# Modeling CD137 signaling in human M. tuberculosis responses using bayesian analysis.

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**Abstract**

**1. Introduction**

Comentario de la intrdoccion:

Esta bien el primer parrafo, El Segundo se va muy de lleno a la senializacion especifica sin hacer mención a la ventaja de la modelización matematica. Esta tiene que aparecer bien pronto no después de tantas líneas. Fijate de mechar la modelización antes.

Tuberculosis is one of the earliest recorded human diseases and is still an unresolved global health problem. Its causative agent, *Mycobacterium tuberculosis* (*Mtb*), produces 2 million deaths annually worldwide despite current drug treatment. Even more, one-third of the world population is infected with Mycobacterium tuberculosis, the causative agent of the disease (WHO, 2010).

Although immunological mechanisms against *Mtb* are not fully understood, protective defense to mycobacterial infections is primarily mediated by the interaction of antigen-specific T cells and macrophages. [[1](#_ENREF_1), [2](#_ENREF_2)]. This interaction often depends on the interplay of cytokines produced by these cells. Even though a wide spectrum of cytokines may contribute to protection, a type 1 response, dominated by interferon (IFN)- secretion, is considered the main mediator of protective immunity against *M.tb* [[2](#_ENREF_2), [3](#_ENREF_3)]. IFN- activates Macrophages to become efficient effector cells that express microbicidal substances and cytokines, of which tumour necrosis factor α (TNF-α) play a fundamental role in controlling mycobacterial infection [[4](#_ENREF_4)] [[5](#_ENREF_5)]. Whereas, the protective role of IFN- in tuberculosis is well established [[2](#_ENREF_2)] [[6](#_ENREF_6)], TNF- exhibits a very complex network of interactions and many of its activities are still not fully understood [[7](#_ENREF_7)]. However it is known that TNF- displays a main function in controlling *M. tuberculosis* infection, activating macrophages early during the immune response and participating in granuloma formation [[8](#_ENREF_8), [9](#_ENREF_9)], excessive levels of TNF- may cause tissue damage *in vivo* [[7](#_ENREF_7)] including hyperinflammation and caseous necrosis.

Several signaling proteins modulate the level and pattern of cytokines produced by immune cells upon *M.tb* antigen stimulation [[10-12](#_ENREF_10)]. In particular, we have demonstrated a key role of CD137 in modulate cytokine human responses against *M. tuberculosis*. Signaling through CD137:CD137L pathway interfered with IFN- and TNF- secretion by innate immune cells, but boost T cell effector functions [[13](#_ENREF_13)].

CD137 (4-1BB), is a TNFR related superfamily signaling molecule that regulate effector functions of most types of immune cells [[14](#_ENREF_14), [15](#_ENREF_15)]. It has been traditionally attributed to CD137 a costimulatory role on T cells, responding to a ligand on activated antigen-presenting cells (APCs) [[16](#_ENREF_16)] [[17](#_ENREF_17)]. However, is now recognized that CD137 is expressed, being present or induced on various types of immune cells far more broadly than first recognized, including expression on DCs [[18](#_ENREF_18)], monocytes [[19](#_ENREF_19)] , and NK cells [[20](#_ENREF_20)].

The main objective of our work was to present a novel method to formalize in mathematical language our experimental data by finding a simplified model that fit experimental with simulation data.

A fundamental challenge in modern biology is the integration of diverse experimental data into a cohesive image of the evolution of a biological system through time. Computational modeling of cellular and molecular pathways plays a key role for this purpose, including the ability to test hypothesis, identify areas of missing knowledge and explore parameters of a particular system. In particular, during recently years, this tool was used to explore diverse aspects of the immunological responses against *M.tb,* reviewed in [[21](#_ENREF_21)] and [[22](#_ENREF_22)], but little was published focusing in costimulation.

The goal of this work was to develop a simplified but representative, Bayesian model of CD137 signaling during human response against tuberculosis and simulate its evolution through time to compare with experimental results. Therefore this computational model must be enough complex (containing enough number of parameters) to fit experimental data.

This idea was previously apply by our group to determine the parameters that define the kinetic of sinaptics receptors [[23](#_ENREF_23" \o "Moffatt, 2007 #97), [24](#_ENREF_24" \o "Moffatt, 2007 #62)].

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The framework from this work is based on the bayesian iteration. Provided that you can write down a statistical model relating the quantities you are interested in to the data you can observe (possibly via many unobserved intermediary variables), then you can (in principle) carry out Bayesian inference to extract the information in the

data to give fully probabilistic information on all unobserved model variables.

In the simplest (continuous) setting, we are interested in making inferences about the parameter vector. In the simplest (continuous) setting, we are interested in making inferences about the parameter vector of a probability (density) model giving rise to an observed data vector y. If we treat the parameters as uncertain, and allocate to them a ‘prior’ probability density *(),* then Bayes theorem gives the ‘posterior’ density



where is the marginal density for  obtained by integrating over the prior. Since is regarded as a function of for fixed (observed) , we can re-write this as so that the posterior is proportional to the prior times the likelihood. [[25](#_ENREF_25)]

The main limiting factor in applying Bayesian methods is computational. For non-trivial problems, analytic approaches to Bayesian inference are not possible, and their numerical solution is often challenging due to the need to solve high-dimensional integration problems (which in the discrete case translate to combinatorial summation problems). To deal with this problem, we use a novel fitting scheme, which combines simulated annealing and Levenberg Marquardt algorithm, to adjust our model to our experimental data obtained of in vitro cultures of human PBMCs.

(Our results identified key factors and parameters values of the system. As we expected, TNF plays a more important role in IFN- decrement than direct CD137 signaling.) Esto hay que ver que da…

treatments

**1.** **Materials and methods**

2.1 Experimental data

Most of the experimental data was from a previous work of our lab [[13](#_ENREF_13" \o "Fernandez Do Porto, 2011 #7)]; we also included some additional kinetics results.. Data were collected from in-vitro cultures of Peripheral Blood Mononuclear Cells (PBMC) of tuberculosis patients stimulated with a cell lysate from the virulent *M. tuberculosis* H37Rv strain, prepared by probe sonication as published before [[13](#_ENREF_13)]. Briefly, cells were incubated in the presence/absence of *M.tb* Ag (10 μg ml–1). At different times, CD137 and CD137L expression was determined by flow cytometry. For blocking experiments, cells were incubated 30 min with blocking mAbs (BD, Bergen County, NJ, USA) against CD137 (5 μg ml–1, 4B4-1), CD137L (5 μg ml–1, C65–485), or isotype control. Then, cells were stimulated with or without *M.tb* Ag. The percentage of IFN-γ or TNF-α-secreting cells and apoptosis were determined by flow cytometry. Cells were also pulsed with [3H]TdR (1 μCi per well), harvested 16 h later and [3H]TdR incorporation was measured in a liquid scintillation counter. In separated experiments, IFN-γ and TNF-α production was evaluated by ELISA (enzyme-linked immunosorbent assay) following the manufacturer's instructions (eBioscience, San Diego, CA, USA).

