A systems perspective of host–pathogen interactions: Predicting disease outcome in tuberculosis

Karthik Raman[1], Ashwini Gurudas Bhat and Nagasuma Chandra*

Bioinformatics Centre, Indian Institute of Science, Bangalore – 560012, India

Author for correspondence:

Dr. Nagasuma Chandra
Bioinformatics Centre
Indian Institute of Science
Bangalore – 560012
INDIA

Phone: +91-80-22932892
Fax: +91-80-23600551

E-Mail: nchandra@serc.iisc.ernet.in

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[1] Present address: Department of Biochemistry, Winterthurerstrasse 190, University of Zurich, CH-8057 Switzerland

Summary

The complex web of interactions between the host immune system and the pathogen determines the outcome of any infection. A computational model of this interaction network, which encodes complex interplay among host and bacterial components, forms a useful basis for improving the understanding of pathogenesis, in filling knowledge gaps and consequently to identify strategies to counter the disease. We have built an extensive model of the *Mycobacterium tuberculosis* host–pathogen interactome, consisting of 75 nodes corresponding to host and pathogen molecules, cells, cellular states or processes. Vaccination effects, clearance efficiencies due to drugs and growth rates have also been encoded in the model. The system is modelled as a Boolean network. Virtual deletion experiments, multiple parameter scans and analysis of the system's response to perturbations, indicate that disabling processes such as phagocytosis and phagolysosome fusion or cytokines such as TNF-α and IFN-γ, greatly impaired bacterial clearance, while removing cytokines such as IL-10 alongside bacterial defence proteins such as SapM greatly favour clearance. Simulations indicate a high propensity of the pathogen to *persist* under different conditions.

Keywords: ***Boolean networks, immune system modelling, tuberculosis disease outcome prediction, host–pathogen interactome, in silico knock-outs***

Introduction

Despite several advances in the treatment of tuberculosis (TB), it remains one of the major killer diseases world over, with nearly two million people dying each year (World Health Organisation, 2008)[1]. *Mycobacterium tuberculosis* (*Mtb)*, the etiologic agent of TB, is among the most persistent pathogens known today. The pathogenesis of *Mtb*, a facultative intracellular pathogen, relies on its ability to survive inside host macrophages[2]. The interaction of *Mtb* with its host is a perfect example of a balanced biological system, wherein *Mtb* uses host macrophages for replication and yet, the macrophages must remain viable to host the mycobacteria[3]. *Mtb* enters the human body via the respiratory tract through the inhalation of respiratory droplet nuclei, which are typically 1-2 μ in size. Once inside the lungs, the dynamic interplay between the host and pathogen can have any of the four outcomes: (a) the initial host response may be completely effective and kill the bacilli; (b) the organisms can grow and multiply immediately after infection, resulting in primary TB, (c) the bacilli may become dormant and never cause disease at all and (d) the latent bacilli can eventually become active and progress to disease condition[4]. The bacilli do not remain in the airways in the lung; they enter the lung parenchyma and replicate in the tissue macrophages and monocyte-derived macrophages that are recruited to the site of infection[5]. Other antigen-presenting cells such as dendritic cells (DCs) are also present in the airways[4]. *Mtb* uptake into the host cell, either opsonically or non-opsonically, is facilitated by the various receptors such as complement receptors (CRs), toll-like receptors (TLRs), mannose receptors (MRs) and scavenger receptors, present on the surface of the host cell. The initial interaction with the surface receptors influences the subsequent fate of *Mtb*. The multiplicity of existing phagocytic receptors, their ability to cross-talk, the presence of multiple receptors for internalisation of the pathogen and their uneven distribution among cells and cell types, introduce a high level of complexity[6]. The various signalling molecules and adhesion molecules of the host play a role in recruiting cells of the immune system to the site of infection. Influx of immune cells to the site of infection leads to the formation of granuloma, which functions to prevent the dissemination of mycobacteria.

Computational models are proving to be enormously useful in understanding the complex interplay between host and pathogenic factors[7]. There have been several advances in the

understanding of the interactions of *Mtb* with the human immune system. Here, we report an extensive model of the host–pathogen interactome of *Mtb*, accounting for several mechanisms of invasion by the pathogen, defence of the host, as well as the various defence mechanisms of the pathogen. Large-scale systems-level models of host–pathogen interactions, integrating information from various levels of abstraction can be of immense use in understanding processes of infection and developing strategies for combating disease. Using our model, some of the questions that we attempt to address are: What are the critical bacterial factors responsible for infection? What are the principal components of the human immune response and how robust is this response? How does the outcome of disease depend upon various parameters, such as bacterial growth rate, delay in onset of adaptive immunity and other cellular processes? How does the system respond to perturbations? In other words, how do variations in system components such as knock-outs or inhibitions or delay in onset of a given event, influence the disease outcome?

