Computational modelling for understanding tuberculosis dynamics in lungs. From latent infection to active disease.

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Summary: Tuberculosis (TB) is an infectious bacterial disease caused by *Mycobacterium tuberculosis* (*Mtb*), which most commonly affects the lungs. In healthy people, an infection with *Mtb* often causes no symptoms, remaining controlled as a non-contagious latent tuberculosis infection. World Health Organization estimates that one third of the world population is already infected by this bacillus. From those, a 10% will probably develop an active disease the next decade. Nowadays, over 1.5 million people die annually because of an active TB.
The mechanisms that maintain a latent infection for a few years or that make it evolving towards an active disease are not fully understood, yet.
In this thesis we propose to build different computational models at different scales to study the dynamics of TB in lungs. The models will be fed with experimental data from minipigs, goats and macaques and will be fit to human clinical and epidemiological data. These models will be used to determine the main biological and physical mechanisms that trigger the disease and to characterize the effect of different preventive therapies.

I. Introduction

A. Tuberculosis

Tuberculosis (TB) is still one of the major humankind threats, being one of the 3 main causes of death by an infectious disease worldwide. TB is a communicable chronic infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*) that every year affects 10 million people worldwide and kills over 1.5 million persons. An 85 % of the TB cases correspond to a pulmonary disease, while the rest are extrapulmonary[1]. Despite the global efforts to fight the disease, its incidence is still stable, being the infectious disease that has killed most people in history. This circumstance is fueled by the progressive urbanization of the population together with poverty-related socio-economic factors. In fact, even though most of the cases are in developing countries, European cities today face a significant challenge to control TB infection and spread. The End TB Strategy by the World Health Organization[2] claims for an intensified research and innovation as the third pillar for achieving the goal of reducing TB incidence by 80 % and TB deaths by 90 % on 2030. This strategy also identifies the Latent Tuberculosis Infection (LTBI) as one of the challenges to overcome in order to accomplish the stated objective.

Natural history

TB infection starts when an *Mtb* arrives at a pulmonary alveolus and it is phagocyted by an alveolar macrophage. These bacilli can resist the bactericidal mechanisms induced by the macrophage and multiply inside the phagosome[3]. After 5-6 days, they cause macrophage necrosis and thereby enter the extracellular milieu, where they are phagocyted by another macrophage, which also fails to control the bacillary growth and is likewise destroyed. This activates the inflammatory response that causes a continuous flow of macrophages and neutrophils toward the infected alveolus. Bacilli continuously lysis the macrophages and may use neutrophils as a support to multiply extracellularly. This cycle ideally ends once the specific immune response appears. T cells activate macrophages to kill bacilli and drain them. If immune response works correctly TB lesions are controlled, encapsulated and calcified. During this process fibroblasts located at the septae lead the encapsulation of the lesions that get in contact with these intrapulmonary membranes, thus helping to stop their growth and isolating them from the surrounding environment. According to the Dynamic Hypothesis[4], there is a certain probability that few bacilli escape from the lesion, typically, inside a foamy macrophage, and start a new infection in other alveoli. This is known as the endogenous reinfection process, and takes place through the bronchial tree (figure 1).

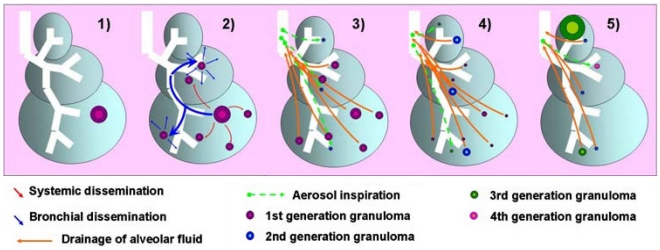

FIG. 1: Latent tuberculosis infection and the generation of active TB according to the Dynamic Hypothesis. Once the initial lesion has been generated (1), bronchial (blue arrows) and systemic (red arrows) dissemination generate new secondary granulomas (2). This process is stopped once the specific immunity has been established, which starts a constant drainage of non-replicating bacilli towards the bronchial tree (solid arrows) to which the inspired aerosols (dotted arrows) can return, thereby generating new granulomas (3, 4). This process implies finding different generations of granulomas simultaneously. In this dynamic process, if one of these reinfections takes place in the upper lobes, it will have the opportunity to induce a cavitary lesion (5). Adapted figure[4].

