Can systems immunology lead tuberculosis eradication?

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Abstract (100-120 words)

25 years after the declaration of a Global Emergency by the World Health Organization, tuberculosis (TB) remains a major enemy to the humankind. During this period, much progress has been done to better understand its natural history, revealing its huge complexity, which highlighted the need for implementing systems immunology approaches. Recent advances focused in understanding the role of macrophage subtypes and dendritic cells role, the importance of cytokine balance, and the antigenic repertoire. Identification of early irruption of polymorphonuclear neutrophils and extracellular growth of the bacilli seem to be the most disruptive factors to understand the evolution towards active TB. Their inclusion in future models will provide new tools for the better understanding of the tuberculosis.

Keywords

Systems biology, Systems immunology, Tuberculosis, Granuloma, Mathematical model, Agent-based model.

Introduction

Mycobacterium tuberculosis (*Mtb*) remains the only bacteria able to cause the highest mortality by itself. Tuberculosis (TB) natural history has an extraordinary complexity and, in fact, there are still too many unknowns [1] on the mechanisms underlying it. Although there was a significant increase in new information in the last years because of the entrance of omics, it remains unclear how to interpret and use such information for the understanding of key questions. The last review on TB FAQS [2] is a clear and strong declaration of humility around the issue, showing up to which degree of ignorance we are still immersed since 1993, when World Health Organization declared for the first time in the history a Global Emergency linked to a sole infectious disease. This lack of knowledge and capability for giving response to these questions is the explanation for the current feeling of "trap" in which we are immerse, to the extreme that we are not ashamed to publicly declare that we are searching a "pink swan" [3]. The numbers are crushing because of the global annual stability on the absolute number of new TB cases (around 9 million), the massive amount of deaths (around 1.4 million), the increase on multidrug-resistant cases (about 0.5 million), the slow annual decrease on the incidence (around 1.5%) and, what is worse, the clear understanding that

these data come themselves from an epidemiological model [4]. This scenario is in clear contrast with, for instance, the recent success achieved in the case of Ebola. Roughly just after 3 years of the epidemics declared in occidental Africa, a vaccine is available giving a 100% efficacy of protection [5].

The purpose of this work is to obtain a more thorough understanding of 14 biological phenomena identified at a cellular level that a systems immunology approach would enable to contextualize and quantify in a time/spatial frame. This will provide a better knowledge of the natural history of the *Mtb* infection and will facilitate progress in the fight against TB.

What is the role of looking at TB challenge from the point of view of an "in silico" systems immunology?

Usually, the systems immunology approach is seen with skepticism by the immense majority of researchers. The complexity of the mathematical language and the theoretical assumptions do not help at all to its understanding and its value to experimental researchers. Even when trying to set multidisciplinary teams [6], the intellectual background of each integrant usually represents a strong handicap for a fluent exchange of ideas. In addition, there are different interests. From one side, experimental researchers want to find new clues and predictions from their work. From the other side, theoretical biologists have a need for new experimental data to confirm their models. All in all, there is a basic point of confluence that will always help both sides: trying to include experimental observations in a relevant time and space frame.

Systems immunology is the right approach for bridging these issues in the context of host-pathogen interactions in infectious diseases, as defended by Kirschner *et al* [7]. In general, computational models offer a platform where experimental data are integrated and used to interpret observed phenomena. In addition, carefully designed simulation series are appropriate for testing hypothesis, exploring the parameters space with a higher precision and in a wider range than experiments, and predict emergent behaviors. Systems immunology cannot substitute experimental observations, however it is the perfect traveling companion to face TB challenges at this level.

The bottom-line of systems biology approaches

Davis *et al.* [6] defines a systems approach as a "broad strategy for understanding how a complex set of interacting components works to produce certain outcomes", giving rise to systems immunology when we deal with the interactions with or between components of the immune system. Systems immunology approaches can make use of different modelling techniques, taking into consideration some common characteristics of the biosystems that are aiming to describe: (1) they are constituted by discrete elements, from chemical signals and molecular structures of the cellular membrane to a population of cells organized in certain structures; (2) their behaviors have stochastic elements; and (3) it is normally necessary to consider a space-time framework in order to understand their dynamics. Continuous models can be used as a first approximation to the whole system and also to describe those parts or components where discrete elements are not relevant. On the contrary, bottom-up approaches are necessary when dealing with the interaction between components, where agent-based models, multiscale approaches and hybrid models reveal strategically interesting.

