# Simulation of the dynamics of tuberculosis lesions in mice lungs

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Abstract: Tuberculosis (TB) is still one of the most important infectious diseases worldwide, killing over 1 million people each year. It is estimated that one third of the world population has already been infected by *Mycobacterium tuberculosis*. The threshold between a latent infection and an active disease is not clear, yet. Experimental evidence in mice show that the area affected by lesions increases exponentially with time, and that the number of lesions initially increases but afterwards oscillates. These phenomena can be explained if the merging of neighbouring lesions takes place. The aim of this project is to simulate the basic dynamics of lesions growth and merging, trying to reproduce both observations.

## I. INTRODUCTION

*Mycobacterium tuberculosis* is a bacterium that causes TB in humans. TB is a disease that primarily affects the lungs, although it can attack other parts of the body.

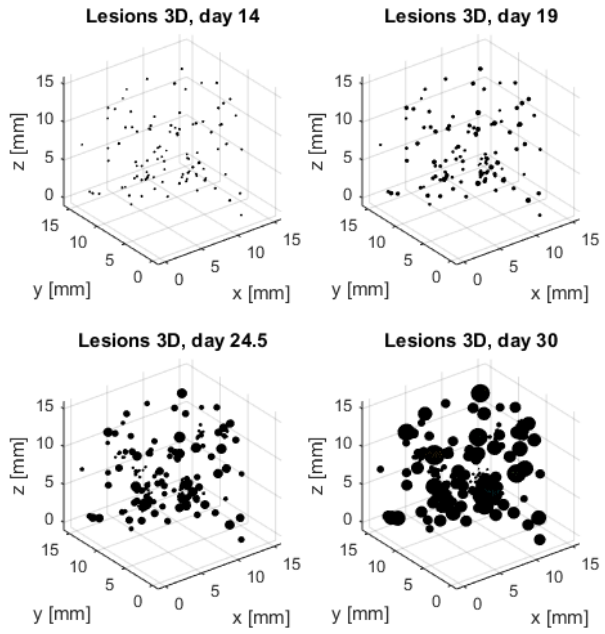


FIG. 1. Evolution 3D of tuberculosis lesions. A total of 100 initial lesions was considered, enclosed in a space of 15x15x15mm.

Latent tuberculosis infection (LTBI) is a state of persistent immune response to stimulation by *Mycobacterium tuberculosis* antigens without evidence of clinically manifested active TB. Someone has latent TB if they are infected with the TB bacteria but do not have signs of active TB disease and do not feel ill. People with latent TB infection do not have symptoms, and they cannot spread TB bacteria to others. However, if latent TB bacteria become active in the body and multiply, the person will go from having latent TB infection to being sick with TB disease, falling into active TB disease. Overall, without treatment, it is estimated that about 5% to 10% of infected people will develop active TB disease at some time in their lives.

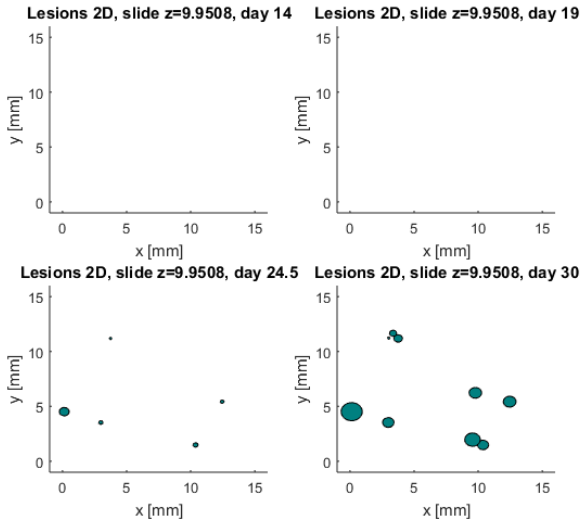


FIG. 2. Evolution in 2D of tuberculosis lesions in a randomly chosen slide. A total of 100 initial lesions was considered, enclosed in a space of 15x15x15mm.

