An agent based modeling approach for the analysis of tuberculosis – immune system dynamics

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***Abstract*— Tuberculosis is one of the world's deadliest diseases that infects one third of the world's population, mostly in developing countries. However, tuberculosis is becoming again very dangerous also for developed countries, due to the increased mobility of the world population, and the appearance of several new bacterial strains that are multi-drug resistant. With the aim to help in finding new therapeutic interventions against tuberculosis, we present the application of a computational modeling infrastructure named UISS (Universal Immune System Simulator) able to simulate the main features and dynamics of the immune system activities. We show a further development of UISS to consider the underlying tuberculosis pathogenesis and its interaction with the host immune system. Even though the model can be further personalized employing immunological parameters and genetic information, based on the available data, we obtained simulation scenarios able to reproduce persistent latent infection or the development of active disease. In particular, UISS is able to simulate those mechanisms in which M. tuberculosis is involved in the early influx of alveolar macrophages and recruited neutrophils until the formation of the tuberculous granuloma, at both cellular and molecular levels.**

Keywords—artificial immune system; immune modeling; tuberculosis; therapeutic strategies

I. INTRODUCTION

Tuberculosis (TB) has existed for millennia and remains a major global health problem. It causes ill-health in millions of people each year and in 2015 was one of the top 10 causes of death worldwide, ranking above HIV/AIDS as one of the leading causes of death from an infectious disease. TB is a contagious airborne disease caused by the bacillus Mycobacterium tuberculosis (MTB). It typically affects the lungs (pulmonary TB) but can also affect other sites (extra pulmonary TB). Like the common cold, it spreads through the air. People who are infected with pulmonary TB (TB of the lungs, the site most commonly affected) can spread the disease by coughing, sneezing or even talking. If the disease goes untreated, each person with active TB infects, on average, 10–15 others every year (Global WHO TB report 2016).

Drug Susceptible-TB can be treated with a course of four standard (first-line) drugs. There is usually an intensive two-month phase of treatment with the use of all drugs, followed by a four-month continuation phase with only two. Due to the extremely long treatment and hepatotoxic effect many people clearly do not finish the course of drugs, and as a consequence resistance to TB drugs may be developed [1]. Thus, and following WHO guidance issued in May 2016, all cases of rifampicin-resistant TB (RR-TB), including those with multidrug-resistant TB (MDR-TB), should be treated with a second-line MDR-TB treatment. Thus, the duration of treatment is much longer for MDR-TB (at least 9-12 months) compared to drug-susceptible TB (between six and nine months), with a significantly higher risk for adverse drug reactions and unsuccessful treatment outcomes, particularly death. The outcome of MDR-TB treatment is poor: of all MDR-TB cases worldwide who started treatment in 2010 only 48% had a favourable outcome.

The current prophylactic vaccine in use against human TB is Mycobacterium bovis Bacillus Calmette-Guerin (BCG) which was developed almost 100 years ago and has been shown to prevent severe forms of TB in children. However, there is currently no vaccine that is effective in preventing TB disease in adults, either before or after exposure to TB infection [2].

MDR-TB is a European public health priority and the WHO Regional Committee for Europe adopted the tuberculosis action plan for the WHO European Region 2016–2020 in 2015.

In 2014, the World Health Assembly adopted WHO's End TB Strategy to eliminate the global TB epidemic by 2035, by reducing 90% of TB cases (compared to the 2015 baseline). To reach such a goal, a comprehensive approach, including new and more effective vaccines, is needed, as well as improved diagnostics and treatment. Vaccines are considered the most efficacious and cost effective means to tackle diseases. For this particular disease, multiple vaccine development strategies are being pursued:

- Prevention of infection: vaccines are administered before MTB exposure, in order to prevent initial infection and therefore disease;
- Prevention of disease: vaccines are administered after exposure to MTB, to people who are infected but may be asymptomatic and at risk of developing disease in the future, in order to prevent manifestation of active disease and therefore reduce transmission.
- Prevention of recurrence: vaccines are administered after infection and treatment of MTB disease, to prevent reactivation and subsequent transmission.