GranSim: a historical hybrid systems immunology approach to granuloma formation

GranSim is hybrid model of granuloma dynamics that was released in 2004 by Segovia-Juarez *et al* [8]. In its original version, it used a combination of a continuous modelling of chemokines with a discrete agent approach of macrophages and T cells in a cellular automata-like environment. This hybrid model has experienced several subsequent modifications and updates to deal with particular questions, as will be detailed in the following section, and it has been used by other groups as a tool for simulating TB host-pathogen interactions in lungs.

A reasonable TB morphological model. Setting a basic phenotypical space-time frame.

As the main driver in the immune response against TB is the cellular immune response, it is logical to draw it at the cellular scale. Figure 1 tries to roughly include the whole progress in 14 steps that should be taken into account in any systems biology approach for the understanding of this disease. In the next paragraphs we look over the different steps, we revise the most recent systems biology approaches to each of them and we highlight the unknowns that still need to be tackled. Table 1 summarizes these published approaches and relate them with the corresponding steps (1 to 14), providing more technical details such as the kind of model(s) used, if they use GranSim or not, and their main purpose and the variables considered.

The long road towards the alveoli (1)

The first step is the entrance of the bacilli through an aerosol drop. Even when this step could be considered out of the scope of this review, our lack of understanding makes worthy to consider it, especially taking into account the data recently appeared about the role of the hydrophobicity of the cell wall of *Mtb* in comparison with other mycobacteria and its influence to remain in aerosol droplets [9]. Also, newest data on the aerosol quality and the capacity of these aerosols to hold *Mtb* bacillary clumps [10] might be relevant to understand the interaction with the alveolar macrophage. At this stage, there is a need for linking the classic data of the frequent observation of TB in the right upper lobes and the periphery of the lung [11] with the respiratory movements and the bronchial tree geometry, taking advantage of newest data on the deposition of particles and with the help of virtual modeling of the lung [12].

The interaction at the alveoli (2)

There is a lot of information about the interaction of *Mtb* with the alveolar macrophages (AM), mostly because the relative easiness of this model *in vitro*. Nevertheless, a model that integrates from the interaction with the receptor until the escape of *Mtb* from the phagosome by triggering necrotic factors and inhibiting the apoptotic ones, has not been built yet. Equally, at this level, the interaction with the epithelial cells once the macrophage is destroyed by the growth of the bacilli should be still clarified [13]. In this regard, *Drosophila melanogaster* appears to be a good model to clarify the interaction of the bacilli with the respiratory and intestinal epithelia [14]. A new recent model built in Netlogo platform has linked the lack of drainage in the alveoli of the upper lobes with the local accumulation of bacilli. This increase in the bacillary charge has to be faced by the new incoming alveolar macrophage, which has been linked with the type of inflammatory response (i.e., monocyte or polymorphonuclear neutrophil (PMN) oriented) [15].

The onset of the granuloma (3&4)

It is assumed that each alveolus holds a unique AM and that its replacement is a physiological phenomenon. AM must phagocyte all the particles and pathogens carried by the inhaled air. Thus, its substitution must be considered as normal. It is thought that, after a sort of interaction with the epithelial cells, interstitial AM are the ones that replace damaged AM. This is a very important point in order to keep the tightness of the alveoli not to disrupt the low tensional surface induced by the constant secretion of surfactant that avoids its collapse. Triggering a local inflammatory response and thus breaking the endothelial integrity to allow the entrance of blood cells and plasma is a not well understood process. This has to be well tuned as it represents the loss of the alveoli functionality. Probably this takes place once several neighbor AM from the same acini (for anatomical reasons) are necrotized [3]. The local accumulation of chemokines, surely helped by the stimulation of epithelial cells, must lead to a critical point that stimulates the entrance of blood cells and plasma, ruining the acini. As mentioned above, inflammatory response induces two sorts of granulomas, the one in which there is a monocytic predominance (proliferative) or a PMN based one (exudative). This phenomenon has been recently defined after carefully studying the murine model with the C3HeB/FeJ strain, also known as Kramnik model [16], [17]. Regarding the monocytic infiltration and its transformation in macrophages, they can differentiate in M1 or M2 class, a fact that is relevant due its differential capacity to react against the bacilli, as recently pointed in a mathematical model [18].

