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Who benefits from cellular immune response during the Chagas disease?

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ABSTRACT

We extend our previous model for the dynamical interaction between a mammal's immune response and the *Trypanosoma cruzi* parasite during the acute phase of Chagas disease. The model here considers both humoral and cellular responses and the different stages of *T. cruzi* (intracellular and extracellular phases) inside the mammal host. We analyze the dynamical time evolution of the populations obtaining phase diagrams of the model results. The steady-state solution of the system yields two outcomes associated to Healing and Chronic stationary cases, the death case obtained when just the humoral immune response alone was considered is not being present. This result implies that, surprisingly, although the immune cellular response is obviously beneficial for the host, it is also evolutionary advantageous for the parasite, as it helps to preserve the host alive and, after transmission to a healthy host, perpetuate the disease. Of course, if the cell damage by the parasite's intracellular stage is high, it may cause the host death. This possibility is accounted in the model by introducing a death criterion related to cell destruction. We present a new phase diagram, that restores the host death case and generates a phase diagram similar to the one arising from the original model.

1. Introduction

Chagas disease is a Latin American endemic infection caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). Nowadays it affects nearly 6–7 million people, mostly in Latin America, and causes on average about 12,000 deaths per year (World Expert Committee, 2002). *T. cruzi* can be transmitted in different ways: blood transfusion, congenital transplacental, organ transplant, oral, laboratory accidental infection and, by the feces of hematophagous triatominae bugs (Canals et al., 2017; Martorano Raimundo et al., 2010). This last way to acquire the infection is the most predominant mode. The process begins when an infected insect ingests a blood meal from a healthy animal or human. At the same time when the insect is biting, it usually defecates, depositing the parasites in its feces. The victim instinctively scratches the skin as the result of the insect bite, allowing the parasites to get into its new host. Once into the mammalian body, the parasites, already differentiated into the circulating tripomastigote stage, reach the host bloodstream, from where they can access and penetrate into a variety of cell tissues (with marked preference for cardiocytes and smooth muscles). Inside a cell that make up the tissue, the parasites transform into amastigotes (the intracellular stage) and replicate by binary fission until it is completely full. Consequently the cell bursts out, releasing new parasites already differentiated again into circulating trypomastigotes. They could continue spreading through the blood stream, succeeding in colonizing other cells of the same tissue or other tissues. They can also be taken in a new insect bite, continuing the infection transmission cycle (Storino and Milei, 1994; Storino, 1998). As this reproduction process repeats, a slow, but continuous, cellular damage is produced, that worsens the health condition of infected people (mainly due to the loss of cardiac ganglion cells), ending in chronic cardiomyopathy.

As its counterpart, the mammal innate immune response works with at least three different mechanisms: (1) direct destruction of parasites by macrophages and dendritic cells, (2) activation of these cells to be antigen presenters cells (APC) and (3) sensing host cells subject of *T. cruzi* invasion (Tarleton, 2007). The antigen recognition process occurs because APCs display a fraction of antigen on its surface coupled with a major histocompatibility class (MHC) II molecule to induce its interaction with specific helper T cells (HTCs). During their interaction cytokines are secreted by HTCs to active the proliferation of B cells and cytotoxic T cells (CTCs). Then the immune response follows two paths, one using CTCs, whose major function is to lyse cells infected with intracellular pathogens such as viruses, bacteria or parasites inaccessible to antibodies. A CTC only lyses a cell when an epitope (part of antigen) bounded to a MHC Class I molecule is displayed on the surface of the infected cell. The other immune system response path is through the B cells. These B cells come in millions of different types

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(called species in our model) and, when mature, they differentiate in plasma cells, which in turn produce the different antibodies that circulate into the blood stream and are capable to bind antigens involved in an infection. A number of these B cells become Memory B Cells so that a greater number of antigen-specific B cells will be available on a second contact with the same antigen (Goldsby et al., 2000; Regueiro et al., 2010; Delves et al., 2017). In order for the immune system to control the Chagas infection the synergic action of several elements is necessary: the action of a strong humoral immune response, a powerful production of Citokyne Type I and the activation of T CD8+ cells, which allows the recognition of host cells infected by the parasite (Tarleton, 1990; Tarleton et al., 1996; Rottenberg et al., 1993, 1995).

Our group has developed a model which makes an easy identification and quantitative assessment of the effects of modifying the relevant parameters involved in the infection and the generation of the humoral immune response possible. The model was developed in Ref. Sibona et al. (2005) considering that the antibody production is a function of parasite number, simplifying the detailed mechanisms that correlate parasites to the antibody source. Consequently, we omitted a detailed discussion of B-cell activation and antibody secretion, focusing instead on providing a different insight into the microscopic competition processes underlying the acute phase of the disease.

As for example, with this model (considering just an extracellular parasite replication) we portrayed a simultaneous infection with two different *T. cruzi* lineages and one antibody species (Vega et al., 2012). The major outcome here was the non-existence of a simultaneous equilibrium between the two parasite strains and the immune response population. This is in agreement with the clinical reports where there is no evidence of a double infection reported in an hemoculture test (Ramirez et al., 2010; Mantilla et al., 2010; Rodrigues et al., 2010). Actually this led us to contemplate the possibility that a first infection could provide immunity in case of a following infection.