In addition to the classical approaches, immunotherapeutic vaccines are being developed for individuals with active TB in conjunction with TB drug therapy, with the aim of shortening the duration of the therapy and/or reducing recurrence rates after completion of treatment.

In this scenario, one of the most promising technology to increase the high likelihood chance of success of these promising vaccines is represented by the application of computational modeling strategies to integrate and empower the standard clinical trials for the testing of treatments in patients affected by M. tuberculosis.

We present here an extension of an agent based model platform named Universal Immune System Simulator (UISS) [3] that was successfully applied to a large number of disease modeling scenarios [4]–[9]. This extension is able to simulate the dynamics and the specific features of the MTB infection and its interactions with the host immune system.

II. SYSTEMS AND METHODS

A. The conceptual model

UISS is a simulation platform based on agent-based modeling (ABM) approach. We used it as a starting point to implement all the cellular and molecular entities needed to model the dynamics of MTB and its interaction with the host immune system. The advantages of this modeling methodology are well known: entities as well as biological functions and interactions can be described very closely to the biological reality; at the same time, this approach allows a mathematician or a computer scientist to describe the scenario using a logic and rational framework. Finally, the ABM approach allows flexibility and further extension and refinement of the model without significant additional effort.

Figure 1 depicts the main infrastructure of UISS platform. Identification concept include all the entities with their functions and activities that play both a role in the immune system dynamics and in the evolution of TB. In particular, we considered all the relevant immune cells and molecules along with their specific properties.

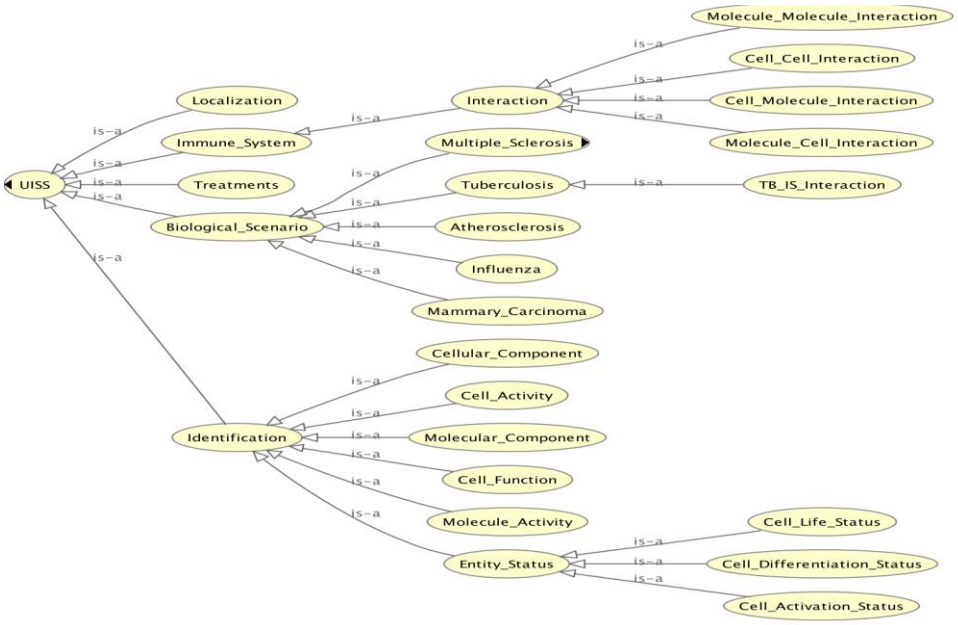

Figure 1. Classes that describe the knowledge developed for immune system – tuberculosis dynamics. The mother class is represented by the simulation framework (UISS, Universal Immune System Simulator). Attached to the UISS there are classes that categorize the main components required for an exhaustive description of the immune system i.e., localization (to identify the site of the entities during their interactions), identification (to describe all the cellular and molecular components), immune system interactions, treatments that may affect the disease course, and the class of biological scenario that the computational framework simulates, in this case tuberculosis.

UISS is a multi-scale, multi-organ, three-dimensional agent based simulator of the immune system with an attached module able to simulate the dynamics of specific biological pathways at the molecular level.