2.2 In vitro signaling of CD137 model

The idea of this article is to create a mathematical/computational model of CD137 signalingwhich could replicate our experimental data of in-vitro responses against *M. tuberculosis*. We focus our model on cytokines regulation and include in it only those variables for which we have experimental data, excluding those cell types or cytokines for which we have not.

The *Mtb*-immune system model of Marino [[26](#_ENREF_26" \o "Marino, 2010 #342)] serve as our background model. We have developed a mathematical model consisting in a system of non-linear ODE containing 17 equations and 83parameters. We also include some accessory equations for the prediction of experimental data.

We approximate solutions of ODE we use Euler method (cita?). Thus, a simulation consists of finite time-steps. Each time-step corresponds to approximately 6 s of “real time”. This was determined by considering the fastest process represented in the model as in [[27](#_ENREF_27" \o "Segovia-Juarez, 2004 #344)]. Our model includes three types of cells populations, as follows: Antigen Presenting cells (APC class), NK cells (NK class) and T cells (T class). We have previously shown that CD137 and CD137L are both expressed on APC and NK cells while only CD137 is expressed in lymphocytes after in-vitro *M.tb* stimulation[[13](#_ENREF_13" \o "Fernandez Do Porto, 2011 #7)]. For simplicity, APC and NK express ligand and receptor simultaneously on the same cell in our model, since it was reported that receptor and ligand can be expressed on the same innate immune cell [[18](#_ENREF_18" \o "Futagawa, 2002 #437)] [[19](#_ENREF_19" \o "Langstein, 1998 #458)]. However, the implication of a single cell expressing both ligand and receptor and bidirectional signaling by CD137/CD137L on immune outcome are not well understood [[15](#_ENREF_15" \o "Wang, 2009 #86)]. As there exists much more bibliography of reverse than direct signaling in monocytes, we only include reverse signal in these cells. On the other hand we include direct signaling through receptor in NK and LT cells, as there are not convincing literature of a role of reverse signaling in these types of cells. We assume that CD137 or CD137L signaling occurs in a single event. Stimulation of CD137 ligand on human monocytes has been shown to induce DC differentiation, and these CD137L-DCs are more potent than classical DCs, in stimulating T cell responses in vitro. That is why, and given our experimental data, we capture Monocytes, Macrophages and Dendritic cells in a single population: APC. During persistent infection, as tuberculosis, CD137 and CD137L expression can be prolonged [[15](#_ENREF_15" \o "Wang, 2009 #86)] so our model doesn`t describe internalization of receptors.

As we focused in cytokine responses we only include NKbright cells in the model since they are the most efficient cytokine producers, excluding the cytotoxic NKdim. Also we didn`t include CTL function in T cells. A single T cell class captures CD4 and CD8 proinflammatory T cells**.** Priors parameters for cells interaction are taking from…

2.3 Treatments.

Simulations were performed in three different virtual treatments for data fitting. “M.tb treatment” simulates experiments in which cells are stimulated with *M.tb* antigen (Ag). “blocking treatment” simulates experiments in which cells are stimulated with *M.tb* in the presence of anti-CD137 blocking antibody (Ab). “Media treatment” simulates control experiments without Ag and Ab.

2.3 APC dynamics

As said in the introduction, the control of tuberculosis infection requires the coordinated interaction of APC and T cells. Macrophages, the preferred habitats of *M.tb* [[28](#_ENREF_28)], and DCs are the major antigen presenting cells involved [[29](#_ENREF_29), [30](#_ENREF_30)].

We describe four different APC subpopulations (Eqs. 1-4): resting (APC0); activated (APCa); activated and signalized by CD137 (APCbo); activated and binding to an anti-CD137 blocking mAb (APCbl) and activated, signalized by CD137 and bind to an anti-CD137 blocking mAb (APCbo\_Ab);.

APC0 includes circulating undifferentiated monocytes and immature dendritic cells. Upon interaction with *M.tb*, dendritic cells undergo a repertoire of phenotypical changes, a process termed as maturation [[30](#_ENREF_30)]. TNF- and IFN- are also required for macrophage activation by the antigen [[31](#_ENREF_31)].

Therefore, in our model, resting APC undergo activation by antigen uptake during *M.tb* stimulation, in a TNF- and IFN independent (DC, term A-Ag2) or dependent (monocytes, term A-Ag1) manner. So, loss of APC0s is modeled with APC0 uptake of the antigen or death at a rate of µA0 (Eq.1). A little ratio of APC0 express receptor and ligand and produce basal levels of cytokines. We considered macrophague classical activation and not alternative.

Eq.2 describes the dynamics of APCa, showing a balance between APC uptake of bacteria (production of APCa) and natural death(µAa) and TNF-induced apoptosis (µA).

As we focus on CD137 signaling, parameters (cytokine production, proliferation and apoptosis rates) define two types of activated cells, considering if they are signalized by CD137 (APCs-APCs\_Ab) or not (APCa, APCAb).

CD137 is expressed by primary monocytes in an activation dependent manner [[32](#_ENREF_32)]. We assume that all activated APC express ligand and receptor and produce TNF-, but only a fraction produces IFN-

Unpublished data of our lab using cultures of purified monocytes stimulated with lysate of *M.tb* antigen, has demonstrated that CD137 interacts with CD137L, both expressed on the APC decreasing TNF- secretion. On the other hand, there are existing reports showing that, CD137 pathway induces activation, migration, survival, and differentiation via direct, reverse and bidirectional signaling on monocytic cells (monocytes, macrophages, and DCs) [[33](#_ENREF_33)] [[34](#_ENREF_34)], but it seemed likely that CD137 would play different roles depending on the infecting bacterial species. In fact, it has been proposed that CD137 plays opposite roles in Gram-negative and Gram-positive bacterial infections [[35](#_ENREF_35)]. (So initial guess for induction of apoptosis, proliferation and cytokine secretion by CD137 is either positive or negative).