Considering the last hypothesis we decided to extend the model and described the interaction between the *Trypanosoma rangeli* and the humoral response (Vega-Royero and Sibona, 2014); afterwards we simulate the dynamic of a mixed *T. cruzi–T. rangeli* infection. We found that a pre-infection with *T. rangeli* induces a protective effect against Chagas disease, due to the increase in the antibody levels. Unfortunately this protection is just a temporarily effect (less than three months) against a possible future infection with *T. cruzi*. Besides, although our model successfully reproduced many aspects of the disease, a combined analysis of both humoral and cellular immune reaction remained to be done.

Independently of our work, Nelson and Velasco Hernández (2001) put forward a model of cell-mediated (not humoral) response to *T. cruzi* to describe the late stages of pathogenesis and the possible role played by autoimmunity in determining the disease outcome. Oliveira (2010) developed mathematical models to describe the humoral or cellular immune responses facing independently a *T. cruzi* infection. Both models yield three possible outcomes: the existence of a trivial solution (related to the elimination of the parasite population), a coexistence of antibodies and parasites population, or the uncontrolled growing of the parasite population. This last result appears as a result of a weak immune system or the lack of interaction between the respective immune response and *T. cruzi*. Galvão and Garcia Vivas Miranda (2010) present a multi-agent-based computational model for the evolution of Chagas disease, that considers the interaction among inflammatory cell, fibrosis, cardiomyocyte, fibroblast, and *Trypanosoma cruzi*. Their results reproduce well the parasite and inflammatory cell populations, and the volume fraction occupied by the myocardial tissue.

More recently, Yang (2015) proposed for the first time a model that describes the combined interaction between both immune responses and *T. cruzi*. His model considers susceptible and infected cell populations, and a simple mechanism of activation of the immune system due to the presence of the circulating *T. cruzi*. Yang's model yields two solutions: a trivial one (associated to an individual free of infection) and a non-trivial one, which is locally asymptotically stable. This non trivial solution is due to the consideration of a finite number of healthy host cells, and an equilibrium between the infection rate and the replenishment rate of these cells. Yang's work analyzes the effect of modifying only the immune reaction parameters, while those associated with the parasite and host cells are kept constant.

Here we present a model that links our previous humoral immune response and a cellular immune reaction similar to that included in Yang's model. This extended version of the model includes also the presence of antibodies (even in absence of parasites), their affinity during the infection and the proliferation of activated T cell lymphocytes, caused by the cloning process due to the recognition of an infected cell. Surprisingly, these simple mechanisms makes it impossible for the parasite to growth without control, even for an infinite number of host healthy cells, obtaining an equilibrium among immune reaction and *T. cruzi* population and eliminating the previous host death case. Nevertheless, as it was studied in a previous work (Cossy Isasi et al., 2009), death due to cell destruction through *T. cruzi* reproductive cycle is one of the observed clinical outcomes. In particular, different *T. cruzi* strains produce different cell damage, with the consequent mouse death, independently of the parasitemia levels (Sibona et al., 2005). It was also observed that the action of the protective ganglioside GM1 decreases cardiocyte destruction, leading to the reduction of death cases in murine experiments.

Considering these situations we propose a simple threshold for death that restores the host death case and generates a phase diagram similar to the one arising from the original model. The model developed here aids to the identification of relevant parameters involved in the parasitic infection and the generation of both immune responses. In particular, the conditions that they must fulfill to cure the disease, giving a direction to concentrate the efforts to find a vaccine or cure against this disease. Furthermore, the results obtained from the model show a similar qualitative description from partial experimental data observed in the literature (Bouhdidi et al., 1994; Sato et al., 1992).

The rest of this paper is organized as follows: In Section 2 we present the model, whose steady-state solutions are discussed in Section 3, where we also present phase diagrams describing the infection outcome in terms of the parasite and antibody generation rates. The dynamic properties are analyzed in Section 4 and in Section 5 we propose a new death case, presenting the modified phase diagram. We conclude with a short discussion of the results, particularly to whom the cellular response benefits.

2. The model

We will work on a previous model that considers only the humoral immune reaction (Condat et al., 2003). We will describe it succinctly first to show how the previous results (Sibona et al., 2005) are modified due the action of the cellular immune system. It only takes into account three different populations: N different antibody species $a_i(t)$ capable of mediating parasite elimination, the parasites in its tripomastigote circulating stage $n(t)$ and the infected cells $r(t)$. As we described in the introduction, the presence of the parasite triggers the production of new antibodies, which in turn are eliminated by their interaction. We assume that the antibody is disabled after binding a parasite in an encounter with a rate α_i. The antibody evolution equation then reads:

$$\dot{a}_i(t) = \gamma_i n(t) - \alpha_i(t) a_i(t) n(t) - \frac{1}{\tau_i}[a_i(t) - a_{i0}] \tag{1}$$

where γ_i is the antibody production rate coefficient due to the parasite population, $a_{i,0}$ is the amount of antibodies in absence of infection and τ_i is the intrinsic antibody lifetime. The removal efficiency coefficient α_i is described by a smoothly increasing function of time (Cossy Isasi et al., 2001), $\alpha_i(t) = \alpha_{A,i} + \alpha_{B,i}(1 - exp(-t/T_i))$, where T_i is the "learning time". It makes reference to the ability of the immune system to produce antibodies with an increased efficiency (Bouhdidi et al., 1994).