Results

Network Assembly

We have constructed a network model of the host–pathogen interactions based on extensive data available from literature. The model includes the various components of the innate response, the adaptive immune response to tubercular infection, as well as the several components of mycobacterial virulence and defence against the host immune system. Such a comprehensive systems-level picture of the host–pathogen interactions has the potential to address several questions, particularly the criticality of the various factors involved in the immune response and bacterial defence. Fig. 1 gives a schematic representation of the complex network of host–pathogen interactions in tubercular infection. Table 1 lists some of the major events in the immune response, which have been incorporated in our model. The model consists of 75 components, spanning across various levels of organisational hierarchy in host–pathogen systems. These include 56 host components, of which 26 are at the molecular level, 11 represent various cellular processes, while 19 correspond to various cells and cell states involved in the immune response. The model also includes 18 virulence factors of the bacterium. A detailed description of the experimental studies used for network construction has been provided as

supplementary material (see Supplementary Text S1). However, for clarity, a brief description of the components of the network is given below.

Innate immune response

The control of *Mtb* infection is mainly through cell-mediated immunity; hence, the humoral immunity has a limited role in controlling the infection[5, 8]. The control of infection requires the co-ordinated interaction of macrophages, DCs and T cells. Macrophages, the preferred habitats of *Mtb*[9] and DCs are the major antigen presenting cells involved[10, 11]. Figure 1 illustrates that macrophages and DCs occupy a prominent role in the model, right from the initiation of infection. The bacterial load is denoted by the parameter B_0. Entry of the pathogen is either by engulfment (via TLRs and other receptors) or sinking of the bacilli into the cell (via CRs)[12, 13]. The role of TLRs is captured in the *TLR signalling* Boolean transfer function, while the role of CRs is incorporated into the *CR_MR_other_signalling* transfer function (see Supplementary Text S2). While the signalling events of TLRs are well understood, the signalling events of CR, MR and other receptors are not well characterised[14-16]. Cholesterol acts as the docking site for the binding of *Mtb* to the surface receptors on the host macrophages[9]. To account for the time taken for phagocytosis to occur, the parameter δ_{PH} has been introduced. Phagocytosis and subsequent signalling, depending on the type of receptors involved, leads to the production of cytokines that are either pro-inflammatory (tumour necrosis factor (TNF), IL-1, IL-1, IL-6, GM-CSF (granulocyte monocyte colony stimulating factor)) or anti-inflammatory (transforming growth factor TGF-β, IL-10, IL-6), with the anti-inflammatory cytokines (AICs) having an inhibitory effect on the production of pro-inflammatory cytokines (PICs)[17, 18] and chemokines (IL-8, CCL2, CCL3, CCL5), which are the major signalling molecules in the host immune response. Due to the redundancy of the chemokine system, the contribution of individual chemokines is difficult to evaluate[19]. For this reason, the individual chemokines have not been encoded separately in the model. A balance between the effects of PICs and AICs is thought to determine the outcome of disease, whether in the short term or long term[20]. Macrophages, upon phagocytosis, can become activated phagocytic cells (APCs), which have increased phagocytic activity, show increase in cytokine production and release the effector molecules, such as reactive oxygen intermediates (ROIs; hydrogen peroxide) and reactive nitrogen intermediates (RNIs; nitric oxide)[21]. The black

arrows in Fig. 1 indicate connections that follow sequentially, while blue arrows, such as those from macrophages to APCs indicate signalling for proliferation or recruitment. TLR signalling also leads to the upregulation of the antimicrobial peptide, cathelicidin, which inhibits the growth of *Mtb*[22].

Simultaneously, a phagosome is formed inside the macrophages and cytoskeleton rearrangement of the macrophage takes place[23], with the phagosomes acquiring the early and late endosome markers, followed by the fusion with the lysosome to form the phagolysosome[24]. Once this organelle is formed, the next step is antigen processing, followed by antigen presentation (either through the classical MHC presentation pathway or through the non classical CD1 presentation pathway[19]. These events are captured through nodes such as Phagolysosome formation, antigen processing and antigen presentation and their interactions encoded in their respective Boolean transfer functions. DCs are the other major antigen presenting cells involved in the control of TB infection; they link the innate and adaptive immunity[25]. DCs mature upon infection with *Mtb*, present the mycobacterial antigens to T cells in the secondary lymphoid organs and not at the site of action[11, 26]. DCs have a special role in antigen presentation due to their ability to present non-protein antigens to T cells via CD1 molecules[25]. The other cells involved in the innate immunity against *Mtb* are the neutrophils and natural killer (NK) cells. Neutrophils are the first cells to arrive at the site of multiplication of the bacilli; and they can transfer their microbicidal granules to the infected macrophages[8, 10]. NK cells, upon stimulation by the cytokines released by APCs or DCs, produce cytokines like IFN-γ and IL-32. The role of NK cells has not been definitively demonstrated *in vivo*[5], and hence they have not been included in the present implementation of the model.