The success on the control of the lesion depends on a correct equilibrium between the inflammatory response, which promotes the growth of the lesion, and the immune response, which controls and stops its growth. If one of these two responses has an incorrect behavior the infection can evolve towards disease.

Computational models

Mathematical and computational models may be used for making progress on the understanding and control of the infection.
In TB, mathematical models have been used for increasing the understanding of TB infections at different levels. For example, to study host - pathogen interactions[5] or to reproduce granuloma's formation[6]. Many of the existing models have been used for understanding the role of antibiotics on the disease dynamics[7,8].
Nevertheless, there are still few publications on models for increasing the understanding of an active disease triggering. As far as we know, none of the published models takes into account the role of the pulmonary structure in terms of bronchial tree (endogenous reinfection) and septae (encapsulation).

B. Previous work

In a previous work, the dynamics of TB lesions during an active disease in mice was described by an Agent-Based Model (ABM)[9]. This model accounted for the growth, coalescence and proliferation of lesions, showing that the most important mechanism for lesions' growth during the active disease was coalescence. In a later work, the dynamics of lesions during a latent infection in minipigs was tackled by implementing a revised version of the previous ABM into a computational model of the bronchial tree[10,11]. The model was fed with Computed Tomography scan data from latent infection in minipigs. In this case, the model showed that the proliferation of lesions through the bronchial tree was essential for maintaining the latent infection[12,13].

Experimental data

Existing data of three animal models will be used for the development phase of mathematical models. The experiments were carried out in the context of other projects where certain biomarkers were analyzed. In this project we will focus on the obtained X-Ray Computed Tomography (CT) images of their lungs in order to analyze location, size and calcification of lesions. There is data from three different animal models:
Minipigs: minipigs are animal models of LTBI with a pulmonary structure similar to humans, including septae. 6 of them were endobronchially infected with 10^3 CFU. They were euthanized after 12 weeks infection, and their lungs were submitted to CT.
Macaques: macaques are animal models of ATB, but they lack septae structure in their lungs. 30 of them were infected with aerosol. Then, alive animals were periodically scanned with CT (weeks 3, 7, 11 and 16 post-infection).
Goats: goats are animal models of ATB with a pulmonary structure similar to humans, including septae. 12 of them were endobronchially infected with 10^3 CFU. Half of them were used as control and half of them were treated with heat-killed *M. manresensis*. They were euthanized after 16 weeks of infection, and their lungs were submitted to CT.
Data from minipigs were already analyzed[11,13] identifying each lesion by its spatial position, distance to pleura, density and diameter, and then used for adapting and parametrizing the computational models.

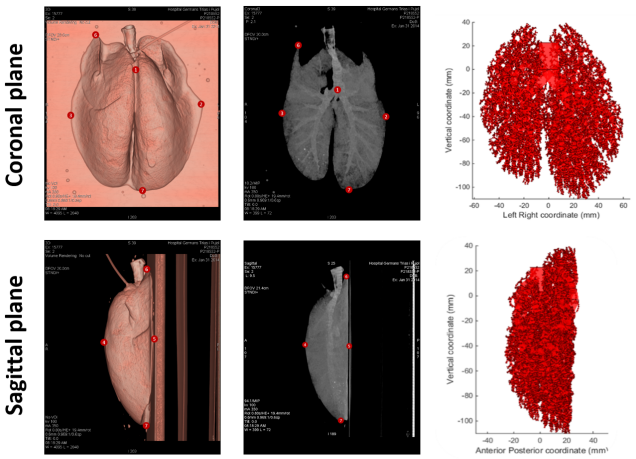

FIG. 2: CT data of a minipg pulmonary surface (left and center), and the corresponding virtual bronchial tree (right).