Due the interaction with the antibodies, the circulating parasite population also decreases with the same removal efficiency coefficient α_i, but it increases due to the burst of the infected cells $r(t)$. Also the process to infect a healthy cell decrease the number of tripomastigotes in blood, increasing in turn the number of infected cells. Denoting N_r as the mean number of trypomastigotes emerging from a cell rupture, η as the probability per unit time that an infected cell will burst releasing new parasites in the blood stream (cytotoxicity), and ξ as the rate at which circulating parasite penetrates into a host cell (infectivity), the circulating parasite population evolution equation is given by

$$\dot{n}(t) = \eta N_r r(t) - n(t) \sum_{i=1}^{N} \alpha_i(t) a_i(t) - \xi n(t). \tag{2}$$

Our previous model considers the intracellular *T. cruzi* amastigote stage, which replicates by binary fission, only through the number of infected cells (Cossy Isasi et al., 2009, 2001; Sibona and Condat, 2002; Vega et al., 2011). The $r(t)$ evolution follows up from the same process described for the circulating parasites and is given by,

$$\dot{r}(t) = \xi n(t) - \eta r(t). \tag{3}$$

If we introduce the effect of the immune cellular reaction, new populations must be included and the previous equations are modified. Following the model proposed by Yang (2015), for the cellular immune response, we introduce two new populations: the non-activated cytotoxic T lymphocyte cells (CTL), $Q_i(t)$, and the activated cytotoxic T lymphocyte cells, $c_i(t)$. We consider that there are M different non-activated cytotoxic T lymphocyte cells species produced in the bone marrow at a constant rate σ_i. This rate has been taken as a constant due to the capability of the human body to maintain an equilibrium with the other immune responses (homeostasis). The evolution equation of each non-activated cytotoxic T lymphocyte species is then given by,

$$\dot{Q}_i(t) = \sigma_i - \frac{Q_i(t)}{\tau_{iQ}} - \phi_i Q_i(t) n(t) \tag{4}$$

Here, the second term represents the natural death of CTL cells with an intrinsic lifetime τ_{iQ}, and the third term indicates the switch from non-activated CTL cells to the activated CTL cell stage. This activation process is due to stimulation by cytokines, as the result of the previous interaction between the T helper cells and antigen presenting cells. This activation process occurs at a rate ϕ_i and is proportional to the amount of circulating parasites that are triggering the reaction. Once the cytotoxic lymphocyte cells $c_i(t)$ are activated, the only way they disappear is by natural death, with an intrinsic lifetime τ_{ic}. As a consequence, the dynamic evolution for the activated CTL cells is described by the equation,

$$\dot{c}_i(t) = \phi_i Q_i(t) n(t) - \frac{c_i(t)}{\tau_{ic}} + \delta_i r(t) c_i(t) \tag{5}$$

The last term denotes their proliferation caused by the cloning process due to the recognition of an infected cell by an activated lymphocyte T cell $c_i(t)$, a process that occurs at a rate δ_i. CTL activated cells $c_i(t)$ target infected cells and are responsible for its elimination. Then the evolution equation of infected cells $r(t)$ has to be modified to include this process:

$$\dot{r}(t) = \xi n(t) - \eta r(t) - \kappa_i r(t) c_i(t), \tag{6}$$

where the last term represents the elimination of infected cells at a constant rate κ_i. The equations for the antibody and parasite populations do not change.

3. Steady states

For simplicity, and to have a better understanding of the model, we first study the $N = M = 1$ case. By setting the time derivatives in the previous equations equal to zero we can study the long time ($t \to \infty$) system behavior. In this way a set of steady state populations are found and classified in two cases:

I Healing: If $N_r < 1 + \alpha a_0/\xi$ (a low number of parasites coming out of the bursting invaded cells), corresponding to a high removal efficiency, both parasite and infected cell populations disappear at long times and the humoral and cellular immune responses return to its initial conditions ($r_s = n_s = 0$, $a_s = a_0$, $T_s = \sigma\tau_T$, $c_s = 0$) for any γ value. Note that the healing borderline depends on the no-disease antibody production (a_0) and not on the production increase induced by the circulating parasite presence (γ). If the parasite numbers are close to extinction then the immune system is poorly stimulated to produce more specific antibodies; then, it has to overcome the disease just with the antibody production that exists for the no-infection case, but with an improved efficiency (α). In other words, the disease is controlled by an enhanced antibody production, but it is completely eliminated thanks to the improved efficiency of the antibodies.