The interaction with the regional lymph node (5)

With the inflammatory response and destruction of the alveoli functionality, the lymphatic drainage starts propelled by the overpressure caused by the plasmatic entrance. Bacilli are drained freely towards the local lymph nodes at the very beginning, and by the newly induced dendritic cells (DC) afterwards. Once there, they are phagocytosed by the first line of macrophages disposed at the narrow subcapsular sinus [19]. Those that are not filtered are finally phagocytosed by DCs. This aspect was addressed experimentally raising the concept of two compartments at the lymphatic tissue: the infected one and the proliferative one, respectively [20], a process that should merit a deep modelling exercise. DCs will then trigger a lymphocytic proliferation depending on the inflammatory media in which they are immersed. Acute mediators, a complex mixture of IL-1, TNF-α, IL-6, IL-10, prostaglandins and leukotriens, to cite some of them, together with cytokines like IL-2, IL-4, IL-12, IL-13, IFN-γ and TGF-β will determine the sort of CD4 T (Th1, Th2 or Th17 basically) together with CD8 T, Treg and B cells. This is a very complex process that was initially addressed and is constantly updated by the GranSim model [8]. In one of its latest versions, as a part of a multi-compartment hybrid model, it explicitly accounts for the DC role on the interaction with the regional lymph node [21], [22]. This update has been raised on the selection of antigens and the selection of memory T cells that would be able to better control the infection [22].

The relation of the granuloma with the adaptive immune response (6 to 8)

The sort of lymphocytic population triggered and proliferated at the lymph nodes will reach the lung parenchyma, pumped by the right ventricle. Basically, Th1 cells will detect and activate infected macrophages through IFN-γ and will kill the bacilli. This action can be also induced by CD8 cells. On the other hand, this action can be moderated by Th2 cells by inducing IL-4 and IL-10. Tregs also can help this anti-inflammatory response by secreting IL-10 and TGF-β and through direct cell to cell interaction. Equally, activated macrophages tend to suppress the action of T cells, raising the typical “doughnut” form, also reproduced by some models [23], [24], being able to stop the local proliferation of lymphocytes (if this takes places, since

it is a controversial issue). Activated macrophages also hold survived bacilli that, entering into dormancy, avoid the destruction. They also phagocyte the cellular debris of death cells, finally becoming foamy macrophages (FM) [25]. FM can either be locally necrotized or drained towards the bronchi escalator [26]. In that case, they may be trapped in the parenchyma and develop a local new foci, or they can reach further and become part of the drained alveolar fluid. Th17 lymphocytes will be responsible for attracting more PMNs. This production would be highly biased by the initial induction of a PMN-based granuloma at the beginning of the infection. The enormous effort done in omics study and the huge information already obtained from it, even coming from blood sampling in humans or from the macaque model, has to push to new algorithms to understand its complexity and finally include them in models like GranSim, where there is a constant update with the new information obtained [21], [24], [27]–[30].

The balance on the size of the granuloma (9)

Induction of TB is characterized by the formation of a large enough lesion able to be seen in a chest X ray, i.e., of around 10 mm. Recent data on the C3HeB/FeJ shows an exponential growth of the granuloma thanks to the massive infiltration of PMNs that fuels the extracellular growth of the bacilli sooner than the previous concept that relegated this phenomenon to liquefacted and cavitated lesions [31]. This phenomenon is helped by the drainage of infected FM that appear heavily surrounded by PMNs, pushing a peripheral growth of the lesion [16]. This lead to think on the coalescence between lesions and raised the TB "bubble model" [32], which demonstrates that the induction of a large lesion requires an exponential growth of the lesion (as seen by the one infiltrated with PMNs), a constant induction of peripheral daughter new foci, and the confluence of all these lesions. This is a very important factor, as usually PMNs role in the TB granuloma is not addressed, even in the GranSim model [8] or in other systems biology approaches [18], [23], [33], [34], which considering an unknown role of these cells, do not include them in their assumptions. Interestingly, data from human lesions obtained from the pre-antibiotic era, clearly point the presence of two sorts of lesions: the proliferative ones, with a controlled size and encapsulated, macrophage-based; and the exudative ones, with a predominium of PMNs, that are the ones that enlarge, become liquefacted and cavitated, or develop large pneumonic processes, both are characteristic lesions of active TB [15].

New tools like the monitoring of lesions in the macaque model thanks to the TAC-PET [35] will definitely help to understand the growth process of the lesions. In fact, the use of *Mtb* bar-coded strains will give a clear clue on it [36].