In contrast to our increasing knowledge regarding the role of CD137 signaling in the induction and development of Th1 responses, our understanding with regard to the effect of CD137 pathway in innate immunity is still in it’s earliest stages. Interactions through CD137-CD137L among APC with other cells which are key players of innate immunity, which are capable of expressing CD137 and/or CD137L, is an unexplored area. As it was demonstrated that APC and NK activate each other during human response against *M.tb* we allow ligand APC to interact with NK receptor.

Once APCa interacts with other APC or NK expresssing CD137 or CD137L they became signalized by CD137 (APCs). If APCa present the antigen to a naïve T cell they can became signalized by CD137 too (if the interaction is not blocked by an antibody). As it was demonstrated that APC can also interact with NK cells we include this possible mechanism.

Traditionally monocytes were assumed not to be able to proliferate, but it was shown that CD137 induces a widespread proliferation of human peripheral monocytes [[19](#_ENREF_19)]. Hence, we only allowed signalized APC to proliferate. Dinamics of these cells also include natural death and TNF-induced apoptosis.

Equation () describes APCAb dynamics. It accounts for the APCa receptor binding to blocking mAb. As these cells are not signalized other parameters are the same of APCa. Although they have the receptor blocked, these cells can be reverse signalized by the antigen (APCs\_Ab). APCs\_Ab also “comes” from APCs that bind the antigen.

2.4 NK dynamics

NK cell activity is regulated by a balance between activating and inhibitory receptors [[36](#_ENREF_36)] [[37](#_ENREF_37)]. Early studies showed that stimulation of mouse NK cells with cross-linking anti-CD137 antibodies or with CD137L-expressing cells induced their proliferation and IFN- secretion [[38](#_ENREF_38)]. However, it was recently demonstrated that CD137 is expressed by activated human NK cells, but surprisingly, this interaction reduces NK-cell activation (and IFN- production). Moreover, it was shown that impaired NK-cell reactivity after CD137 triggering is not due to survival but rather to inhibitory signals [[39](#_ENREF_39)]. As there is no evidence of CD137L reverse signaling on NK cells we exclude this possibility.

Two major subsets of NK cells have been recognized in peripheral blood based on the differential expression of CD56 receptor [[40](#_ENREF_40)] . The vast majority of circulating NK cells (CD56dim) are cytotoxic and do not produce IFN-Only 5–10% of NK cells are IFN--producing cells (CD56bright). We have previously shown that CD137 and CD137L are expressed only on CD56bright NK cells. As we focus our analysis on cytokine modulation by CD137, we only include CD56bright NK cells in our model.

Similar to APC, the model include five NK cells stages: resting (NK0), activated (NKa), activated and signalized by CD137 (NKs), activated, signalized and blocked by anti-CD137 mAb (NKs\_Ab) and activated and blocked (NKAb).

CD56bright NK cells activation requires IL12, NK cell–APC interaction and *M. tuberculosis*–NK direct contact [[41](#_ENREF_41)] [[42](#_ENREF_42)]. IL-12 is indirectly model as activated APC presence. So, loss of NK is modeled as NK activation (at rate of KaNK) and death (μNK0). During NK activation, NK-CD137:CD137L-APC interaction could take place, but there is no evidence how this interaction occurs. So, for clarity and simplicity, we preferred to include activation and signaling in two steps. As NK-NK interaction was also shown and NK express both ligand and receptor, we did not discard this interaction too. Therefore NKa dynamics include CD137 signaling by APC or NK cells, natural death, TNF induced-apoptosis and proliferation.

Eq. () describes NKs, incorporating IFN-/TNF induction by CD137. Eq() describes NKAb, defined by same parameters that NKa, but with the receptor bind to anti-CD137 blocking mAb. Eq() describes NKs\_Ab that behaves as NKs, but the receptor is also blocked.

We assume that all activated NK cells produce IFN-, but only a fraction produce TNF and express ligand/receptor pair.

2.5 Lymphocytes dynamics

Adaptive immune responses mediated by T cells play a vital role in the elimination of *M. tuberculosis* [[43](#_ENREF_43)].

We model four different T cells population: non-specific-antigen-T cells (Tn), specific-antigen naïve T cells (T0), activated with CD137 costimulation cells (Ts) and activated but not signalized CD137 Th1 cells (Tb).

Non specific T cells (Eq. ) are the main component of PBMC in vitro. So they were included because, its quantity is of significant importance in the total cell count. They can either proliferate at rate of PTns or die at rate of μTns. Naïve T cells are described in equation LT0. They proliferate and die at the same rates of LTns, but they include activation/differentiation that depends on activated APCs (APCa, APCs, APCs\_Ab and APCAb). This activation could happen with LT receptor free (at a rate of…) or blocked (by anti-CD137 mAb) depending on mAb concentration.

We include activation and differentiation in a single step. LT0sare activated with a rate of A\_T , but in the presence of mAb in media they can be activated by all activation signals (including CD137) (LTs) or have CD137-CD137L interaction blocked. Equation (14) models Ts dynamics. It accounts for the differentiation of naïve LT0, proliferation (with a rate of PTs), natural death (μTs) and TNF-induced apoptosis. Equation (15) describes TAb cell dynamics, incorporating CD137 blockage stimulation of apoptosis, and inhibition of proliferation and IFN- and TNF- production. CD137 was proposed as an effector T cell marker [[44](#_ENREF_44)]. Therefore, we assume that all activated LT express receptor. Unpublished data of our lab demonstrate that only 40% and 23% of CD137+ LT are IFN-+ and TNF-+respectively (so % and % priors where set for this values).

Extensive evidence has shown that signals through CD137 delivered by agonistic antibodies or by overexpressed ligand can augment T-cell activation or survival [[45](#_ENREF_45)] [[46](#_ENREF_46)] [[47](#_ENREF_47)] [[48](#_ENREF_48)]. CD137 is not expressed on resting T cells, but rather induced with antigen (Ag)-receptor signaling [[46](#_ENREF_46)] [[48](#_ENREF_48)] [[49](#_ENREF_49)]. So we assume that CD137 blockade in LT induce apoptosis and inhibits proliferation and cytokine production.