TB infection begins when the mycobacteria reach the alveolar air sacs of the lungs, where they invade and replicate within endosomes of alveolar macrophages. Macrophages identify the bacterium as foreign and attempt to eliminate it by phagocytosis. During this process, the bacterium is enveloped by the macrophage and stored temporarily in a membrane-bound vesicle called a phagosome. The phagosome then combines with a lysosome to create a phagolysosome. In the phagolysosome, the cell attempts to use reactive oxygen species and acid to kill the bacterium. However, M. tuberculosis has a thick, waxy mycolic acid capsule that protects it from these toxic substances.

A macrophage is a type of phagocyte, which is a white blood cell responsible for detecting, engulfing and destroying pathogens and apoptotic cells. The ones that

encourage inflammation and therefore are the object of study in this project are called M1 macrophages.

An infected macrophage happens when the pathogen lives inside the macrophage instead of being destroyed by it, thus making the pathogen hide from the immune system and allowing it to replicate.

The inflammatory response is a great part of the process simulated in this project, and it consists on the massive accumulation of macrophages, among others, in the site of the infection, thus forming which will be called a lesion.

The specific immune response is the body's response caused by its immune system being activated by antigens. While the inflammatory response is taking place, a signal is sent and the specific immune response comes. It may happen that this specific immune response is not capable of countering the disease if the inflammatory response is too big.

The laboratory mouse is a powerful tool that scientists use to model human diseases and conditions in the search for better treatments and cures for humankind's most devastating genetic diseases. The ability to model human disease in the mouse makes it such a valuable experimental system. Genetically and genomically, the human and the mouse are very similar: many of the disease-related genes are nearly identical.

Assuming the surface of a lesion follows a logistic growth

$$\frac{dS}{dt} = \alpha\ S\left(1 - \frac{S}{S_{max}}\right)$$

Then one can obtain an equation for the radius, assuming the lesion has spherical shape

$$\frac{d\left(4\pi r^2\right)}{dt} = \alpha\left(4\pi r^2\right)\left(1 - \frac{4\pi r^2}{4\pi {R_{max}}^2}\right)$$

$$2r\frac{dr}{dt} = \alpha r^2\left(1 - \frac{r^2}{{R_{max}}^2}\right)$$

$$\frac{dr}{dt} = \frac{\alpha}{2}r\left(1 - \frac{r^2}{{R_{max}}^2}\right)$$

$$\frac{dr_i}{dt} = v_i r_i\left(1 - \frac{{r_i}^2}{{R_{max,i}}^2}\right)$$

## II. HYPOTHESES

Three main hypotheses and one basic hypothesis are considered initially:

ZERO (Spherical shape): TB lesions may be considered individually as spheres of a given radius which do not move from their initial sites at all.

FIRST (Logistic growth): TB lesions grow logistically due to the inflammatory reaction, the maximum being given by physical limitations of the space.

$$\frac{dr_i}{dt} = v_i\ r_i\left[1 - \frac{{r_i}^2}{{R_{max,i}}^2}\right] \tag{1}$$

Where $v_i$ is the growth rate of lesion $i$ and $R_{max,i}$ is is the maximum radius that lesion $i$ could eventually achieve. It can be $R_{max,1}$ by default or $R_{max,2}$ if the lesion is the result of the merging between lesions (see Table I). Note that these maximum radii model somehow the specific immune response, since they limit the inflammatory response up to some point.

SECOND (Reproduction): New lesions can appear, caused by extracellular bacilli or infected macrophages that escape from older lesions, according to the endogenous reinfection theory.

The number of new lesions arising from a given old lesion linearly decreases with mother's age and linearly increases with mother's radius.

$$N_{daughter,i}(t) = K_i\ r_i(t)\left[2 - \frac{T_i(t)}{14\ days}\right] \tag{2}$$

Where $K_i$ is the reproduction constant (see Table I).

Equation (2) only applies to lesions aged between 14 (the amount of macrophages is large enough so that some infected macrophage may be dragged to other areas of the lung) and 28 days (the lesion is considered to be already so well encapsulated that this dragging is not able to occur anymore).