UISS takes care of both cellular and molecular entities. Cellular entities can take up a state from a certain set of suitable states and their dynamics is realized by means of state changes. A state change takes place when a cell interacts with another cell or with a molecule or both of them. We considered the relevant lymphocytes, i.e., B lymphocytes, helper, cytotoxic and regulatory T lymphocytes and natural killer cells. Monocytes are represented as well and we take care of macrophages and dendritic cells. For what concerns molecules, the model distinguishes between simple small molecules like interleukins or signaling molecules in general and more complex molecules like immunoglobulins and antigens, for which we need to represent the specificity.

At the same level of entities, we implement immune system activities. They include both interactions and functions. Functions refer to the main immune system tasks. In particular, UISS takes care of the diversity of specific elements, major histocompatibility classes restriction, clonal selection by antigen affinity, thymus education of T cells, antigen processing and presentation (both the cytosolic and endocytic pathways are implemented), cell–cell cooperation, homeostasis of cells created by the bone marrow, hypermutation of antibodies, cellular and humoral response and immune memory.

Our computational modelling framework represents receptors and ligands as bit strings and use a string matching rule to model affinity. This clever idea was introduced by Farmer et al. [10] as a way to perform calculations for determining molecular complementarity and predicting the optimal size of an epitope. From immunology, we know that binding is a threshold effect consisting of two components: the affinity of a single receptor and ligand, and the total binding, or avidity of multiple binding pairs. Binding is modeled by a string matching rule by counting the number of positions in the string at which the symbols are complementary (known as Hamming distance). Repertoires are represented in the model as sets of strings. By adopting bit strings, many binding events can be simulated quickly, making it feasible to study large-scale properties of the immune system. Character strings produced accurate models when benchmarked to experiment, suggesting that the abstraction captures important features of receptor/ligand binding.

In particular, specificity is implemented in UISS by a bit-string polyclonal lattice method. Bit-string refers to the way the molecules and the specificity among molecules is represented, polyclonal indicates that more clones of different specificity of lymphocytes are represented and lattice means that we use a discrete lattice to represent the space, that is, the space is discrete. The set of lymphocytes receptors is represented by bit-strings of length “h” which then forms the so called shape space. A clonal set of cells is characterized by the same clonotypic receptor, i.e., by the same bit-string of length l. The potential repertoire of receptors scales as 2^l. The receptor–coreceptor binding among the entities are described in terms of matching between binary strings with fixed directional reading frame. Bit-strings represent the generic binding site between cells (through their receptors) and target molecules (through peptides and epitopes). An interaction between two entities is a complex action which eventually ends with a state change

of one or both entities. Specific interactions need a recognition phase between the two entities; recognition is based on Hamming distance and affinity function and is eventually enhanced by adjuvants. When two entities, which may interact, lie in the same lattice site then they interact with a probabilistic law. All entities which may interact and are in the same site have a positive interaction. Physical proximity is modeled through the concept of lattice-site. All interactions among cells and molecules take place within a lattice-site in a single time step, so that there is no correlation between entities residing on different sites at a fixed time. The simulation space is represented as a L X L X L cubic lattice, with periodic boundary conditions to the left and right side, while the top and bottom are represented by rigid walls. All entities are allowed to move with uniform probability between neighboring lattices in the grid with equal diffusion coefficient (Brownian motion).

B. Extending UISS platform to include TB dynamics

To include into the simulation platform all entities, interactions and target tissues needed to represent and mimic TB dynamics and its interaction with the host immune system, we used the developed ontology and the methodology followed by Motta and Pappalardo [11]. TB infection started with the establishment of the MTB in the airways and lung parenchyma [12]. Here, the bacilli are phagocytosed by the alveolar macrophages and are taken up by neutrophils and dendritic cells. The fate of the infection is decided just in this early phase. Macrophages and neutrophils determine the outcome of the immune response to MTB infection influencing the subsequent immune response toward potential clearance or containment of the pathogen. This can lead either to a persistent latent infection or the development of active disease. This also strongly depends on the specific virulence of the MTB strain. Different MTB strains are able to modulate the immune system thanks to their ability to inducing LXA4 (pro-necrotic) and inhibiting PGE2 (pro-apoptotic) production, leading to macrophage necrosis and inhibition of macrophage apoptosis, ultimately resulting in mycobacterial spread [13]. This mechanism has been implemented in UISS; moreover, UISS distinguishes different Mycobacterium tuberculosis strains as well, as the outcome of the disease depends also on the genetics of both the pathogen and the host. This was obtained developing subroutines in the code that allow specific interactions with the innate immunity depending on the selected strain.