II (a and b) Chronic disease: $N_r > 1 + \alpha a_0/\xi$, the parasite infection is controlled but not eradicated. All populations reach values different from zero for any γ value. The expressions for the steady state values are very long and complicated, so we will not include them explicitly here. Nevertheless this state can be split in two cases, according to the antibody steady state population. If the antibody production rate is larger (smaller) than a threshold value (αa_0), the antibody steady state population is larger (smaller) than its initial value. Although the other cell steady state populations have no differences between both chronic cases, the final size of the antibodies steady state population alone is enough to differentiate both chronic cases. Lets recall that Chagas disease diagnosis is performed through the ELISA test of blood, looking for antibodies formed against *T. cruzi*. This means that, according to the clinical observations, the mammal common case is IIa, and not IIb.

The stability of the steady state solutions is proved using the Routh–Hurwitz Criterion (Murray, 2002). The preceding results of the parasitic invasion are best described by constructing a phase diagram in the plane defined by the parameters γ and N_r. In Fig. 1(a) we can observe the phase diagram obtained by considering just the humoral reaction (adapted from Sibona et al., 2005), and the new phase diagram considering both humoral and cellular reactions in Fig. 1(b).

In both figures, and in the following, the parameters were chosen arbitrarily for a better visualization of the phase diagram an the dynamical evolution of the populations. Of course a different parameters set will produce different populations evolution, but with the same

Table 1
Parameter values arbitrarily chosen for a better visualization of the phase diagram depicted in Fig. 1.

Parameters		
Symbol	Definition	Values
α	Parasite removal rate	3
κ	Infected cells removal rate	0.5
τ_a	Antibody intrinsic lifetime	10
a_0	Antibody initial amount	2
τ_Q	Non-activated CTL intrinsic lifetime	10
Q_0	Non-activated CTL initial amount	2
τ_c	Activated CTL intrinsic lifetime	10
c_0	Activated CTL initial amount	2
ξ	Parasite penetration rate into a host cell	10
η	Probability of breaking cell	2
σ	CTL production rate	3
ϕ	CTL activation rate	2
δ	Activated CTL proliferation rate	0.2

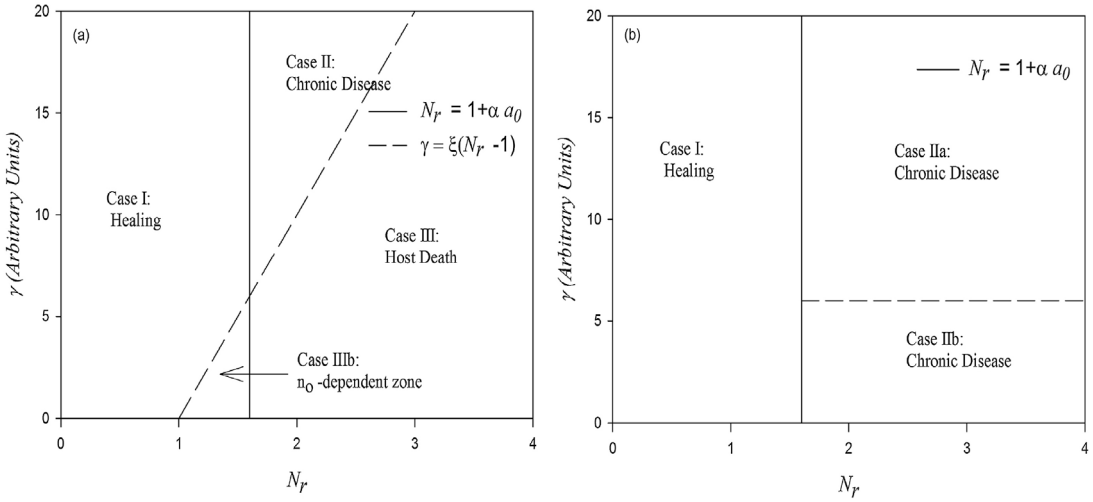

Fig. 1. $\gamma - N_r$ phase diagram describing the outcome of the parasite infection. (a) Considering just the humoral immune reaction (figure adapted from Sibona et al., 2005). (b) Considering both humoral and cellular immune reaction, the host death case (III) disappears. The parameter values are given in Table 1.

characteristics, according the corresponding phase diagram. In Fig. 1(a) we observe four different cases for the previous model: (I) Healing, similar to the previous description. (II) Chronic Disease, that includes both subcases a and b. (III) Host Death, in which parasites grow without control. And (IIIb), where the outcome depends on the parasite load inoculated. Comparing both phase diagrams we can observe that if we include the cellular immune response, the host death case (case III) disappears, but the borderline among healing and chronic disease does not change. This means that the cellular immune response helps the system to control the invasion, reducing parasitemia levels, but it cannot eliminate the infection. As a consequence, if we want to get a vaccine or a cure for the disease, improving the humoral immune response is the key. Another aspect that is also interesting to note is that the elimination of the death case is not only beneficial to the host, but also to the invading parasite. Thanks to the cellular immune reaction the parasite can reproduce during a long time without losing its "habitat", even if the host has a weak immune reaction.