THIRD (Coalescence): Lesions can merge when they are close enough, showing a coalescence behaviour.

Two lesions $m$ and $n$ are said to stick if $d_{mn} < r_m + r_n$, and they keep growing individually, only that one lesion is removed from the total count. They merge when $d_{mn} < max(r_m, r_n)$, so both lesions disappear for creating a new one of radius $({r_m}^3 + {r_n}^3)^{1/3}$, located at the center of mass of the original lesions. This new lesion is considered to have the age of the oldest of the original lesions.

All the initial parameters considered in this project are shown in Table I. They are quite similar to those in [2], which in turn were calibrated taking experimental data into account.

## III. SIMULATION

The codes for simulating are written in MATLAB as follows. Firstly, a definition of all parameters is needed. Those are the size of the lung, considered to be a cube; the mean initial radius of lesions; the mean initial age of the lesions, since the simulation is performed to see only a certain phase; the mean value for the growth rate; the mean maximum radii, which are the limits of the logistic growth for a individual common lesion and for a

lesion which is the result of the merging of two lesions, respectively; and the mean reproduction constant, which reflects the probability for a lesion aged between 14 and 28 days to reproduce. All those mean parameters are going to be multiplied by a set of Gaussian random numbers centered at 1 with standard deviation 0.2, in order for every lesion to initially have different values near the corresponding mean value.

| Parameter | Value |
|---|---|
| $x_{max}$ | 15 mm |
| $y_{max}$ | 15 mm |
| $z_{max}$ | 15 mm |
| $r_{init}$ | 0.075 mm |
| $T_{init}$ | 14 days |
| $v_i$ | 0.15 days$^{-1}$ |
| $R_{max,1}$ | 1 mm |
| $R_{max,2}$ | 10 mm |
| $K_i$ | 5 mm$^{-1}$ |
| $\delta_0$ | 1 mm |

TABLE I. Parameters for the simulation. Gaussian noise will be applied to those which are mean values.

Other crucial parameters for the simulation are the number of initial lesions, which is always a number between 50 and 150 (some different values are tested); the time step of the simulation, taken as 0.5 days; the number of time steps, taken as 33 such that the simulation goes from day 14 to day 30; and $\delta_0 = 1$, which is a constant related to how far from the mother lesion a daughter lesion appears, in each case being multiplied by a random number which is exponentially decreasing with distance.

A matrix with 8 columns is created, each column being one property of a lesion, where each row of the matrix corresponds to a lesion. The initial coordinates of the center of each lesion are determined randomly, by means of a uniform distribution, and they make up for the first 3 columns. Column 4 is for the radius, column 5 is for the age, column 6 is for the growth rate, column 7 is for the maximum radius and column 8 is for the reproduction constant. With all this, the main temporal loop starts and the dynamics are simulated.

First, column 5 (age) is updated, and equation (1) is integrated by means of a simple explicit Euler method. The latter step allows to update column 4 (radius).

Right after, the cases of sticking and merging are considered. All possible pairs of lesions are compared. Those pairs whose distance is smaller than the sum of their radii are susceptible of being stuck or merged, according to the process explained in Section II, Third hypothesis.

Afterwards, reproduction is considered. Only those lesions with an age between 14 and 28 days are able to produce daughter lesions, so after taking that restriction into account, some new lesions may emerge according to the process explained in Section II, Second hypothesis (see equation 2).