Another important feature that was implemented into UISS platform is represented by autophagy. Autophagy is a mechanism induced for example by interferon gamma cytokine (IFN-γ) that leads to macrophage phagosome maturation and an increase in its acidification and, as a consequence, MTB killing.

Next step, following the way of innate immunity processes, is represented by the activation of specific CD4+ T cells during MTB infection. Lung neutrophils could promote this activation providing the bacilli to dendritic cells (DCs) in a form that makes DCs more effective initiators of CD4+ T cell activation. Some types of MTB strains are able to inhibit neutrophils apoptosis, leading to a delay in CD4+ T cells activation. UISS implements both neutrophils ability to capture and deliver MTB for CD4+ T cells activation and their possible apoptosis inhibition through a set of computational rules that translate into stochastic probabilities the ability of different MTB strains to behave when interact with neutrophils.

All the remaining immune system machinery related to DCs MHC class I and II presentation of MTB bacilli and consequent CD8+ T cells activation (along with the cytokines cascade) is already implemented in UISS and will be used to model specific MTB immune system interactions regarding cellular immune response.

About humoral immune system machinery, B-cells not only produce immunoglobulins and present antigens to T-cells, but also have additional key roles in the immune system. Current knowledge on the role of B-cells in infections caused by intracellular bacteria is fragmentary and contradictory [14], and this reflects also for MTB in which B-cells have long been ignored as their primary product, immunoglobulins, are unlikely to recognize intracellular bacteria. Recent studies suggest that B cells could be involved in modulating immune activation and susceptibility to infection via immune regulation by the induction of cytokines such as IL-10, possibly by engagement of distinct FcγR by antibodies produced by B cells during M. tuberculosis infection [15]. UISS already implements all the B cells machinery.

Multiple physiological compartments are relevant to TB. In UISS we implemented three main compartments. Two were already present i.e., peripheral blood and peripheral lymph nodes where most of the immune system activities take place. The third one we added is the lung parenchyma compartment to simulate the main organ target of MTB. Antigen presenting cells from the lungs travel to lymph nodes, where T cells were activated and recruited to lungs. Here the formation of granulomas can occur [16]. Granulomas have been made of caseous necrosis surrounded by a ring of macrophages, neutrophils, giant cells, and an outer ring of lymphocytes including T and B cells [17]. Within granulomas, bacteria are either residing inside the caseous center (non-replicating bacteria), within macrophages (intracellular bacteria) or in extra- cellular spaces. It has been established that the outcome of all granulomas likely determines whether the host's disease is chronic or active. Granulomas formation dynamics has been implemented in UISS and first results are shown later in the Results section.

III. RESULTS

Actual extension of the UISS simulation platform is able, at the end of the game, to reproduce and simulate two specific MTB scenarios i.e., the one in which the host immune system is able to recognize and clear the infection and the other one in which, instead, MTB establishes a chronic infection with some granulomas formation as a reservoir of MTB infection. The simulation space is 3 cubic millimeters for the lung compartment and lymph nodes compartment, while for the peripheral blood compartment we simulate three microliters. The observation period is 2 years.

Figures 2, 3, 4 and 8 are related to a simulation scenario in which the immune system of the host reacts in a strong way to a mycobacterium strain that is not virulent. Figures 5, 6, 7 and 9 deal with, instead, a virulent strain of the mycobacterium tuberculosis.