Adaptive immune response

The onset of adaptive immunity in infected patients occurs several weeks after initial infection[27], a factor accounted for by the parameter δ_{AI}. The innate immune machinery is only the first line of defence against the pathogen. The adaptive immune response is more specific and more potent, involving several complex mechanisms. T cells are the main components of the adaptive immune response. T cells can recognise the antigen presenting cells loaded with the peptides on the MHC molecules and can differentiate into CD4+ cells (Th cells) or CD8+ cells (Tc cells) or

γ/δ T cells, depending on the cytokines that stimulate the naive T cells. The Th cells can differentiate into Th1 cells, Th2 cells, or the newly characterised Th17 cells. The cytokines released by each subset negatively regulate the cytokines released by the other subset. The Th2 related cytokines can inhibit the production of PICs produced by the macrophages[28], can activate the eosinophils, basophils and mast cells, which release potent inflammatory molecules like ROI and cytokines (IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-13, TNF-α), and can express cell adhesion molecules on their surface, thus playing a role in the formation of granuloma to contain the infection[29]. γ/δ T cells play a role in the apoptosis of the infected cells, but their role has not been definitively determined *in vivo*[5], and hence they have not been included in the present implementation of the model. The Tc cells or the CD8+ cells are involved in the killing of the infected target cells, by releasing the Tc related cytotoxins (Perforin, Granulysin) and Tc related cytokines (IFN-γ, TNF-α) or by the activation of the Fas-FasL pathway. Apoptosis is an effective mechanism of killing the infected cells containing *Mtb*[30].

Fibroblasts play a role in maintaining the extra-cellular matrix during granuloma formation. Though humoral immunity is not prominent in *Mtb* infection, B cells do play a role in the granuloma formation. They release cytokines and chemokines, which attract T cells. B cells are found in large numbers in the granuloma[5]. The infected macrophages, multi-nucleated giant cells (fused macrophages), T cells, fibroblasts, other cells of the immune system, cytokines, chemokines and adhesion molecules are the important components of the granuloma, the characteristic feature of *Mtb* infection, where the bacilli become latent. The granuloma prevents the dissemination of *Mtb* and thus contains the infection.

Mtb virulence factors

The model contains 18 different bacterial virulence factors that are important in bacterial defence against host immune responses. All these virulence factors are indicated in red or green typeface in Fig. 1, depending on whether they promote or inhibit a particular process. Bacterial virulence factors, such as ManLAM, Phosphatidyl-myo-inositol mannoside (PIM),19kDa lipoprotein, FAP (fibronectin attachment protein), Ag85 complex, LprG, SecA2, LAM, SodA, SodC, KatG, BpoB, NuoG, PknE, PknG, SapM, urease, have been captured in our model through 18 nodes and their corresponding transfer functions. During *Mtb* infection, the balance between the bacterial growth

and survival and the magnitude of the host immune response determines the final outcome of the disease.

Boolean network model

The network of host–pathogen interactions described above was translated into a Boolean network model. Given the paucity of mechanistic data, detailing kinetic parameters for the various immune processes, a Boolean network model can be sought to obtain insights into the host–pathogen system. Boolean network modelling has been shown to be useful for the modelling of host–pathogen interactions in *Bordetella pertussis* and *Bordetella bronchiseptica* infection[28]. Our model consists of 75 nodes; each node is represented by a Boolean transfer function. 12 quantitative parameters have also been integrated into the model. The nodes represent various components of the host immune system and the bacterium and also include the critical components of bacterial defence. The state of each node in the network can be either 'on' or 'off', a qualitative description of the concentration or activity. The change of each state is represented by a Boolean rule, a transfer function involving simple logical constructs such as 'AND', 'OR' and 'NOT'. For example, an activation is indicated by an 'OR' operator, while an inhibition is indicated by an 'AND NOT' operator. An 'AND' is used to highlight a scenario where more than one of the components need to be present concurrently to cause an activation. Where there is uncertainty in the control of a node, 'Random' variables are used, to account for poorly understood phenomena. The system with its states initialised, corresponding to t = 0, is then simulated, or evolved, by an iterative procedure, where the state of each of the nodes of the system is computed based on the update rule. The asynchronous update method, as described by Albert and co-workers has been used[28].

