Report on the extensions implemented into the modeling simulation platform to reproduce the immune system – TBC – vaccines interactions and dynamics.

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The present document contains the report of Deliverable 3 WP2.

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Topic

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Deliverable: The goal of this deliverable is to integrate into UISS modeling framework the capability to simulate the dynamics and the specific features of the tuberculosis mycobacterium infection. Moreover, the UISS modeling framework will be enabled to simulate the artificial immunity induced by two vaccines i.e., Ruti vaccine and ID93 vaccine.

Discussion

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During months 8-16 UISS was extended to implement the needed features to allow UISS modeling framework to simulate the artificial immunity induced by two vaccines i.e., Ruti vaccine and ID93 vaccine, provided by AF partner and IDRI partner, respectively. To do so, a detailed knowledge about the components that are used in Ruti and ID93 vaccines was required. Vaccines use several mechanisms to be able to induce a proper immune response and each of them will be added to the simulation platform. Antigens structure, liposome recognition by the immune system, adjuvants stimulation of the innate immunity and the delivery dynamics of each vaccine will be implemented accordingly. Moreover, as the clinical trial we set in the project for the validation of the in silico clinical trial will make use of antibiotic strategy based on isoniazid, we further extended the UISS framework to take into account the M. tuberculosis – isoniazid interaction. Both bactericidal and bacteriostatic mechanism was implemented in the UISS model. The model is able to distinguish the rapidly dividing mycobacteria from the slow one as UISS follows the single entity dynamics.

To model the entire dynamics of MTB and interactions with the immune system machinery, we selected all the players that have a role in TB in order to include the disease model into the simulation framework. The starting point was the implementation of all the biological entities involved into the dynamics of MTB, both at cellular and molecular level. To analyze the behavior of a possible therapeutic intervention, we selected the two vaccines we are going to test inside the STriTuVaD project i.e., RUTI and ID93 vaccines. To this end, we implemented inside UISS the main mechanism of action (MoA) of these immunotherapeutic vaccines.

Our model takes into account both innate and adaptive immunity (both cellular and humoral) and immune memory. Figure 1 depicts all the entities implemented within the simulation framework, especially immune cells, cytokines and specific biological processes along with their peculiar properties in TB dynamics. All the entities of the TB disease model interact each other, and are appropriately located in two specific compartments: the lung (pulmonary alveoli) and the peripheral lymph nodes.

The starting point of our TB disease model consists on the aerosol droplets of MTB that reach lung alveolar macrophages (AMs) on one side and neutrophils (N) on the other one. When an AM becomes infected, it secretes IL-1, TNF-alpha, IL-12, IL-6 and chemokines. Depending on MTB strains and their virulence, infected AM can play a different role in determining the downstream

pathways leading to the induction of either apoptosis or necrosis and to the final outcome of the infection. In this context, lipoxin A4 (LXA4) promotes necrosis, while prostaglandin E2 (PGE2) is a proapoptotic factor. When necrosis process is favored, the AM becomes necrotic and contributes to the MTB spread. Otherwise, when the AM becomes apoptotic, simultaneously three specific scenarios can occur. a) In the first-case scenario, AM apoptotic can interact with a lung resting macrophage (M) and lead to efferocytosis of macrophages, in other words an engulfment of AM apoptotic by M, essential for tissue homeostasis and immunity. This means switches from "resting" to "active" status. b) In the second scenario, AM apoptotic cells can encounter a lung dendritic cell (DC). AM apoptotic can be taken up by DC that capture antigens (Ag) through a process called nibbling; then, DC will process and present the resulting fragments to antigen-specific T lymphocytes in the context of molecules of the major histocompatibility complex of class I (MHC-I) or related proteins. From this point forward, MTB–antigen processing DCs, migrate to the local lung-draining lymph nodes (by 8–12 days post infection) driving naïve T cell polarization. This migration is influenced by IL-2 release and other chemokines, except when IL-10 is present and is able to block this moving. c) The last scenario considers the secretion of MTB debris from AM apoptotic: MTB debris will interact with DCs, in status resting, that will process and present the resulting fragments to antigen-specific T lymphocytes in the context of molecules of the major histocompatibility complex class II (MHC-II) or related proteins.

When MTB infects a lung N, N produces and secretes IL-1 and other chemokines. Just like AM, also for N the MTB strain can lead to a different role in the induction of either apoptosis or necrosis and to the final outcome of the infection.