Figure 2 depicts the dynamics of CD4+ T cells population. Both TH1 and TH2 arms are activated at the time of the TB challenge inoculation (day 33). The CD4+ T cells activation persists until day 500, peaking at day 60 i.e., about 30 days post-infection. CD4+ TH2 cells are also present due to the double nature of the TB mycobacterium. It is, in fact, an intracellular bacterium, behaving as both extracellular and intracellular agent. Figure 3 depicts the immunoglobulins and mycobacterium tuberculosis antigens dynamics over time. First immunoglobulins type M (IgM) appears, just few days after infection challenge. Then when the adaptive immunity takes place, immunoglobulins type G (IgG) appears (along with a very small fraction of immunoglobulins type E, IgE). IgG are specifically directed against the mycobacterium strain and provide the clearing of the antigen. MTB infection (from MTB bacilli presence point of view) is cleared about 20 days after the challenge. Figure 4 refers to the dynamics of alveolar macrophages (AM), the main MTB cellular target inside lung compartment. At the cellular level, the lung tissue is completely recovered after more or less four months after infection challenge. Also, necrotic tissue (due to necrotizing AM) is cleared. CD8+ T cells are the main actors of such a recover.

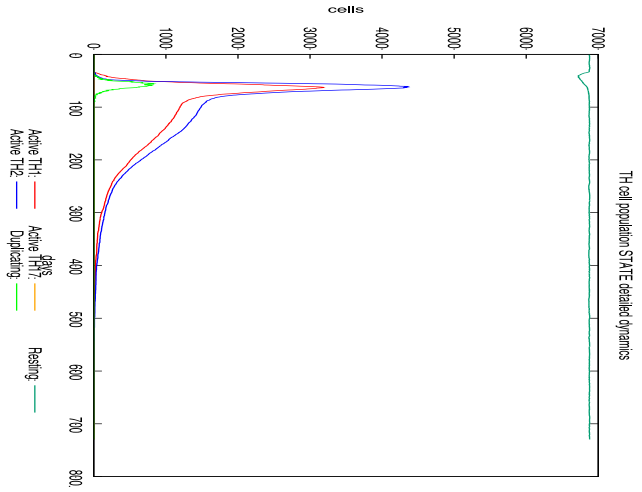

Figure 2. CD4+ T cells dynamics. Both Th1 and Th2 dynamics is depicted.

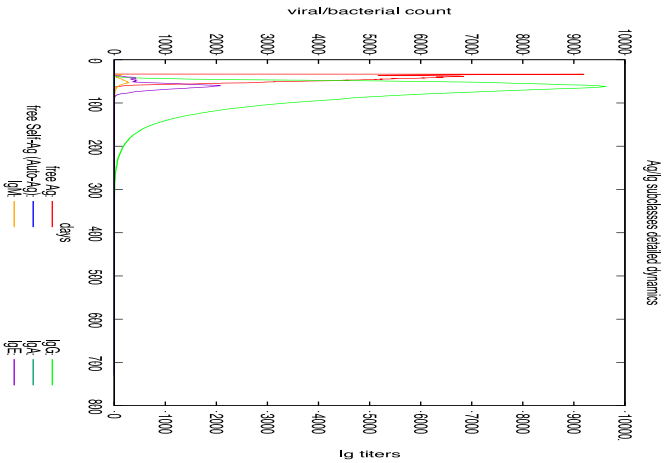

Figure 3. Immunoglobulins and MTB bacilli dynamics. All the main immunoglobulins subclasses (IgM, IgG, IgA and IgE) dynamics is depicted.

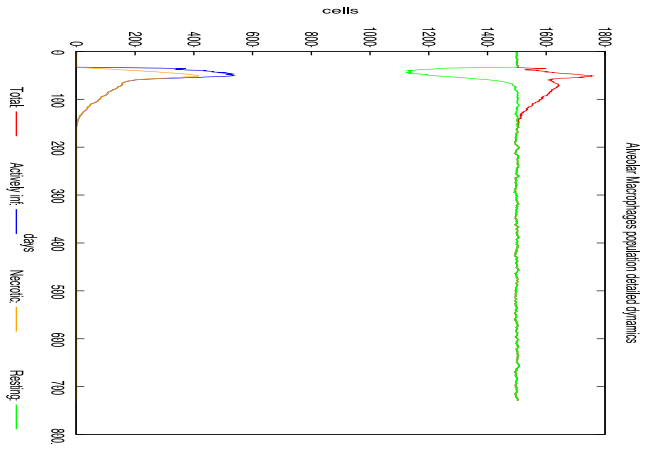

Figure 4. Alveolar macrophages population dynamics inside the lung compartment. The graph shows the behavior of total AM, actively infected (that means MTB intracellular bacterium infected the AM) and the necrotic differentiation that affects AM.