We also include an equation for annexin incorporation by LT describing the percentage of cells that enter apoptosis in tA (time duration of apoptosis) before than experiment time.

2.6 Culture media dynamics

Cytokines are produced by a large variety of cells involved both in innate and adaptive immunity [[50](#_ENREF_50)]. As we measure IFN- and TNF- levels and intracellular expression by single cells in our experimental data, we modeled these two key cytokines. TableS (Supporting Information online) lists cytokine production by cell types.

Each equation has a degradation rate for each cytokine represented by a μ coefficient. TNFα (, equation (25)) is mainly secreted by activated APC (APCA, α1 term). Since, it has been proposed that CD137 plays opposite roles in Gram-negative and Gram-positive bacterial infections 11Priors estimates for the induction of TNF by CD137 are neutral. The presence of the antigen enhances TNF production by APCA. Lymphocytes and NK cells also secrete TNF (α3 and α4), but prior estimates indicates a low contribution of these cells to total TNF- levels.

Human natural killer (NK) cells are a major source of early gamma interferon (IFN-γ) upon *M. tuberculosis* stimulation in vitro. [[42](#_ENREF_42)]. Macrophages were found to produce small levels of IFN- during *M. tuberculosis responses* (Cooper 2009, *Mycobacterium tuberculosis* infection and inflammation: what is beneficial for the host and for the bacterium?)

*Prior estimates indicate that i*nfected macrophages produce a small amount of IFN-γ (α7 terms). *(AI)* [[51](#_ENREF_51)]. Once adaptive immunity has developed, IFN-γ is mainly secreted by lymphocytes (α5 and α6 terms) (Barnes et al., 1993; Tsukaguchietal.,1999).

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IFN-g is predominantly secreted by innate cells (mainly natural killer cells, sg terms in Eqs.(26)and(30)intheSupportingInformationonline) right after infection and upon signalingbyinterleukin12(IL-12) and by Tcells upon instruction byinterleukin12(IL-12) and IL-18 (Korbel etal.,2008). Infact, a second wave of IFN-g production occurs when adaptive immune cells are generated

Si bien el IFNg es producido por las LT en presencia de IL-12 nosotros incluimos el porcentaje de T activadas que producen IFNg

As we are working with lysate of bacteria we asume that there are not infective macropage. So we estimate INF-g values

Si bien las NK producen IFN en presencia de IL-12 nosotros lo incluimos en su estado de activación

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*2.7 Prior parameter estimates*

It is hard to determine prior parameters values of the system, since experimental data comes from a variety of sources obtained in different experimental contexts (including several models in vivo and in vitro). Therefore we choose to seek order of magnitude estimates for prior parameters with reasonable biological value. Such order of magnitude estimates come from both modeling and biological literature (table X).

In some cases it has not been possible to obtain in the literature one estimate for a parameter, and hence we have included order of magnitude estimates for different cells so as to achieve a lower (or upper) bound.

To set prior parameters we did consider experimental data we set out to fit.

2.8 Novel Bayesian fitting approach

Our goal is to recapitulate experimental data with the model (modelfitting) and second, to use the model to determine the key mechanisms that affect specific cellular processes, including recruitment, activation, infection, and trafficking (using uncertainty and sensitivity analysis).To accomplish these goals we use a novel fitting approach which combines simulated annealing and Levenberg Marquardt algorithm, to adjust our model to our experimental data obtained of in vitro cultures of human PBMCs (see Fig. 2 for a schematic diagram of the main steps of the algorithm). Due to parameters vector is larger than experimental values vector, it is necessary to initialize the algorithm with an adequate prior parameters vector for the posterior parameters we want to estimate. We accomplish this task by prior parameters estimates (see 2.7).

Results

Se encuentra el modelo

Se restringe el universe de parametros

Cinéticas

Algún cambio

Discussion

In this work we have describe a method to formalize, and better understanding experimental data. More cynetics data are necessary than habitualmente. Esto no genera un costo materialy en horas muy mayor. Los papers are presented as pictures of a moment, but nothing says about kinetic.

Previous mathematical models have been developed to consider macrophage dynamics, for example: partial differential equation (PDE) models in tumor biology

In this paper we developed a spatio-temporal model of the initial, innate immune

response to *tuberculosis*.We have extended temporal models ofWigginton and Kirschner

[42] and Tran *et al.* [37] to incorporate spatial effects. Our model consists

of coupled reaction-diffusion-advection equations governing dynamics of macrophages

(resting and infected), bacteria (extracellular and intracellular) and a bacteria-

released chemokine each of which affect the final granuloma size.

[25,26]; ordinary differential equation (ODE) models in Mtb infection [42],

phagocytosis [37], HIV infection [16,43] and in the immune response to an unspecified

disease or infection [18, 29

Previous mathematal models has been developed to address important questions regarding both the immune response and *M.tuberculosis* infection that have been difficult to approach using traditional experimental methods *(7–12).*

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In this paper we have use an inverse logic. We are looking forward to demostrated that not only it is posible to

Formalize in mathematical language experimental data obtained in vitro but is a good thing

se busca demostrar la forma en que se puede formalizar en lenguaje matemático resultados obtenidos in vitro. Creemos necesario que la biología y en particular el campo de la inmunología avance hacia una formalización matemática de hipótesis, presunciones, conclusiones que los experimentalistas poseen cualititativamente. Creemos que de esta manera se podrá integrar de mejor manera los datos obtenidos de diversas fuentes experimentales. Una vez definido (y validado el modelo) y encontrado los parámetros que definen dicho modelo, se podrá utilizar el mismo para integrarlo con otras fuentes experimentales de manera de poder simular los mecanismos de interacción in vivo.

Costimulatory signals represent a complex network of ligand-receptor interactions that quantitatively and qualitatively influence immune responses and affect all its major participants including effector lymphocytes and innate cells. [[52](#_ENREF_52)] Among TNFR superfamily members, CD137 has emerged as an important costimulatory receptor.

We have previously demonstrate that CD137:CD137L interactions work differently on different cell populations, inhibiting or increasing a particular cytokine secreted by a particular cell type.