Both AM and N effector functions can be negatively modulated by IL-10 induction during MTB infection. Respectively, IL-10 can lead to the inhibition of macrophage and neutrophil effector functions, with reduction of bacterial killing and impairing of secretion of cytokines and chemokines.

As previously said, IL-10 can also block chemotactic factors that control DC moving to the lung-draining lymph nodes.

The scenario inside the lymph node depicts the DC cells in antigen presenting cell status secreting IL-12, Type 1 IFN, IL-6 and IL-23 and driving naïve T cell differentiation toward a Th1, Th2 or Th17 phenotype. Th cell population differentiation can be negatively modulated by IL-10 and regulatory T cells (TReg). Protective antigen-specific Th1 cells migrate back to the lungs about 14–17 days after the initial exposure and infection to MTB and under a chemokine gradient (except when IL-10 blocks this process). In the lung, activated Th1 cell population, produce and secrete IFN-γ, causing macrophage activation, relative cytokine production (IL-12 and TNF-alpha) and bacterial control. It is worth to mention that in this context, IL-10 can block macrophage activation and consequent cytokine secretion. Also, TReg negatively modulate Th1 population effector functions.

For the sake of completeness, the Th1 cell population interacts also with B cells leading to three specific processes at the same time: after a successful interaction, B cells duplicate, differentiate in memory B and secrete immunoglobulins type G (IgG).

Similarly, Th2 cell migration and interaction with B cells leads to B cells duplication, differentiation in memory B cells and secretion of immunoglobulins type A (IgA). For what concern Th17 cell population, their migration and interaction with B cells will finally lead to cell duplication and secretion of immunoglobulins type E (IgE). Inside the lymph node, B cells interact also with MTB; after that B cells become active and secrete immunoglobulins type M (IgM).

After we implemented all of the specific immune system machinery – MTB dynamics, we started the implementation of Ruti MoA. To this end we added the effects of RUTI vaccination strategy, putting into the model the liposome dynamics and how the way it interacts with the immune system. The immunostimulatory activity of liposomes depends on diverse mechanisms: antigen delivery, particle size-dependent tissue penetration and access to the lymphatics. We retrieve this information from the data coming from AF partner. Also depot effects, promoting persistence, stability, conformational integrity and gradual release of vaccine antigens have been added to the UISS model. Moreover, additional mechanisms of liposomes include Toll-like receptor (TLR)-dependent and TLR-independent signal transduction, along with cross-presentation into major histocompatibility type I (MHC-I) pathways, caused by nanoparticle mediated leakage of antigens into the cytosol after phagosome uptake. Hence, to take into account the characteristics of RUTI vaccine, we extended UISS framework to include: activation of PRRs that triggers the initiation of the innate immune response; activated CTLs that recognize peptides bound to the major histocompatibility complex class I and II molecules (MHC-I, MHC-II), which express antigenic peptides on APCs and bind to T cells via the T-cell receptor. TH cells provide help to antigen-specific B cells, resulting in antibody production.

ID93 is a vaccine that consists of a fusion of four MTB proteins: Rv1813, Rv2608, Rv3619, and Rv3620. The vaccination strategy includes also an adjuvant, GLA-SE. Strains particular features have been implemented in UISS as we needed to exactly know the specific mechanism of action of each considered M. tuberculosis strain. We also implemented the specific mechanism of action of the GLA-SE adjuvant. GLA-SE contains the synthetic TLR4 agonist GLA formulated in a stable oil-in-water nano-emulsion. TLR4 and, in general, the toll like receptors machinery, is already present in the UISS computational framework. We implemented the GLA-SE adjuvant component of the ID93 vaccine in terms of specific stimulation of TLR-4 in cells expressing this toll like receptor.

We extended the UISS framework to take into account the M. tuberculosis – antibiotics therapy interaction. The clinical trial will use different antibiotics i.e., Rifampicin, Isoniazid, Ethambutol, Pyrazinamide and Fluoroquinolones. Both bactericidal and bacteriostatic mechanism has been implemented in the UISS model.

Validation

To validate what we implemented into the UISS computational framework, we started to evaluate some scenarios that could arise during the MTB – immune system – vaccines interactions and antibiotics. We analyzed three simulation scenarios. The first one is represented by the establishment of MTB latent chronic infection with some typical granuloma formation as a reservoir of MTB infection. The second one deals with a reactivation phase during latent chronic infection where a possible breakdown of the granuloma may lead to the spread of the bacilli and to the reactivation of the disease, with an increased necrotic burden. The third consists on the latent chronic disease infection scenario during RUTI or ID93 vaccine administration: here we show the evolution in time of M and CD4 T cell populations, in conjunction with the temporal evolution of IFN-γ that owns an important role as a biomarker of protection.