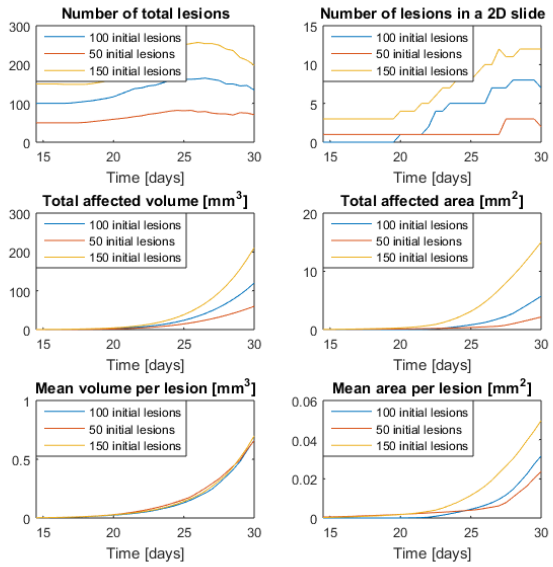


FIG. 3. Effects of varying the initial number of lesions. In yellow, 150 initial lesions; in blue, 100 initial lesions; in red, 50 initial lesions; all of them enclosed in a space of 15x15x15mm.

A 2D slide is randomly chosen, and the area of the lesions on that slide is computed by means of a Monte Carlo method. Other important quantities, such as the number of lesions both in 3D and 2D, or the volume, as well as the mean volume and area per lesion are also computed.

Apart the general code, two different additional codes are created: one for repeating the simulation without endogenous reinfection and another one for repeating the simulation without coalescence.

## IV. RESULTS

Some snapshots of the evolution of lesions in 3D are gathered in Figure 1. As it is straightforward to see, lesions grow with time; and they also reproduce.

In Figure 2 one can see the evolution of lesions in a randomly chosen slide of the lung. This is most likely the situation that one would find in laboratories, where only a certain slide of the lung can be analyzed. As in the 3D case, lesions grow; and at a point new lesions appear, either because of reproduction or because of growing of lesions that were initially not large enough so as to reach that slide.

Figure 3 shows that the initial number of lesions does matter. The more initial lesions considered, the more affected area and volume during all the simulation. Also, the evolution of volume and area seem to somehow follow an exponential law. It is remarkable that the number of lesions firstly increases and at a point it starts oscillating, most probably due to coalescence. This reflects the experimentally observed process of immune response,

where first the inflammatory response appears and at a point the specific immune response appears.

In Figure 4 one can see the effects of making the initial lesions be closer, with the same number of initial lesions. The closer they are, the faster the number of lesions decreases, again because of the fact they are near and coalescence is fomented. Regarding volume and area, they still follow exponential laws, being the $6x6x6mm$ setup the most growing one.

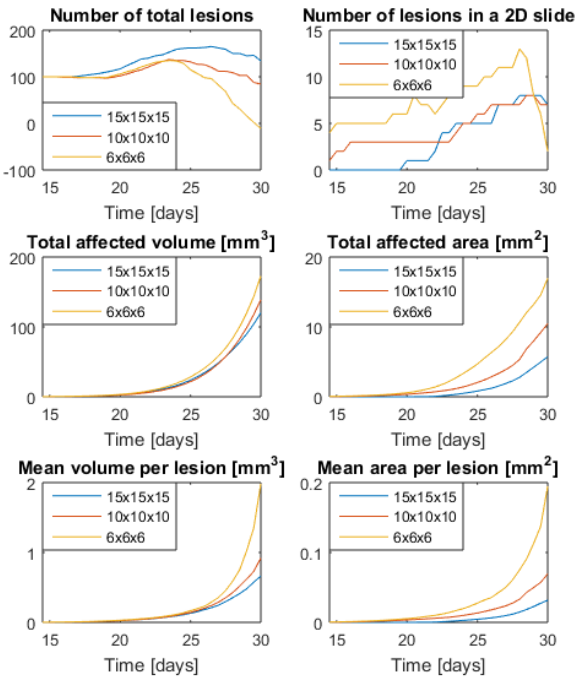


FIG. 4. Effects of varying the mean distance between initial lesions. A total of 100 initial lesions was considered, enclosed in spaces of 15x15x15mm in blue, 10x10x10mm in red and 6x6x6mm in yellow.

The most important outcomes of this project are perhaps contained in Figure 5. In it, the effects of considering some modifications to the hypotheses of the problem are showed. The only results considered here are for 2D slides, since an eventual testing of medicines would also be checked in 2D slides. The 3D results are not showed.