Figure 5 depicts the dynamics of CD4+ T cells population. Both TH1 and TH2 arms are activated at the time of the TB challenge inoculation (day 33). In this case it appears more useful having a look at the memory CD4+ T cells dynamics. One can observe that CD4+ T cell memory persists for a long time after infection (until day 500 i.e., much more than one year). However, it eventually disappears indicating the establishment of the infection, in a chronic scenario (the immune system "tolerates" the infection). Figure 6 depicts the immunoglobulins and mycobacterium tuberculosis dynamics over time. IgM appears, just few days after infection challenge. Then when the adaptive immunity takes place, IgG appears (along with a very small fraction IgE). In this scenario, it appears, after the peaking of IgG that eventually clears the acute episode of the infection, a constant establishment of a low concentration of specific IgG that controls the chronic establishment of the MTB infection. This is also an indication of a B cells derived memory mechanism that is, however, unable to wipe out completely the infection, probably due to reservoir of MTB bacilli inside the necrotic AM. Figure 7 depicts the dynamics of AM. In this case, one can notice that a constant establishment of necrotic tissue is present, eventually increasing in a very slow way. In this case, MTB virulent strain promotes necrotic AM differentiation keeping a constant infection reservoir.

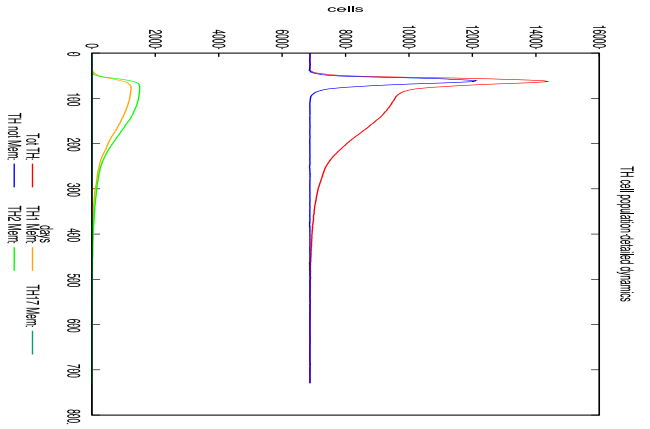

Figure 5. CD4+ T cells memory dynamics. Both Th1 and Th2 arms are depicted.

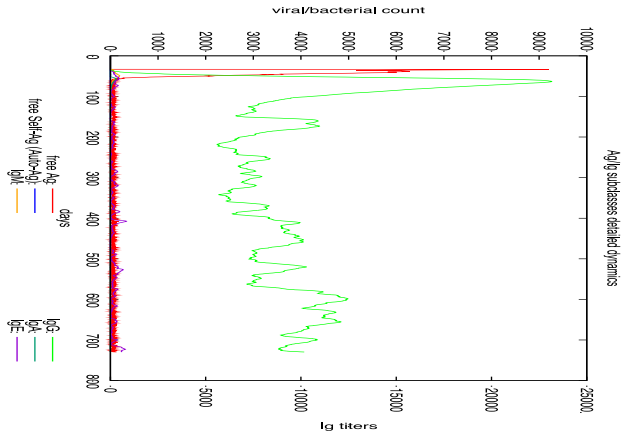

Figure 6. Immunoglobulins and MTB bacilli dynamics. All the main immunoglobulins subclasses (IgM, IgG, IgA and IgE) dynamics is depicted. In this case a constant level of IgG is present indicating the chronic establishment of the infection. One can also notice a very small fraction of MTB antigens showing the activity of immune system machinery against the chronic infection.