For the sake of completeness, we used a “mean” virtual patient. This means that given a set of random virtual patients used to represent a virtual population, a “mean" patient can be described as a patient whose mean entity behaviors fall within the 50 percentile of all the simulation results. This of course allowed us to obtain a level 2 validation i.e., to reproduce a population level dynamics. In D2.4, we will present the virtual patients library creation, in order to obtain a level 3 modeling infrastructure. To this aim we are going to ask Archivel Farma and IDRI partner raw patients’ data in order to personalize simutions.

In Figure 1 panel A, an initial infection of a virulent strain of MTB is supposed to happen at day 40. MTB rapidly infects both AM and M that become infected (blue lines). A similar behavior (not shown here) also occurs for N. Then, MTB grows inside them eventually leading to either apoptosis or necrosis of the infected cell, in accord to the levels of LXA4 and PGE2 in the site of infection. In particular, the virulent strain will drive towards the production of the pro necrotic LXA4 (panel D) and thus to higher percentages of necrotic cells that will become part of the granuloma mass (red lines). After the formation of the granuloma that represents a reservoir of MTB infection, the infection remains mostly latent, and also the CD4 T response that is recruited at the initial stages of infection (panel B) tends towards baseline levels. This is also present in the Interferon-gamma plots (panel C) where after an initial peak, its level drastically drops.

In Figure 2 a breakdown of about the 50% of the total granulomatous burden is supposed to happen approximately after 400 days after primary infection. This translates in a rapid drop in the number of entities that are part of the granuloma (yellow lines) and in a reactivation of the disease with a final worsening of the total granuloma burden, even if an immune response is present (see panels B and C), that is however unable to deal with the spread of the bacteria.

In figure 3 we show the effects of the vaccination based on RUTI vaccine in an individual with latent TB infection. Even if the vaccine seems to be mostly ineffective against the existing granuloma burden (panel A), a strong Th1 induced response is elicited with the induction of immunological memory (panel B). High levels of IFN-γ are present, in good agreement with the results presented in data gathered from literature for a 25ug dosage of the RUTI vaccine in latent patients.

Finally, in figure 4 we show the effects of the vaccination based on ID93+GLA vaccine in an individual (the same virtual patient in Figure 3) with latent TB infection. A strong Th1 response is induced with a down-regulation of Th2 response, with the induction of immunological memory (panel B). High levels of IFN-γ are present, in good agreement with the results presented in specific literature.

Overall, the simulations results are in very good agreement with available data coming from clinical trials. In particular, the in silico framework shows reliability in capturing the main landmarks of both induced immune response mirroring the IFN-γ, CD4 T cells, LXA4 and PGE2 dynamics. Moreover, form the spatial point of view, the granuloma dynamics with MTB spread were appropriately reproduced during the two most widespread scenarios characterizing pulmonary tuberculosis i.e., latent and latent with reactivation.