The encapsulation process (10)

The growth of lesions has a physical constraint, which is the interlobular *septae* that divides the lung parenchyma [37]. This was first described in the minipig model [38], and also takes place in mice when the lesion touches the pleura. The role of fibroblasts and a balanced anti-inflammatory immune response (both triggered by Th2 or Treg cells) might help the encapsulation, induction of miofibroblasts and control of the size of the lesion. The role of TGF-β and fibrosis has recently been addressed in the GranSim model [28], while the encapsulation process has been explored from the modelling perspective in [15].

The airway drainage of the bacilli, a way to the gastrointestinal or to the parenchyma (11 and 12)

Thanks to the bronchial drainage of FM, bacilli have the opportunity to reach the alveolar fluid. Most of them will be drained towards the gastrointestinal tract. Actually, a well-known way to diagnose TB in children, who do not develop cavitated lesions, is through gastric lavage. However, some of the bacilli will become part of the aerosols induced at the final bronchi, thanks to their constant expansion and retraction with the respiratory movements. Even when there is some experimental data and modeling on this aspect, this should be integrated in a deeper model for better understanding the aerosol movements in the lung, as pointed above. Bar-coded *Mtb* strains [36] will clearly give a clue on it.

The way back to the alveoli through the capillary net (13)

Once bacilli reach the lymph node it is reasonable to predict that some of them might escape from both macrophages and DCs, which may move towards the efferent vessels that reach the cava vein, the right auricle and ventricle, and are pumped again to the lung parenchyma. In this case the possibility to reach previously infected foci is higher, as there is a higher irrigation. This phenomenon was highlighted in the zebra fish model [39], but the actual relevance and the probability that these bacilli might induce new foci has not been clarified yet. It is logical to suppose that clumped bacilli would have a better chance to induce a blockage in the capillary end, disrupt the endothelial parenchyma and enter the alveoli as seen experimentally [40]. Remaining bacilli will reach the left ventricle and induce extrapulmonary lesions.

The second systemic wave (14)

With the consolidation of the granulomas, and especially in the enlarged ones, new processes take place. One of them is the progressive formation of necrosis and the appearance of hypoxic zones. This aspect has been addressed in several models due to its interest in the understanding of the mode of action of chemotherapy [23], [34], [41]. Another important process is the neo vascularization. This has a special interest because of its fragility, which will facilitate the direct entrance of the bacilli in the blood and its extrapulmonary distribution through the left ventricle.

Discussion

Since 2004, with the building of the GranSim TB model by the group of Kirschner [8], the understanding of the natural history of TB has experimented a new impulse. In the last two years, this group has greatly updated it by including the role of several cytokines, DCs and fibroblasts. It is expected that they will also include PMNs, the local dissemination of lesions and the encapsulation process in order to have a better model. GranSim and other systems biology approaches have made great progress in the understanding of some of the stated 14 steps, as summarized in Table 1. Furthermore, it will be important to build new models to better understand the dynamics of the infected aerosols and the possibilities of the bacillus entrance in the capillary net to reach extrapulmonary sites.

Conclusion

Identification of early irruption of the PMNs and extracellular growth of the bacilli seem to be the most disruptive factors to understand the evolution towards active TB that have appeared

in the last two years. Their incorporation in the current models will surely be a hallmark in understanding the progression towards active TB and provide new tools to fight against it.