Minipig bronchial tree model

A 3D computational model of the minipig bronchial was already build[11]. Data about the size and shape of the minipigs' lungs were obtained from the reported experiments. The size of each pair of lungs was determined using the maximum coordinates obtained with CT measurements. The corresponding images were used for setting the shape of one specific pair of lungs. We built a bronchial tree inside the computed surface using a set of rules that were developed for simulating a human bronchial tree[10], with the appropriate redimensioning[11]. In figure 2, one of the computational models obtained from simulations is shown.

C. Objectives

The overall goal of this project is to explore the transition between latent tuberculosis infection (LTBI) and active disease (ATB) by developing a virtual lung where different computational models can be employed in order to: (1) identify physical and biological factors that facilitate such transition, (2) elucidate the specific patterns and

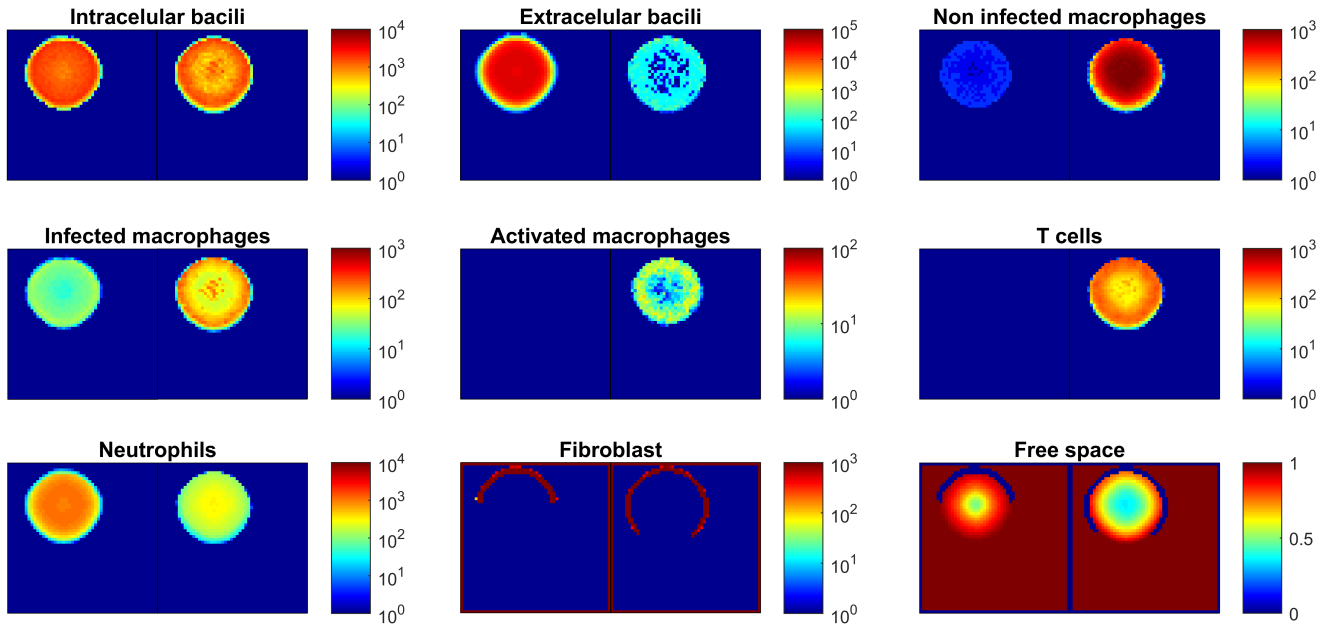

FIG. 3: Reaction diffusion model results at the 32nd day after initial infection. At each subfigure there can be seen the number of elements present in each alveolus considering two different simulations, one with no immune response (left) and the other with immune response (right). As shown, both lesions are being encapsulated by fibroblasts from surrounding septum. In non immune response case there can be seen that most of the bacilli are in the extracellular space while on the immune case most of them are inside the macrophages, which is in accord with experimental observations[15].

dynamics of some patients' categories including children, coinfections with AIDS and infections with multiresistant strains, and (3) characterize the effect of some preventive therapies in the identified factors.
The specific objectives of this thesis are:

O1. To build a model for the undestanding of the disease at scale of a single secondary pulmonary lobule and the formation of granuloma at this level.