In Fig. 2 we can observe the size of the different populations as a function of N_r for three different values of the antibody production rate ($\gamma = 0$, 1 and 10). They correspond to the cases of no humoral reaction, case IIb weak humoral reaction and case IIa strong humoral reaction, respectively. We can observe that for $N_r < 1 + \alpha a_0/\xi$, parasites, infected cells and activated T cells are eliminated, while the rest of the populations steady states are at their initial values. If $N_r > 1 + \alpha a_0/\xi$ the steady state populations related with the parasite emerge (n, r and c), increasing with N_r, while the non activated T cells decreases from the initial value. As we mentioned before, the only population presenting differences between cases IIa and IIb are the antibodies, increasing from the initial value a_0 in case IIa and decreasing in case IIb.

It is interesting to note that, even in the absence of the humoral immune response, the cellular immune response is able to control the infection. Nevertheless it can not always cure the disease alone. This will occur only when high specified antibodies (high α) are produced ($1 + a_0$. $\alpha > N_r$). In this case the innate immune action, represented by the no-disease antibody production a_0, is responsible for completely removing the parasites.

4. Dynamics

Time-dependent solutions to Eqs. (2.1), (2.3)-(2.6) are obtained numerically using a standard Euler method implemented in MATLAB. In Fig. 3 we can observe the time evolution of all populations for both healthy and chronic cases. The parameters were chosen to visualize properly the characteristics of each evolution curve. The dynamics of case I ($N_r = 1$) is similar to the same case in the previous version of the model (Sibona et al., 2005). i.e., the parasite population decreases over time until vanishing while the antibodies and inactivated T cells increase or decrease, depending on the immune reaction parameters, at the beginning of the infection to later return to the initial populations. The infected cell and the activated T cell populations first increase until they reach an intermediate maximum to decrease later over time and then disappear. The increasing and decreasing slopes depend on the model parameters. As for example, a strong immune reaction will make the parasites disappear faster, while the parasite population starting slope will increase with N_r. For case II the situation is quite different. As in the former model, parasites population increases, activating in turn the immune system that increase the antibody population, which can control the disease at the end. In this way an intermediate peak of circulating parasites appears, as occurs in the acute phase of the disease. The curves show also oscillations to the steady state value after an intermediate state in which the parasite invasion is almost controlled. These oscillations are visible due to the parameters chosen. If we look for population evolutions similar to those clinically observed, the parasite steady state population will be much lower than the peak value, vanishing the oscillations. Also the antibodies population will drastically increase in the acute phase before reaching the asymptotic value larger than the initial numbers. The dynamics depicted in Fig. 3 do not change if we modify the parameters governing the immune cellular reaction. Of course, if we increase (decrease) the CTL cells activation, proliferation or recognition rates (ϕ, δ and κ respectively), the parasite populations will decrease (increase), but the general evolution characteristics will be the same. While the dynamics are similar to cases I and II of the previous model (Sibona et al., 2005), here the evolution of populations over time does not depend drastically on the inoculum size neither grows without control. It is evident that the cellular immune system helps to control the parasite making a decisive contribution to stop its growth, but nevertheless could not help to eliminate them completely.

5. Partial cellular destruction death condition

The phase diagram obtained in this work for the full immune reaction seems to be a drawback regarding the original model, as it does not reproduce the clinically observed outcome of the host death as a result of the acute infection (World Expert Committee, 2002). It is also observed in many experiments of murine models that mice die due to the cellular destruction caused by parasite reproduction with high parasitemia levels (Condat et al., 2003). Nevertheless, it would be a