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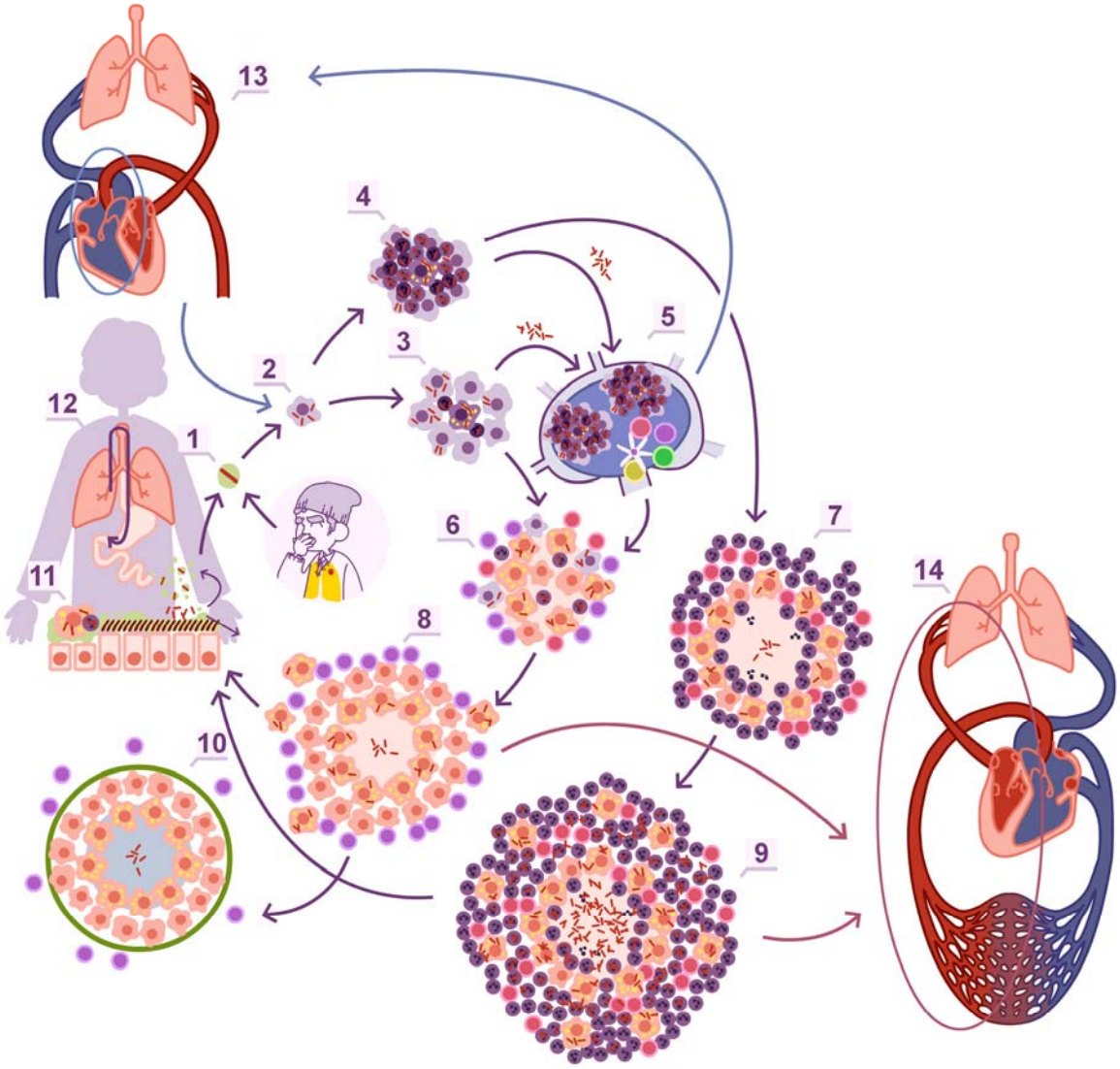

Figure 1. Fourteen steps to frame *Mycobacterium tuberculosis* immune-modelling
The long road towards the alveoli (1). The interaction at the alveoli (2). The onset of the granuloma, which depending on the infiltration percentage of PMNs can be identified as proliferative (3) or exudative (4) when it is low or high, respectively. The interaction with the regional lymph node (5). Once the adaptive immunity is triggered there is a control in the bacillary load in the case of the proliferative granulomas thanks to a predominium of the Th1 response (6 and 8) or a progressive increase thanks to the enhanced entrance of PMNs that allows an extracellular growth of the bacilli (7 and 9). Proliferative lesions are rapidly encapsulated (10). In both lesions there is a constant drainage of dormant bacilli carried by foamy macrophages towards the bronchial tree (11) which majoritarily are drained towards

the gastrointestinal tract (12) but can reenter the lung parenchyma again (1). Systemic reinfection can be done through an over infected lymph node, which drains towards the right heart and it is pumped again into the lung (13), or directly from the lesions, reaching the left heart and being able to induce extrapulmonary lesions (14).

Table 1: Review of the systems biology approaches for the understanding of TB-related issues on the period 2016-2018