O2. To build a computational lung using CT imaging data to reproduce minipig, goat, macaque and human pulmonary structures.

O3. To build, parametrize and validate computational models that correctly reproduce TB dynamics in animal models (goat, macaque and minipig), taking into account processes related with lungs structure.

O4. To adapt the computational model to human anatomy and physiology, using existing CT and X-Ray data from public databases.

O5. To identify key physical and biological factors that facilitate the development of an active disease in animals and humans.

O6. To determine the infection and disease patterns in some patient's categories like children, coinfections with AIDS and infections with multiresistant strains, among others.

O7. To characterize the effect of some preventive therapies in the model's parameters.

II. Preliminary results

Bubble model

Bubble model is a mathematical model that aims to describe the evolution of the TB lesions from an initial infection. It was initially designed for studying an active TB disease in mice[14]. It is an Agent-Based Model (ABM), where each lesion is an autonomous unit that can perform some actions.
It was later modified to reproduce experimental minipigs latent TB data[11,12] tacking into account minipig lungs anatomy and bronchial tree.
Each lesion is an autonomous unit with five properties: 3D spatial position, age and radius. This unit can grow increasing its radius, merge with other units (this process is called coalescence) or trigger an endogenous reinfection process which causes new lesions to appear. New lesions location is computed tacking into account the computational bronchial tree.
This model was successfully fitted to minipigs data[10,11].

Reaction diffusion model

In the last year we have been working on a reaction diffusion model to reproduce tuberculosis infection (O1).
This model is formed by 10 elements: b_I (**intracellular**

TABLE I: Work plan

		Semester						
		1st	2nd	3rd	4th	5th	6th	7th
OBJECTIVES	O1	X	X					
	O2			X				
	O3			X	X			
	O4				X	X		
	O5	X				X	X	
	O6							X
	O7							X

bacilli, bacilli contained inside macrophages), b_E (**extracellular bacilli**, bacilli outside macrophages), m_U (**uninfected macrophages**, macrophages with no bacilli inside), m_I (**infected macrophages**, macrophages with bacilli inside), m_A (**activated macrophages** macrophages that are activated and can kill bacilli), n (**neutrophils**), T (**T cells**), f (**fibroblasts**), s (**inflammatory response signal**) and V_{nc} (**necrotic volume**, volume occupied by dead cells). The model consists of 10 partial differential equations that determine the evolution of each element from an initial state.

All these elements and reactions are considered to occur inside each alveolus. Our model is implemented in a 52x52x52 grid that represents a secondary lobule where each point is an alveolus. In figure 3 the results of the evolution of an initial infected macrophage with one bacillus after 32 days of infection are shown, considering immune or no immune response.

III. Work plan

This thesis project is a part of an interinstitutional and multidisciplinary collaboration that involves computational biophysicists from *Universitat Politècnica de Catalunya*, medical doctors from *Institut d'investigació Germans Trias i Pujol* (IGTP) and epidemiologists from *Agencia de Salut Pública de Barcelona*.

Preliminary computational models have been built and parametrized using minipigs data. Next years we will adapt and fit the models to goats, macaques and humans. Connections between different scales will be explored in order to relate microscopic with macroscopic properties, as well as to establish different infection and disease patterns.

In table I there can be seen when the goals of this project are expected to be achieved.

First year The reaction diffusion model was built according to experimental observations. It was parametrized with biological bibliography[15] and validated by tuberculosis experts from IGTP. The remaining months will be devoted to the update of the Bubble Model in order to incorporate the mian processes described by this reaction-diffusion model, as well as their implementation in the minipigs's virtual lung.

Second year The main goal for the second year is to adjust the models to goats and macaques data. Then, the models will be adapted to human characteristics, comparing latent and active tuberculosis cases. Previouly, their virual lung will be computed by building the bronchial tree and the lungs structure models for each of the animals.

Third and fourth year Once the computational models are built and parametrized, the goal is to identify the parameters that cause a latent tuberculosis infection to evolve into an active one. These parameters should be related with biological and physical mechanisms, medical therapies or other factors that must be identified.

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