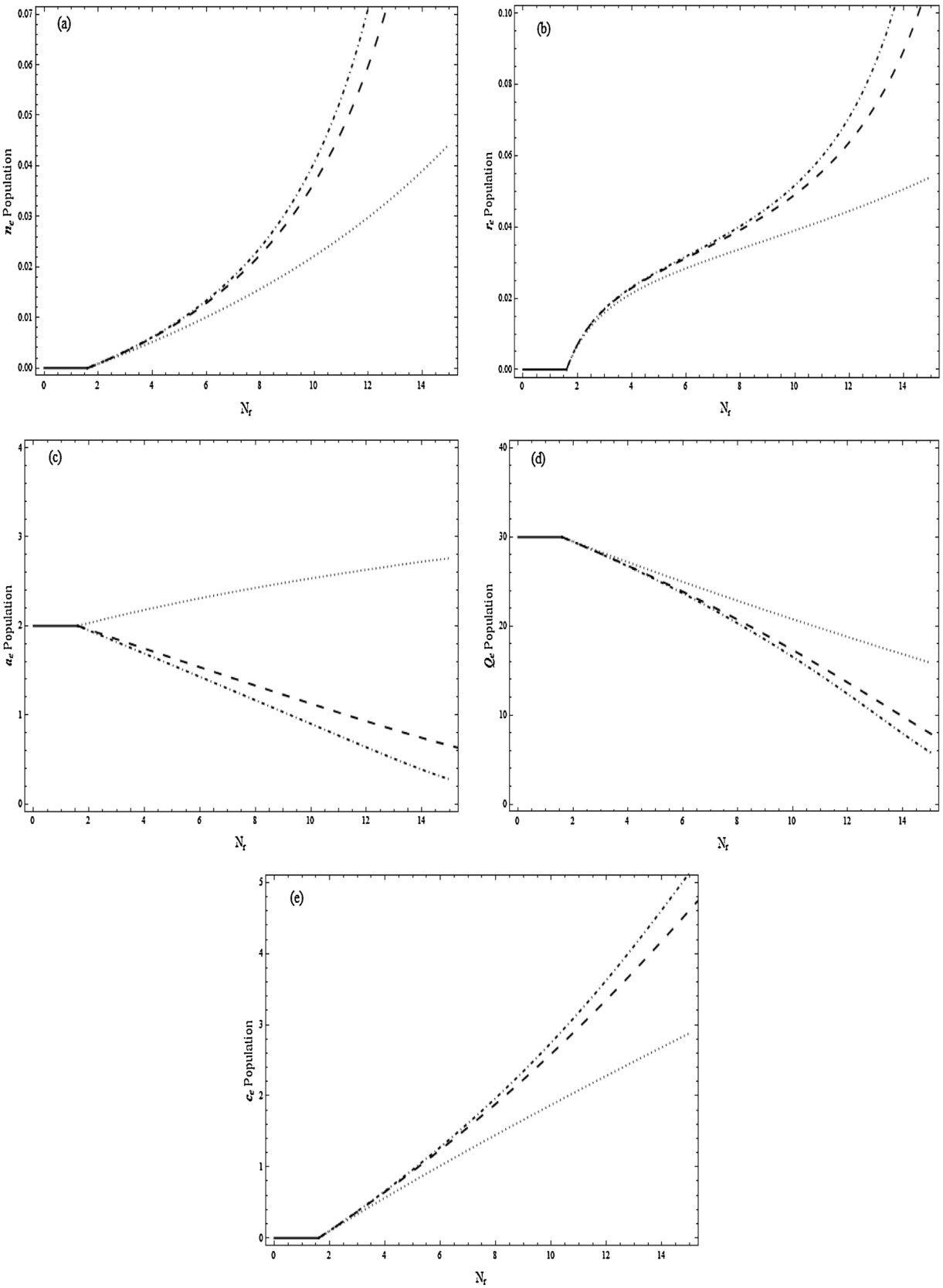

Fig. 2. Steady state populations of circulating parasites n_e (a), infected cells r_e (b), antibodies a_e (c), non-activated CTL Q_e (d) and activated CTL c_e (e) as a function of the mean number of trypomastigotes emerging from a cell rupture (N_r) for different antibody production rate values (γ). The parameters are given in Table 1, except for $\kappa = 2.5$. Each line corresponds to a different immune reaction: $\gamma = 0$ (dotted-dashed line) corresponds to the absence of humoral response, $\gamma = 1$ (dashed line, chronic disease case IIb)represents a weak humoral response and $\gamma = 10$ (dotted line, chronic disease case IIa) a strong humoral response. The solid line belongs to the healing region (case I) of the phase diagram, $N_r < 1 + \alpha a_0/\xi$.