When modifying the first hypothesis, that is, considering the growth rate to be one order of magnitude smaller, the regime is of small inflammatory response. This regime allows to reduce drastically the increase in the number of lesions (in fact, it remains constant during all the simulation in the 2D slide), perhaps due to the fact that reproduction is directly proportional to the radius of a lesion, and it also allows to reduce the increase in the mean area, which is insignificant when comparing with the original simulation.

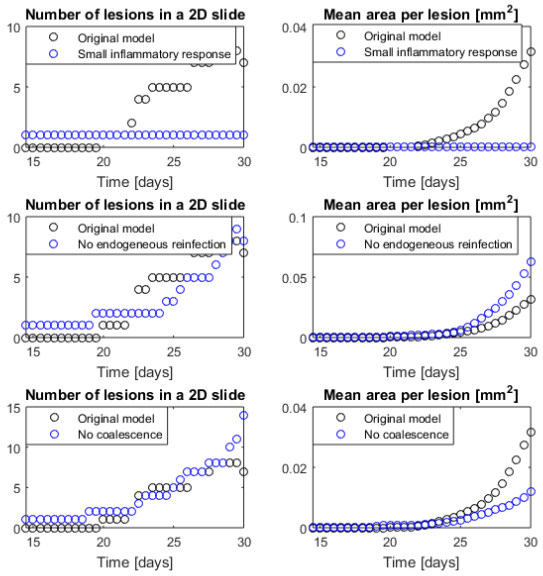


FIG. 5. Effects of considering small inflammatory response (first row of graphs), no endogenous reinfection (second row) and no coalescence between neighboring lesions (third row). A total of 100 initial lesions was considered, enclosed in a space of 15x15x15mm.

No endogenous reinfection does not reduce significantly the increase in the number of lesions. It is clear that, without reproduction, the number of lesions should be lower. However, for this particular 2D slide, the luck makes the simulation host initially one lesion whereas the original simulation did not host a single one. Apart from that, the eventual increase in the number of lesions in the slide may be slightly affected by reproduction of lesions in the original case, but these results suggest that the growing of close lesions that did not initially intersect the slide may be even more decisive. Also, the mean area per lesion is growing at a higher rate than the original one. This can be explained the following way: since the number of lesions in the slide is roughly the same, but in the latter case they are necessarily coming from other older lesions instead of coming from new small daughter lesions, and the area of a lesion increases exponentially (it will increase faster if it is already big), the area is higher than in the original case. All in all, the results of considering the model without endogenous reinfection are not completely satisfactory because the initial random spatial distribution of lesions may affect more than the fact of not considering reinfection, but still some sensible conclusions can be extracted.

When removing the third hypothesis, that is, considering the system without coalescence, the number of lesions increases monotonically, which is obvious since lesions are allowed to reproduce but are not allowed to stick nor merge. The number of lesions in the 2D slide is remarkably higher than in the original case. In terms of mean area per lesion, a certain growth is shown, but

growing at a clearly lower rate than the original case. This makes sense because the lesions that can reach the highest radii are those resulting from a merging (recall $R_{max,2} >> R_{max,1}$), therefore in a simulation where there is no coalescence at all, lesions are more limited in terms of area.

## V. CONCLUDING REMARKS

It is feasible to simulate the immune response for tuberculosis by assuming some simple hypothesis. The tendencies of the simulated results match the experimental ones, thus confirming that the model is valid.

Furthermore, the model is so powerful in the sense that it is relatively easy to modify the initial parameters in order to see how they affect the dynamics of the system, thus aiming the objective where medicine may attack.

Any drugs able to reduce the growth rate of the disease will for sure be great for reducing the effects of tuberculosis. Other drugs able to inhibit coalescence could also serve, although it may not be anybody's preferred choice. Finally, from the results of this work it is not possible to conclude whether inhibiting endogenous reinfection could actually reduce the disease, so more advanced and complete simulations may be performed in order to answer that.

To sum up, the therapeutic drug that I would choose would be one that reduces the inflammatory response to TB by one order of magnitude.

## VI. ACKNOWLEDGEMENTS

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