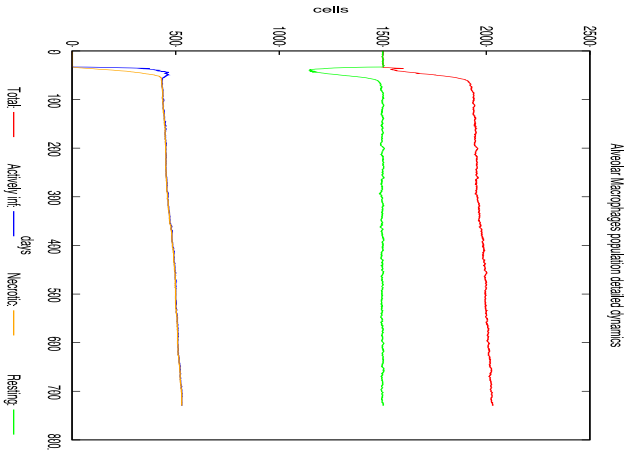

Figure 7. Alveolar macrophages population dynamics inside the lung compartment. The graph shows the behavior of total AM, actively infected (that means MTB intracellular bacterium infected the AM) and the necrotic differentiation that affects AM. In this specific case, necrotic tissue is persistent and, eventually, slowly increasing.

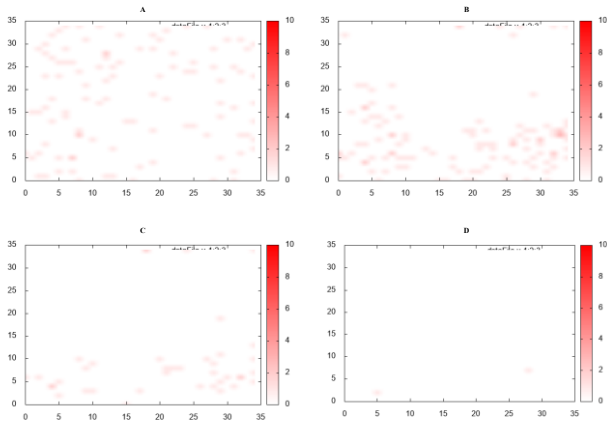

Figure 8. Sequence of the spatial dynamics of alveolar macrophages inside the lung compartment. The four shots depict at time 40, 80, 120, 150 (days), panel A, B, C, D, respectively, the dynamics of necrotic AM, showing the complete recover from the MTB infection.

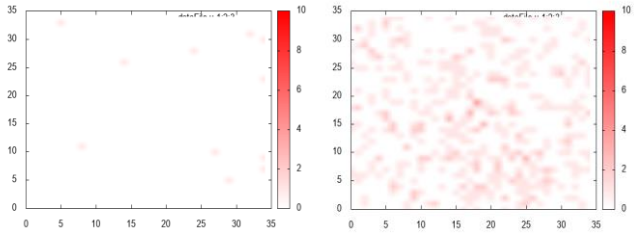

Figure 9. Sequence of the spatial dynamics of alveolar macrophages inside the lung compartment. The two shots depict at time 40, 400 (days) the dynamics of necrotic AM, showing the complete chronic establishment of the MTB infection.

IV. Conclusions

The StriTuVaD multidisciplinary consortium, will deliver an in silico trial platform to simulate the relevant individual human physiology and physiopathology in patients affected by mycobacterium tuberculosis. The StriTuVaD in silico trial will predict, explore and inform of the reasons for failure should the vaccinations strategies against M. tuberculosis under testing found not efficient, which will suggest possible improvements, which can be rapidly explored on the StriTuVaD in silico platform. In this context, we started to develop the extensions needed to be applied to UISS in order to model and simulate the MTB – immune system interactions. We have shown the simulations related to a couple of possible scenarios: persistent latent infection and the active, acute disease (with complete recover). Present results show the UISS capability to simulate the intrinsic immune system behavior that elicit the complete clearance of the infection or, eventually, the chronic establishment of MTB reservoir inside the host.

Next steps will be to further extend the UISS platform in order to take care of the reactivation of latent infection; to insert the mechanisms of action of both antibiotics treatments and vaccination strategies. Inside STriTuVaD project, UISS will contribute in a significant way to provide a level 3 simulation platform (i.e., each individual of the reference population will be simulated and represented using biological and physiopathological data coming from real patients) able to achieve the necessary statistical power (eventually integrated by a number of virtual patients) to offer an effective way to estimate time to inactivation of M. tuberculosis with a standard phase II clinical trial, and also obtain an in silico prediction of the effect of recurrence.

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