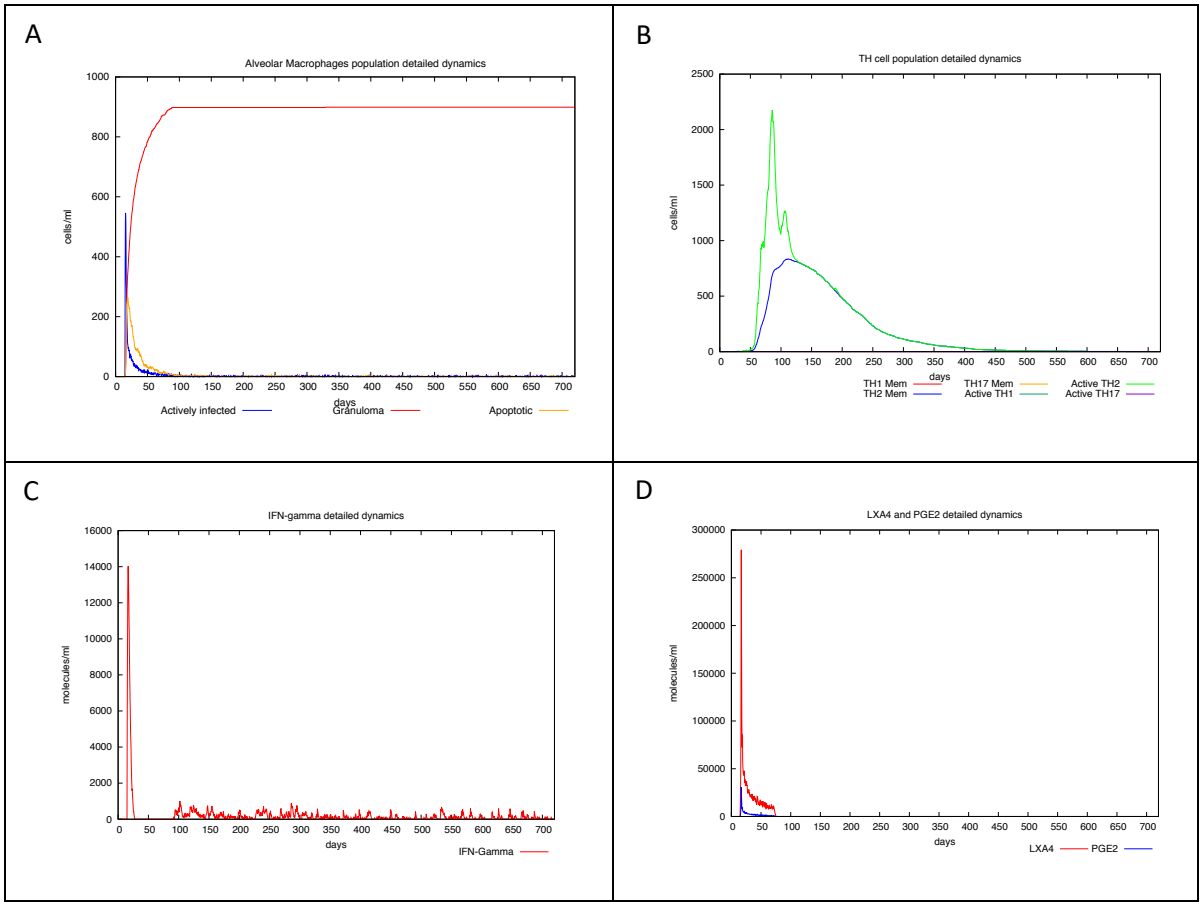

Figure 1. In silico latent tuberculosis infection scenario

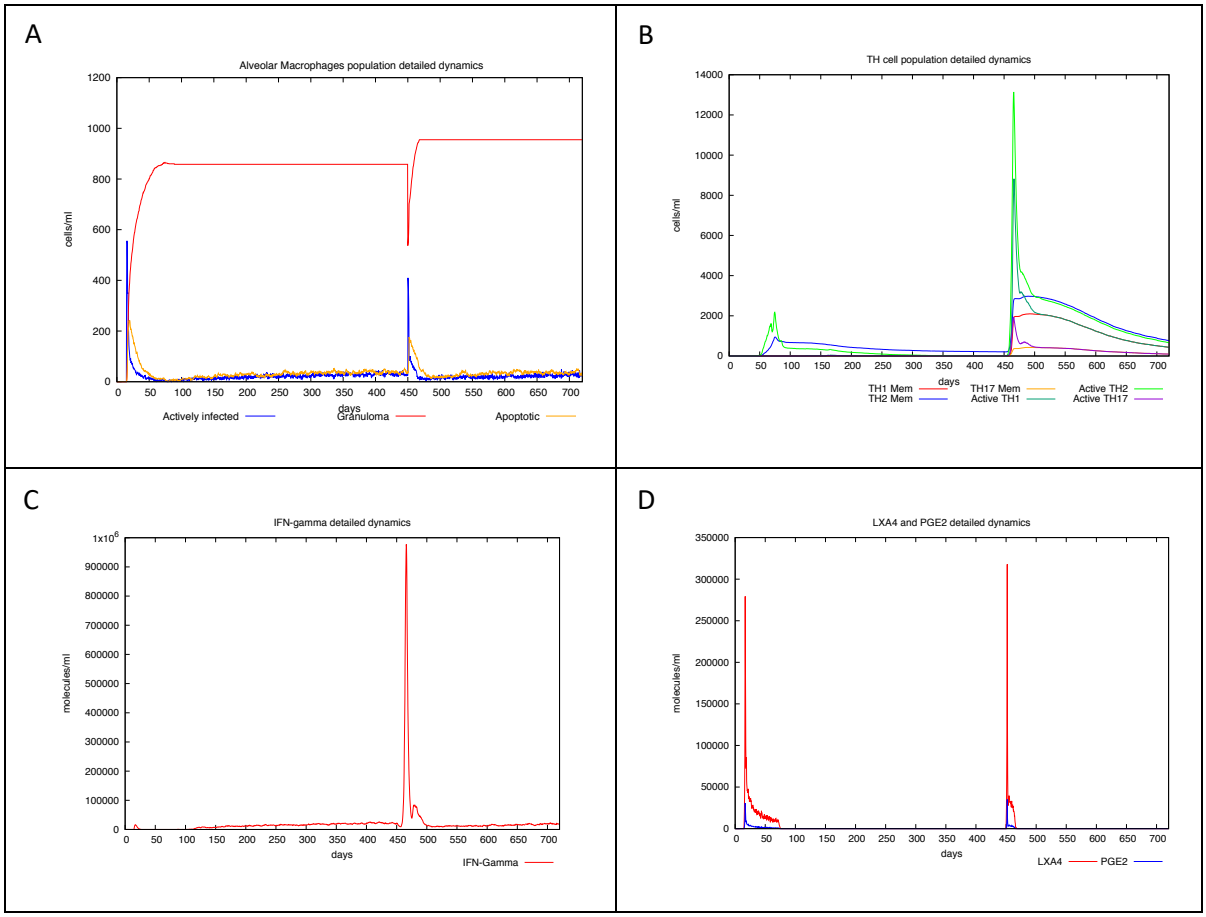

Figure 2. In silico latent tuberculosis infection with reactivation event scenario