We have identified a simplified model of chemokines based on three classes that affect recruitment of macrophages

Una celula (APC o NK) que expresa CD137 y CD137L y es bloqueada solo por CD137

**This model can now be exploited in future work to study coestimulation processes in vivo during human immune response against M. tuberculosis.**

*Furher analysis could differentiate recetor-ligand*

This model can be further exploited to investigate key questions about lympho cyte dynamics and effects on control of infection and pathology, and trafficking of cells between LN and lungs. Additional data will be necessary for these investigations, which are crucial to our understanding of the dynamics of tuberculosis.

**Como no todas las NK expresan CD137 se explica un cambio menos dramático en la producción de IFN**

**Las implicancias de la señalización bidireccional needs to be furher explore.**

**No se tienen en cuenta las interacciones que no modifican el cambio de estado de una célula.**

**Nosotros proponemos/estimamos que la inmunología experimental debe avanzar hacia la formulación matemática de hipótesis, presunciones y conclusiones. Lo que permitirá avanzar hacia la formalización matemática de la misma, aunar criterios, databases e integrar data de diversidad experimental, etc**

**We estimate that experimental immunology should move forward to the mathematical formulation of hypotheses, assumptions and conclusions. Thereby advancing to the mathematical formalization of it, build consensus, databases and integrateexperimental data diversity, etc.**

Mathematical models are emerging as important tools

in the study of microbiology.

Different mathematical models

were formulated on the basis of assumptions regarding

system–component interactions, enabling us to explore

specific aspects at diverse biological scales (e.g. intracellular,

cell–cell interactions, and cell population

dynamics). In addition, we were able to examine both

temporal and spatial aspects. At each scale, there were

consistent themes that emerged as determinative in

infection outcome. Factors we identified include both

host and microbial characteristics. The use of the

models lies in generating hypotheses that can then be

tested experimentally. Here, we outline the primary host

and bacterial factors that we have identified as key

mechanisms that contribute to the success of

M. tuberculosis as a human pathogen. Our goal is to

stimulate experimentation and foster collaborations

between theoretical and experimental scientists.

Here, mathematical modeling becomes an important integrative experimental tool (Figure 1). Mathematical models provide a unique approach for representing and studying the integrated behavior of complex biological systems. A strength of the modeling process is that it can lend insight and clarification to existing data and theories, as well as enabling one to compare and contrast existing hypotheses.

Mathematics has long been relegated to the closet in microbiology studies. The emergence of new computational

tools and other technologies provides a perfect opportunity to dust off old notions of mathematics and welcome it into the biological arena. Here, we show that it can be applied in many ways to study relevant problems in biology, using the example of M. tuberculosis infection.

A key to successful conversations between theory and experiment is the language. If theoreticians will put their work in the proper context of the problem being studied, then experimental biologists can more easily see how mathematics can be used as an additional tool to stimulate and address important questions in microbiology. New experimental technologies [8,27] will eventually produce

in vivo time series cell population dynamics data to support and validate mathematical model results and hypotheses. Combinations of experimental measurements and mathematical models will ultimately yield fundamental insights into biological phenomena. A new generation of students is now being trained to this end.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | APCa | APCbo | APCbo\_Ab | APCbl | NKa | NKbo | NKbo\_Ab | NKbl | LT0 |
| APCa | 2APCbo | 2APCbo | 2APCbo |  |  |  |  |  |  |
| APCbo |  |  |  |  |  |  |  |  |  |
| APCbo\_Ab |  |  |  |  |  |  |  |  |  |
| APC\_bl |  |  |  |  |  |  |  |  |  |
| NKa |  |  |  |  |  |  |  |  |  |
| NKbo |  |  |  |  |  |  |  |  |  |
| NKbo\_Ab |  |  |  |  |  |  |  |  |  |
| NKbl |  |  |  |  |  |  |  |  |  |
| LT0 |  |  |  |  |  |  |  |  |  |

In conclusion, our model provides a framework for the development of spatiotemporal

models of the immune response, not just for *Mycobacterium tuberculosis*.

In particular we are studying the effects of cell-mediated immunity on granuloma

formation with the aim of developing a more complete description of the immune

response to Mtb.

# Proliferacion en monocitos: Tener en cuenta? En nuestro sistema funciona al revés

# For example peripheral monocytes in man consist of two subpopulations of which the CD14high, CD16−cells constitute to 90–95% and the CD14dim, CD16+ cells constitute 5–10% [[2]](http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0016129#pone.0016129-Passlick1). (Species Difference of CD137 Ligand Signaling in Human and Murine Monocytes)

We have identified a simplified model of chemokines based on three classes that affect recruitment of macrophages and T cells to the granuloma via binding of appropriate chemokine receptors on the cell surface

Each of the four roles of TNF (cellular migration, induction of chemokine/TNF secretion, macrophage activation, and apoptosis) may contribute separately to establishing and maintaining control of Mtb infection at the level of a single granuloma. Currently, it is impossible to study these separate TNF functions

using in vitro or in vivo models. In this study, we use a specific type of computational model known as an agent-based model (ABM) to study the contributions of these immune effectors on granuloma formation

We build on our previously described ABM (38), which predicted the emergence of two-dimensional spherical structures (granulomas) without any rules specifying such spatial behavior to occur.

As we work with saturating concentrations of antigen we did not include

As we work with experiments In vitro we assign the same probability of cells to interact with other types de acuerdo a la velocidad de movilidad cellular.

This is an acceptable model since

Anotherkeycytokine for triggeringinflammationisIFN-g. IFN-g is predominantly secreted byinnatecells(mainlynaturalkillercells, sg terms in Eqs.(26)and(30)intheSupportingInformationonline) right afterinfectionanduponsignalingbyinterleukin12(IL-12) and byTcellsuponinstructionbyinterleukin12(IL-12)and IL-18 (Korbel etal.,2008). Infact,asecondwaveofIFN-g production occurswhenadaptiveimmunecellsaregenerated((LM Kirchner)

The mainassumptionofthemodelisthatwithoutinfection (no livebacteria,i.e.CFUlung¼CFUln¼0), thereisessentiallyno cytokine production,thereforenoinflammationoccursandthe MPFs arezero(i.e., d¼dLN¼0).(LM KiRchner)

TNF-induced apoptosis is a shared regulatory mechanism for effector T cells in both compartments.