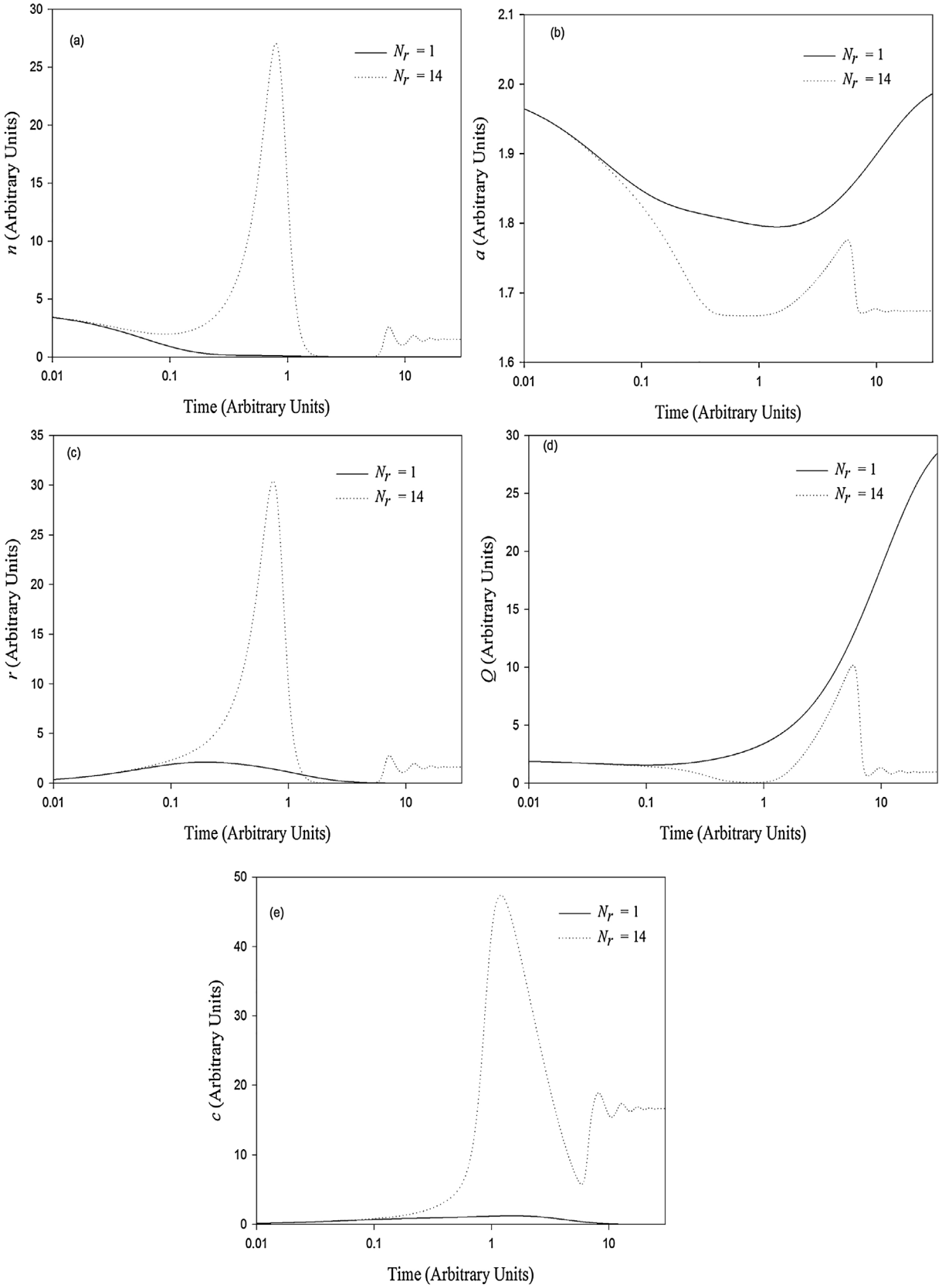

Fig. 3. Dynamic evolution of system populations. In all cases the parameters are given in Table 1 and $\gamma = 3$. The solid line indicates the Case I: Healing, while the dotted line indicates Case II: Chronic disease.

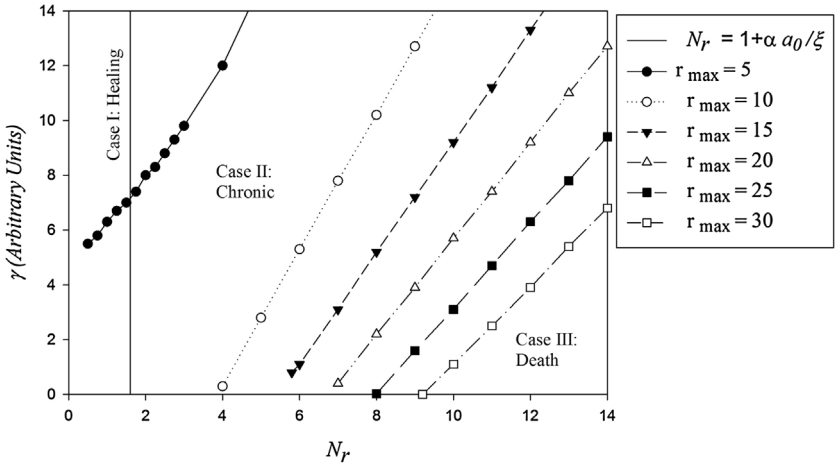

Fig. 4. $\gamma - N_r$ phase diagram describing the outcome of the parasite infection. Simulations performed with the parameters given in Table 1.

mistake to consider that the previously obtained death case, where the parasite population grows without control, is the only possible path to a fatal outcome for the host. If in an intermediate stage the infected cell population is large enough, as to compromise the function of the organ where they belong, that organ could fail, producing the host death. Let us recall that the heart is one of the principal organs affected by Chagas disease. Then, it is interesting to study how the phase diagram changes if we consider that there is a threshold population for the infected cells, r_{max}, over which the host die. We have performed simulations of the dynamical evolution in the entire phase diagram, looking for those cases where $r(t) > r_{max}$, obtaining the threshold curves observed in Fig. 4.

Comparing them with the previous chronic-death borderline, we found that the behavior is similar, a linear growth with a shift towards higher N_r values as we increase r_{max}. Obviously, the parasite levels have to be higher to reach a larger number of infected cells. On the other hand, it is interesting to note that the slope of the linear growth is also different for different r_{max} values. In Fig. 5 we show that the slope is a monotonically decreasing function of the maximum allowed number of infected cells, tending to a minimum slope for high r_{max} values. In the other limit, when this new threshold gets closer to the health-chronic borderline, the slope decrease, loosing the linear behavior that has for large N_r values.

