shape of the functions $M(x)$, $\varphi(x)$ and $P(x)$), but also on the initial condition. Starting point belonging to different basins of attraction of different periodic solutions would result in different behaviors.

Thus the assessment of the global stability of the cycle has an interesting biological meaning: independent of the initial pathogen burden and of immune system effectors, two different pathogen-immune system systems having the same kinetic characteristics oscillate not only with the same period, but also with the same "law". More interestingly, if there is a GAS limit cycle, in a host also large random perturbations of the concentrations (due, for example to the inoculation of further dose of pathogens) do not destroy the behavior before the perturbation, since the orbit will tend to the same limit cycle.

In our family of models we did not explicitly include therapies, although some extremely idealized constant therapies might easily be modelled by simply increasing the parameter η. This increase has the effect of both moving upward the nullcline y_i and also the y coordinate of the PFE. As a consequence, the increase has the effect of allowing not only local but also global eradication, since a sufficiently large therapy dose may act so that: $\forall x > 0 \quad y_i(x) > y_C(x)$. However the inclusion in the model of more realistic time-varying therapies might be not only interesting *per se*, but might produce suggestions on the schedule of their delivering. Regarding this last point, it would of course be very important to apply the methodologies of optimal control theory, but also the theory of nonlinear resonances and chaos. In fact, in case of oscillating patterns the delivering of periodic therapies might lead to interesting phenomena that might contribute to the classification of response pattern to drugs. For example, what might be the effect of the delivering of a

periodic therapy of period T on a host organism whose response to an equivalent constant therapy would be T-periodic?

As far as delays are concerned, here we only (and briefly) analyzed some quite simple, although important, delaying kernels. Of course a further investigation on both general kernels or some more realistic kernels might be of interest. However, a really challenging investigation would be to infer the real delay distributions from suitable biological data.

As far as the pathological conditions shortly illustrated in section 6, a detailed theoretical study on them should give some insight of interest on many diseases involving the activity of immune system. For example, when delivering a contant therapy $\eta_{ther} > 0$ in the pathological case of absence of influx, may easily be read as restoring the system to a physiological condition.

Finally, as in all models of populations based on ODE or DDE (or on equivalent nonlinear stochastic processes) that are aimed at stressing the dynamical effects of the interaction of populations at a macroscopic level, here we disregarded a wide spectrum of important phenomena. For example spatial effects were not taken into account, although they can be important [42, 43], but we did also not model the role of the specific 'immune activities' [44] of both pathogens and effectors that can be considered as active particles [45] for which both stochastic integral modelling [45] and multi-scale modelling [46, 42] might reveal finer details of the interaction.

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E-mail address: alberto.donofrio@ifom-ieo-campus.it