4-1BBL is quite sensitive to downmodulation in the

presence of 4-1BB, whereas the reciprocal is not true. As

shown in Fig. 3, on DCs derived from mouse bone marrow

treated with granulocyte-monocyte colony-stimulating factor

(GM-CSF) and IL-4 and then a TLR stimulus, one can readily

detect surface 4-1BB but not surface 4-1BBL. However, if one

uses 4-1BB-deficient DCs, 4-1BBL is now detectable on the

mature DCs, implying that 4-1BBL is downmodulated in the

presence of 4-1BB. On the other hand, 4-1BB levels change

only slightly in the presence of ligand (Fig. 3). (complexities and challange)

Tuberculosis (TB) remains an enormous global health problem despite

current drug treatment. The disease causes nearly 9 million new cases

and 2 million deaths annually worldwide, and is among the most

common causes of morbidity and mortality in patients with HIV

infection. Bacillus Calmette-Gue´rin, the only available vaccine, is of

variable efficacy, especially in TB-endemic regions, such as Argentina.

Development of a more effective vaccine depends on a better understanding

of the human immune response to this pathogen.

Tuberculosis (TB)4 kills more people per year than any

other single infectious disease. Infection by its causative

agent, *Mycobacterium tuberculosis* (Mtb), results in active

disease in only a minority of cases (\_10%); the majority of

infections are controlled and clinically silent, although the host

often remains infected (reviewed in Ref. 1).

The

power of this approach lies in the emergence of behaviors that

arise from interactions between agents that would otherwise be

impossible to know a priori.

it was

estimated that T cells move with an average velocity of

11 mm= min; i.e., approximately 10\_5 m= min

The rate of macrophage movement wascalcul ated

from the data of Webb et al. (1996), based on cloned

macrophage mouse cell line BAC1.25F. They found

macrophage velocitiesin the range of 0.12–0.5 mm= min

using a Dunn chemotaxis chamber. We set the speeds to

1, and 0:0007 mm= min for resting ðMrspÞ; and infected

macrophages ðMispÞ respectively. For activated macrophages

ðMaspÞ we use a range of values between 0.0125

and 1 mm= min :

Of course, actual velocities of T cells in lung tissue

likely vary over a wide range depending on states of

individual T cellsand local environmentsin which they

are moving. Similarly, the frequency with which all

other rulesare executed could vary depending on the

state of the entities involved. Future refinements of this

model could include more detailed and heterogeneous

treatments of time such as these.

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(A) (B)

Fig. 1. Histopathologic comparison of solid (A) and caseous

(disseminated) (B) pulmonary granulomas in the lungs of M.

tuberculosis-infected cynomolgusmacaqu es. Solid granulomas (A)

consist of a densely populated collection of inflammatory cells that

include centrally located macrophages and histiocytes with surrounding

rings of T cells. Caseous granulomas (B) are characterized by a

central area of necrotic material, an outer later of macrophages,

histiocytes and giant cells ringed by T cells. Hemotoxylin and eosin

stain, total magnification 100 \_ : Both granulomass hown are 2mm \_

2mm in size. (Photos donated by Dr. P. Ling Lin, in the Flynn

Laboratory at the University of Pittsburgh).

**Interferon-g (IFN-g) is critical for an effective innate immune tokine-induced NK cell production of IFN-g and TNF-a, also**

**response against infection. A combination of interleukins decreased cytokine-induced NK cell apoptosis. Costimula-**

**(ILs) derived from activated T cells (IL-2) and monocytes tion of a CD3**ì**CD56**" **NK leukemia cell line with IL-2 and**

**(IL-12), ormonocytes alone (IL-15 and IL-12), induces optimal IL-12 or IL-15 and IL-12 induced apoptosis in vitro, which**

**production of IFN-g from natural killer (NK) cells.**

La estimulación de producción de citoquinas de NK por LT no es tenida en cuenta

These issues could be solved by reconstitution experiments employing CD137 or CD137L deficient cellular components in the near future.

NK cells are large granular CD3−CD56+ lymphocytes, constituting approximately 10% of peripheral blood lymphocytes. NK cells lyse autologous infected cells and tumor cells without prior sensitization.10,11 NK cells secrete IFN-g to activate macrophages to kill viruses and intracellular parasites.10–12 NK cells have been found to play an essential role in immune defenses against cancer and infectious diseases in various experimental systems.13 The vast majority of NK cells (90–95%) are cytotoxic and do not produce IFN-g. Only 5–10% of NK cells are IFN-g-producing cells.( Innate and adaptive immune responses to human *Mycobacterium tuberculosis*

infection

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*.* Futures papers may include bidirectional signaling…

*CD137 as an activation marker of LT so all activated cells express CD137 35% de blastos???*

**APC cells dynamics**

Resting (uncommitted) APC (A0)

APC0 activation (inflammatory context independent) (DC)

APC0 activation in inflammatory context (macrophages)



APC0 death



TNF- induced apoptosis of APC

Activated APC (Aa)

APCa death





APCa CD137 signaling by APC

APCa CD137 signaling by NK

APCs binding to

blocking anti-CD137 mAb (mAb values are constant)

APCa CD137 signaling by LT

Activated APC with the receptor bind to blocking anti-CD137 mAb (AAb)

TNF- induced apoptosis of APC

AAb death



APCAb CD137 signaling by APC

APCAb CD137 signaling by NK

CD137:CD137L

induced proliferation of APCa

APCAb CD137 signaling by LT

Activated APC and signalized by CD137L (As)





TNF- induced

apoptosis of APC

CD137:CD137L

induced death of APCa

APCs binding to

blocking anti-CD137 mAb (mAb values are constant)

Activated APC, signalized by CD137::CD137L (As) and the receptor bind to blocking anti-CD137 mAb



TNF- induced

apoptosis of APC

CD137:CD137L

induced death of APCa

CD137:CD137L

induced proliferation of APCa

**Other APC equations**

Total number of APC



Percentage of APC producing IFN- and TNF-

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Percentage of APC expressing receptor and ligand



**NK cells dynamics**

NK0 activation induced by the antigen, APC and IL-12

Resting (uncommitted) NK (NK0)

NK0 death

NK0 proliferation



Activated NK (NKa)