Model type	Uses GranSim (yes/no)	Main purpose	Main variables	Steps totally/partially considered by the model*	Reference
Agent-based Model	no	To explore the role of drainage activity, encapsulation, macrophage tolerability to bacillary load and quality of the immune response on the lesions' patterns	Interstitial and resident alveolar macrophages, activated macrophages, neutrophils, intracellular bacilli, growing and non-growing extracellular bacilli, dormant bacilli, fibroblasts	2,3,4,6,7,8,9,10, 11	[15]
Convection-diffusion partial differential equations	no	To evaluate the efficacy of anti IL-10 and anti IL-13 treatments	M0, M1 and M2 macrophages, infected macrophages, extracellular and intracellular bacilli dendritic cells, Th1 and Th2 cells, naive CD4+ T cells, cytokines (IL-1β, IL-2, IL-10, IL-12, IL-13, IFN-γ TNF-α, GM-CSF)	3,5,6	[18]
Multi-compartment hybrid model (including ABM, PDEs, ODEs)	yes	To study antigen presentation, T cell priming, differentiation and trafficking in the context of TB granuloma formation	Macrophages, T cells, bacilli, cytokines and chemokines, dendritic cells	3,5,6	[21]
Multi-compartment hybrid model (including ABM, PDEs, ODEs)	yes	To explore how properties of LN environmental conditions, T cells and antigens affect the amounts and types of memory T cells, as well as successive vaccinations	Ag-specific naïve T cells, central memory T cells, effector memory T cells, dendritic cells, effector cells, macrophages, bacilli, chemokines, cytokines, and other soluble ligands	3,5,6	[22]
Integrated Multiscale Model	no	To demonstrate how hypoxia can occur in the human response to granuloma formation	Macrophages and T cells as particles, oxygen, chemokines; cytokines and bacilli as field variables	3,6,14	[23]

Multi-scale hybrid model (including ABM, PDEs, ODEs)	yes	To show that pairing computer modeling, statistics and mathematics with datasets derived from non-human primate studies can accelerate biomarker discovery	Macrophages, T cells, bacilli, cytokines and chemokines	3,5,6,13	[24]
Multi-scale hybrid model (including ABM, PDEs, ODEs)	yes	To investigate the regulatory role of TGF-β1 in granulomas' control	Macrophages (resting, activated, infected, and chronically infected), T-cells (cytotoxic, inflammatory, regulatory), bacilli, cytokines and chemokines	3, 6	[27]
Multi-scale hybrid model (including ABM, PDEs, ODEs)	yes	To elucidate the dynamics that lead to fibrosis identifying factors that distinguish central and peripheral fibrosis and the role of cytokines and cells	Fibroblasts, myofibroblasts, macrophages (resting, activated, infected, and chronically inected), T cells (cytotoxic, inflammatory, regulatory), bacilli, cytokines and chemokines (IL10, TGF-β1, TNFα...), collagen	3, 6, 10	[28]
Multi-scale hybrid model (including ABM, PDEs, ODEs)	yes	To address the bacterial phenotypes emergence and their role on the granuloma dynamics and to predict the potential of in vitro screening to identify bacterial metabolic targets for treatment of TB	Macrophages (resting, activated, infected, and chronically infected), T-cells (cytotoxic, inflammatory, regulatory), bacilli, cytokines and chemokines, other compounds (oxygen, glucose, triacylglycerol)	3,6,14	[29]
Multi-scale hybrid model (including ABM, PDEs, ODEs)	yes	To explore the role of low T cell exhaustion on TB granulomas	Macrophages (resting, activated, infected, and chronically infected), T-cells (cytotoxic, inflammatory, regulatory), bacilli, cytokines and chemokines	3,6,14	[30]
Agent-based Model	no	To study the role of lesions coalescence as an essential growth factor in active TB	Lesions' size, age and position	4,7,9	[32]
Non-linear system of ordinary differential equations	no	To assess the impact of the competition among intracellular and extracellular bacteria on the infection prevalence	Non-infected macrophages, infected macrophages, bacilli and T-cells	3, 6	[33]

Hybrid cellular automaton	no	To investigate the role of bacterial cell state and of initial bacterial location (distance to blood vessels) on treatment outcome	Bacilli, macrophages and T cells; oxygen, drugs and chemokines	3,6,14	[34]
Reaction-diffusion model	no	To offer a predictive tool for characterizing oxygen concentration profiles and the amount of hypoxia and necrosis	Oxygen concentration	8	[41]

* See Figure 1 for details: 1. The long road towards the alveoli; 2. The interaction at the alveoli; 3&4. The onset of the granuloma, that may be proliferative (3) or exudative (4); 5. The interaction with the regional lymph node; 6&8. Control in the bacillary load in proliferative granulomas thanks to a predominium on the Th1 response; 7&9. Progressive extracellular increase in the bacillary load thanks to an enhanced entrance of PMNs; 10. Encapsulation of proliferative lesions; 11. Drainage of dormant bacilli inside foamy macrophages towards the bronchial tree; 12. Drainage towards the gastrointestinal tract; 13&14. Systemic reinfection through an over infected lymph node (13) or directly from the lesions (14).