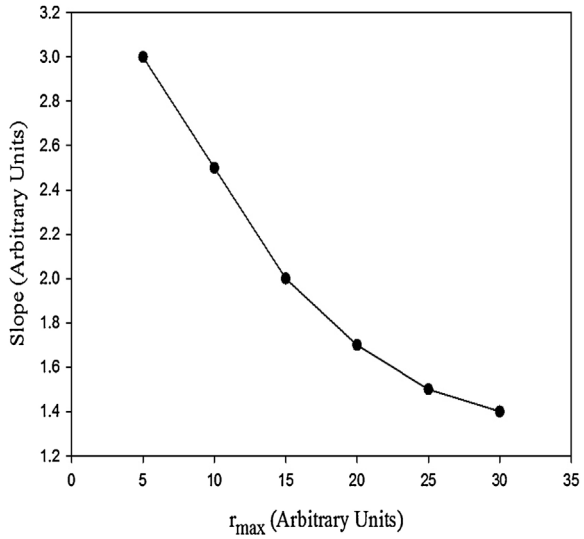

Fig. 5. Slope of the linear threshold among death and chronic cases as a function of the maximum allowed number of infected cells.

6. Discussion

We have extended the analysis of our model for the interaction between parasites and both, humoral and cellular, immune responses acting simultaneously during the acute phase of Chagas disease. The model developed is useful to analyze the parameter dependence of the parasite-immune system interaction and the quantitative assessment of the relevant parameters for an external intervention. The new phase diagram is divided in two regions: Healing and Chronic case (Fig. 1). Interestingly, the joint immune action produces the elimination of the death and inoculation-dependent cases resulting when only the humoral reaction is considered. These consequences could be expected as we are increasing the eradication mechanisms of the disease. Nevertheless the borderline among health and chronic cases is exactly the same as in the previous model, depending only on the humoral response parameters. This means that the cellular immune reaction alone could not remove the infection, and it works as a helper for the humoral immune system. As a consequence, a strengthening of the humoral response has to be obtained if we are looking for a cure to the disease.

At first sight, the cellular immune response benefits the host as it eliminates all possibility of exponential growth of the parasite population, extending the host life. However, this situation also helps the parasite, which may now reach a coexistence state with its host for a long time. The ensuing limitation in the parasite number means that it will not destroy its habitat, increasing the chances of being transmitted to another mammal. *T. cruzi* may have thus evolved to be able to take advantage of the cellular reaction to establish itself firmly in an ecosystem. It is well known that Incas suffered from Chagas disease (Urton and Von Hagen, 2015), and our model could explain how a deadly disease could coexist with humans for so long.

It is interesting to note in Fig. 2 that, even in the absence of humoral reaction, the behavior of the steady state populations are all similar. This observation makes us analyze further the borderline between chronic and healthy cases, that do not depend on the immune reaction properties, humoral or cellular. For a parasite with a certain replication rate (defined by N_r and ξ), we have to improve the parameters governing the immune action in absence of parasites (a_0 and α) to shift the health-chronic borderline to the right, and including the parasite state in the health case region. We conclude that the elimination of the parasite is only obtained by an increase in the removal efficiency, α, or the base antibody population, a_0. Coincidentally, in this direction are the last efforts to find a vaccine or cure against this disease. Recently a chimeric antigen was tailored to increase the specificity of the humoral response (Sanchez Alberti et al., 2017), achieving not only an effective protection against *T. cruzi*, but also showed a reduction in parasite load and chronic inflammation across the course of infection.

Reaching the healthy steady state could take a very long time and an intermediate stage with a very high parasite population could exist. In these cases the immune reaction has to control the infection as fast as possible to avoid other complications, death included. Then we have to make the distinction here that the real clinical cases are not the ones presented in the phase diagram, but the final situation obtained if we have an infinite system evolving for an infinite time. Moreover, the parameter choice of our work is to present a clear visualization of the characteristics of our model and the implications on a parasite infection evolution. To get a proper phase diagram and population evolutions experimental data, during a *T. cruzi* infection of all the populations considered in the model, it is needed. We have no knowledge of such a complete experiment, and then we could not fit the parameters. Any suggestion made by us regarding such a set of parameters real values is not responsible.

Even though the death case does not appear in the phase diagram, we suggest that the host death may occur as the result of the cumulative cellular damage during the disease process, as it is observed clinically or in the murine models. Taking this into account we propose the existence of a critical value for the infected cells, r_{max}, above which the host dies. By simulations we found the minimum value of γ for a given N_r for the system to reach r_{max}. We found then an intermediate death case as a product of the accumulation of the cellular damage made by the parasite to the host tissues. Interestingly, the borderline between chronic and death cases is similar to the one obtained previously without considering the cellular immune response. Nevertheless the location of the boundary will depend on the value of r_{max} and the other model parameters. It is difficult to define a general expression for this curve as the maximum tolerance to the cellular damage could change from host to host; nevertheless work along these lines is in progress.

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