NKa death

NKa proliferation



NKa CD137L signaling by NK

NKa CD137L signaling by APC

NKa binding to

blocking anti-CD137 mAb (mAb values are constant)

NK0 death

TNF- induced

apoptosis of APC

Activated NK and signalized by CD137L (As)

NKs proliferation





TNF- induced

apoptosis of APC

NKs death



Activated APC, signalized by CD137::CD137L (As) and the receptor bind to blocking anti-CD137 mAb

TNF- induced

apoptosis of APC



NKs\_Ab proliferation

NKs\_Ab death



Activated NK with the receptor bind to blocking anti-CD137 mAb (NKAb)



TNF- induced

apoptosis of APC

NKAb proliferation

NKAb death

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**Other NK equations**

Total number of NK



Percentage of NK expressing receptor and ligand



Percentage of NK producing IFN- and TNF-



****

**LT cells dynamics**

Non-antigen-specefic T cells

Tns death

Tns proliferation



T differentiation (induced by activated APC)

Tns death

Tns proliferation

Naïve specific T cells



TNF- induced

apoptosis of APC

T cells activated by APC (with CD137:CD137L signaling) (Ts)

Ts death



Ts proliferation

T differentiation (induced by activated APC)

TNF- induced

apoptosis of APC

T cells activated by APC (without CD137:CD137L signaling) (Tbl)

Effect of CD137 blockade in Ts death



Effect of CD137 blockade in Ts proliferation

T differentiation (induced by activated APC) with CD137::CD137L interaction blocked

**Other LT equations**

Total number of LT



Percentage of LT expressing receptor



Percentage of LT producing IFN- and TNF-





Percentage of LT undergoing apoptosis/necrosis



**Media dynamics.**

*Cytokine production*





Antigen concentration



Total number of cells



Proliferation ratio



### [triated thymidine](http://www.google.com.ar/url?sa=t&rct=j&q=triate+thymidine&source=web&cd=1&ved=0CCEQFjAA&url=http%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2F0027510769901146&ei=L0NFT4quL8KyiQL368m5Dg&usg=AFQjCNEmIeh8UrHSZP4nXVdW_d-oCzaI7Q&sig2=WPa8Q2RI2uaxH3ydcEOQFQ)incorporation