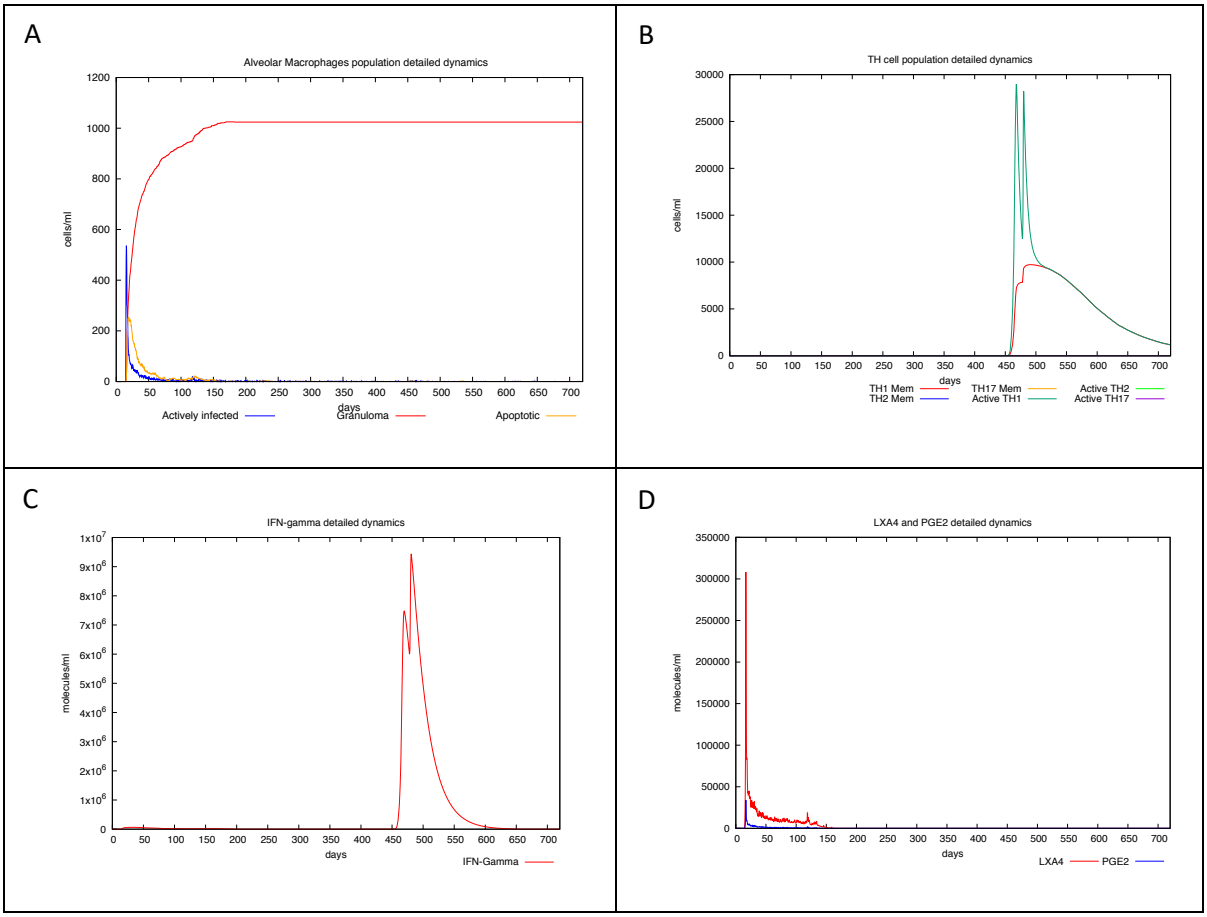

Figure 3. In silico latent tuberculosis infection with RUTI vaccine administration.