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| --- | --- | --- | --- | --- | --- |
| Name | Prior parameters | Posterior Parameters | Reference | units | Description |
| rA | 0.085-0.091 |  | [[53](#_ENREF_53)] | ------ | Ratio of APC in PBMC |
| rN | 0.005-0.015 |  | [[54](#_ENREF_54)] | ------ | Ratio of NKbright in PBMC |
| rT | 0.58-0.82 |  | [[54](#_ENREF_54)] | ------ | Ratio of LT in PBMC |
| rTsp | 2.0e-4-2.0e-3 |  | [[55](#_ENREF_55)] [[56](#_ENREF_56)] | ------ | Ratio of LTspecific to M.tb |
| A0 | 5.000-11-5.000-5 | 5.000-11-5.000-5 | Estimated | (ng A0)-1 h-1 | IFN production by APC0 |
| Aa | 5.000-11-5.000-5 |  | Estimated | ng Aa-1 h-1 | IFN production by APCa |
| IA | 2-4 |  | [[57](#_ENREF_57)] | ------ | Induction factor of IFN production by CD137::CD137L |
| A0 | 6.387-7-6.387-4 |  | [[26](#_ENREF_26)] | ng Aa-1 h-1 | TNFproduction by APC0 |
| Aa | 6.387-7-6.387-3 |  | [[26](#_ENREF_26)] | ng Aa-1 h-1 | TNF production by APCa |
| IA | 2-10 |  | [[19](#_ENREF_19)] [[57](#_ENREF_57)] | ------ | Induction of TNFproduction by CD137::CD137L |
| rA0 | 0.01-0.10 |  | Estimated | ------ | APC0IFN+/APC0 ratio |
| rAa | 0.25-0.75 |  | Estimated | ------ | APCaIFN+/APCa ratio |
| rA0 | 0.01-0.10 |  | Estimated | ------ | APCaTNF+/APCa ratio |
| μA0 | 2.8875-5-2.8875-3 |  | [[26](#_ENREF_26)] | h-1 | Death rate for A0 |
| μAa | 1.4583-4-14583-2 |  | [[26](#_ENREF_26)] | h-1 | Death rate for Aa |
| μIA | 1.0-5.0 |  | [[58](#_ENREF_58)] [[59](#_ENREF_59)] | ------ | Induction of death by CD137::CD137L |
| μαA | 4.167-4-4.167-2 |  | [[60](#_ENREF_60)] | h-1 | Maximum rate of TNF-dependent apoptosis of APC |
| KαμA | 1.0E-3-1.0E1 |  | [[26](#_ENREF_26)] | ng | Half-sat for TNF-dependent apoptosis of APC |
| A | 4.1E-3-4.1E-1 |  | [[61](#_ENREF_61)] | h-1 | Proliferation rate of APCs |
| k(AxAg)1 | 1.0-6-5.0E-1 |  | [[26](#_ENREF_26)] | μg-1 h-1 | APC activation rate (dividir en M y CD?) |
| k (AxAg)2 |  |  | [[26](#_ENREF_26)] | μg-1 h-1 | APC-APC interaction rate (inflammatory context) |
| k(AxA) | 3.05E-8-3.05E-4 |  | [[26](#_ENREF_26)] | cell-1 h-1 | APC-APC interaction rate |
| k(AxN) |  |  | Estimated | cell-1 h-1 | APC-NK interaction rate |
| k(AxT) |  |  | [[26](#_ENREF_26)] | cell-1 h-1 | APC-LT interaction rate |
| k(AxAb) | 1.0E-3-1.0 |  | Estimated | μg-1 h-1 | Ab binding rate for APC |
| KA0,Aa) | 0.1-10.0 |  | [[26](#_ENREF_26)] | μg-1 | Half-sat of IFN- on APC activation |
| K(A0,Aa) | 10e3-10e5 |  | [[26](#_ENREF_26)] | μg-1 | Half-sat of TNF- on APC activation |
| rRLA0 | 0.1-0.10 |  | Estimated | ------ | APC0 CD137+:CD137L+ / APC0 ratio |
| N0 | 4.167E-8–4167E-4 |  | [[60](#_ENREF_60)] | μg-1 h-1 | IFN production by NK0 |
| Na | 4.167\*E-8–4.167E-4 |  | [[60](#_ENREF_60)] | μg-1 h-1 | IFN production by NKa |
| IN | 2.0-10.0 |  | [[39](#_ENREF_39)] |  | Induction of IFN production by CD137 |
| N0 | 3.75-12-3.75-8 |  | Estimated | μg-1 h-1 | TNF production by NK0 |
| Na | 3.75-10-3.75-8 |  | [[62](#_ENREF_62)] | μg-1 h-1 | TNF production by NKa |
| IN | 2.0-10.0 |  | Estimated | ------ | Induction of TNF production by CD137 |
| rN0 | 1.0-5.0 |  | [[63](#_ENREF_63)] | ------ | Ratio NK0 TNF+/NK0 |
| rNa | 10.0-50.0 |  | Estimated | ------ | Ratio NKa TNF+/NKa |
| rN0 | 3.0-5.0 |  | [[64](#_ENREF_64)] | ------ | Ratio NKa IFN-+/NKa |
| N0 | 3.086E-5-3.086E-3 |  | [[65](#_ENREF_65)] | h-1 | NK0 Maximum proliferation rate |
| Na | 3.086E-5-3.086E-3 |  | [[65](#_ENREF_65)] | h-1 | NKa Maximum proliferation rate |
| μN0 | 4.0E-3-4.0E-1 |  | [[65](#_ENREF_65)] | h-1 | Death rate for NK0 |
| μNa | 4.0E-3-4.0E-1 |  | [[65](#_ENREF_65)] | h-1 | Death rate for NKa |
| μαN | 4.167-4-4.167-2 |  | Estimated | h-1 | Maximum rate of TNF-dependent apoptosis of NK |
| KμN | 1.0E-3-1.0E1 |  | Estimated | μg-1 | TNF half-sat for TNF-dependent apoptosis of NK |
| k(N0,Na)A | 1.00e-006-1.00e-003 |  | Estimated | μg-1 cell-1 | Maximum NK activation rate, dependent on APC (and indirect IL-12 |
| k(NxN) | 1.00e-007-1.00e-005 |  | Estimated |  | NK-NK interaction rate |
| k(NxAb) | 1.0e-003 -1.0e-001 |  | Estimated |  | Ab binding rate for NK |
| KA(N0,Na) | 10.0-1.0e003 |  | Estimated |  | APC half-sat on enhancement of NK activation |
| K(N0,Na) | 6.0-600.00 |  | Estimated |  | Half-sat of IFN- on NK activation |
| K(N0,Na) | 6.0-600.00 |  | Estimated |  | Half-sat of TNF- on NK activation |
| rRLN0 | 1.0-17.0 |  | [[39](#_ENREF_39)] |  | NK0CD137+:CD137L+/NK0 ratio |
| rRLNa | 9.0-49.0 |  | [[39](#_ENREF_39)] |  | NKaCD137+:CD137L+/NKa ratio |
| T0 | 8.333E-7-2.75E-6 |  | [[66](#_ENREF_66)] | μg-1 h-1 | IFN-production rate by LT0 |
| Ts | 4.166E-5-4.166E-3 |  | [[60](#_ENREF_60)] | μg-1 h-1 | IFN-production rate by LTs |
| IT | 1.0-8.0 |  | [[67](#_ENREF_67)] [[68](#_ENREF_68)] | ------ | Induction of IFN-production by CD137 |
| T0 | 2.5E-11-3.33E-8 |  | Estimated | μg-1 h-1 | TNF-production rate by LT0 |
| Ts | 2.5E-10-3.33E-7 |  | [[60](#_ENREF_60)] [[69](#_ENREF_69)] [[70](#_ENREF_70)] | μg-1 h-1 | TNF-production rate by LTs |
| IT | 1.0-3.0 |  | [[68](#_ENREF_68)] | ------ | Induction of TNF-production by CD137 |
| rT0 | 1.0-2.0 |  | [[12](#_ENREF_12)] |  | LT0 IFN-+/LT0 ratio |
| rTs | 39.6-52.0 |  | UD |  | LTs IFN-+/LTs ratio |
| rT0 | 1-5 |  | Estimate |  | LT0 TNF-+/LT0 ratio |
| rTs | 20.1-27.8 |  | UD |  | LTs TNF-+/LTs ratio |
| T0 | 7.71E-5-7.71E-3 |  | [[65](#_ENREF_65)] | h-1 | Proliferation rate of LT0 |
| Ts | 0.0083-0.83 |  | [[26](#_ENREF_26)] | h-1 | Proliferation rate of LTs |
| IT | 2-5 |  |  | ------ | Induction of proliferation rate by CD137 |
| μT0 | 1.0E-3-1.0E-1 |  | [[65](#_ENREF_65)] [[26](#_ENREF_26)] | h-1 | Apoptosis rate of LT0 |
| μTs | 2.0E-3-2.0E-1 |  | [[26](#_ENREF_26)] | h-1 | Apoptosis rate of LTs |
| μIT | 2.0-5.0 |  |  | ------ | Induction of apoptosis rate by CD137 |
| K(T\_Ab) |  |  |  |  |  |
| rRT0 |  |  |  |  | LT0CD137+/LT0 ratio |
| μαT | 4.167-4-4.167-2 |  | [[26](#_ENREF_26)] |  | Maximum rate of TNF-dependent apoptosis of LT |
| KαT | 1.0-10.0 |  | [[26](#_ENREF_26)] |  | TNF half-sat for TNF-dependent apoptosis of LT |
| Ap | 2.0-20.0 |  | [[71](#_ENREF_71)] |  | Duration of Apoptosis |
| μ | 0.041-0.16 |  |  |  | Degradation rate of TNF- |
| μ | 0.041-0.16 |  | [[72](#_ENREF_72)] [[73](#_ENREF_73)] |  | Degradation rate of IFN- |
| μAg | 0.041 0.15 |  |  |  | Degradation/internalization rate of Ag |
| Tym | 0.0001 0.1 |  | Estimated |  | Scaling factor [H3]timidine-proliferation |
| nCells | 1000000- 3000000 |  | Estimated |  | Maximum number of cell supported by the media |
|  |  |  |  |  |  |
|  |  |  |  |  |  |