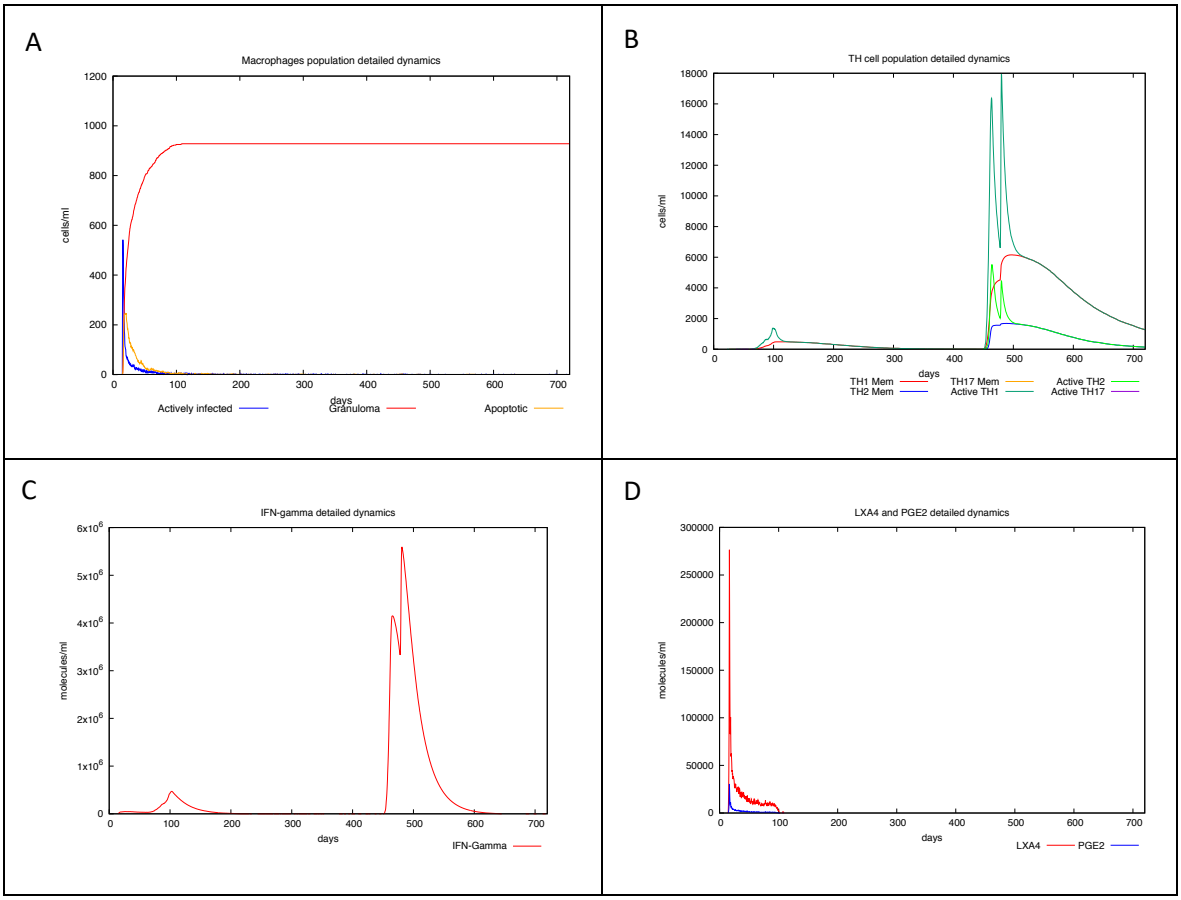

Figure 4. In silico latent tuberculosis infection with ID93+GLA vaccine administration.