The interaction events are encoded as Boolean operations on network nodes. For example, Reactive oxygen intermediates (ROIs) are released by Antigen presentation cells, under the influence of pro-inflammatory cytokines. The detrimental effect of ROIs on the bacteria is mitigated by the various bacterial defence components, such as SodA, SodC, BpoB, KatG, SecA2 and ManLAM[4, 31, 32]. Hence, the Boolean transfer function is represented as *ROI* = Activated_phagocytic_cells and Pro_inflammatory_cytokines and not (SodA or SodC or BpoB or KatG or SecA2 or ManLAM)*, the asterisk denoting the state that is updated. Similarly, Th1 cells

are important in the control of TB infection as they produce the cytokines IFN-γ and TNF-α[4, 6]. T cells in the presence of bacteria, upon stimulation by IL-12 or IL-18 differentiate into Th1 cells. Macrophages, in the presence of chemokines increase the population of Th1 cells[29, 33-35]. The Boolean transfer function for Th1 cells will thus be *Th1_cells* = (Bacteria and (T_cells and (IL_12 or IL_18))) or (Macrophage and Chemokine_signalling)*. In this way, we can interpret Fig. 1, which displays the interactions graphically. Detailed descriptions for each of the Boolean transfer functions are given in Supplementary Text S2.

Quantitative parameters for simulation

Though the model is essentially qualitative, parameters have been encoded for certain important aspects, such as bacterial load, delayed onset of adaptive immunity, delay in progress of phagocytosis and apoptosis and rates of bacterial growth and clearance. Each time-step in the model has been taken to roughly correspond to a day. The doubling rate of *Mtb* in macrophages has been reported to be in the range of 28–96 hours[36]. This would translate into a growth rate (λ) in the range of [0.17, 0.59], which has also been reported in[36]. α, which represents the incremental change in *Mtb* population, would therefore be in the range [0.19, 0.81]. The efficiency with which the host cells can clear the bacteria from the host is defined by the parameter, η. Intake of drugs to cure the infection can result in a change in η, the clearance rate.

For complex processes such as phagocytosis and apoptosis, which operate to clear bacteria from the system, a delay factor indicating the length of time for which a process has to be active to achieve the result of clearing can be specified. This essentially describes the delay of onset of the process, once the conditions necessary for its activation are fulfilled. This was approximately taken to be seven time steps, for both phagocytosis (δ_{PH}), and apoptosis (δ_{AP}), to account for the fact that the onset of phagocytosis or apoptosis is not a simple one-step event.

The initial bacterial load, B_0 was taken as 5. B_{min} was taken as one, and B_{max} was taken as 10^5. These parameters have been varied to analyse different scenarios. For example, B_0 has been varied between 1 and 100. B_{max} has been chosen arbitrarily, but it may be envisaged that beyond over three orders of magnitude (from B_0), the response of the system may be much more unpredictable, as the effect of the unaccounted parameters may increase. Unless otherwise

specifically stated, we have used $\alpha = 0.7$ and $\eta = 0.4$ in our simulations. The delay in the onset of adaptive immunity δ_{AI}, has been chosen as 14, based on reports in literature[27]. To model pathogenesis in vaccinated individuals, δ_{AI} can be taken as zero. These parameters have also been varied, and the results have been provided as supplementary material (See Table S1). Table 2 lists the parameters in this model, and a range in which they have been varied.

Simulations of pathogenesis

The pathogenesis of TB has been simulated using the Boolean network model. The model is first initialised to indicate the states of the various components, at the onset of infection. Following this, the model is evolved for t_{max} time-steps, 105 in our case (corresponding to 15 weeks), at the end of which the outcome of infection is predicted. If the bacteria present in the system at the end of the simulation is greater than the threshold B_{max}, the outcome is active disease. If no bacteria are present, it represents successful bacterial clearance. If bacteria still remain, albeit below the threshold of B_{max}, it represents persistence. Persistence may also be 'triggered' if bacteria remain in the interval of $[P_{min}, P_{max}]$ for longer than δ_{per}.

100 simulation runs were performed for the parameter set $B_0 = 5$, $B_{min} = 1$, $B_{max} = 10^5$, $P_{min} = 5$, $P_{max} = 10^3$, $\delta_{per} = 14$, $\delta_{PH} = 7$, $\delta_{AP} = 7$, $\delta_{AI} = 14$, $\alpha = 0.7$, $\eta = 0.4$, which resulted in the outcomes of active disease in 15 runs, complete bacterial clearance in 42 runs and persistence in 43 runs. The state map for a representative run of the simulation is illustrated in Figure 2A, while the bacterial load is shown in Figure 2B. The same parameter set was used to carry out an extended simulation of 1200 runs, for which the results were similar, with 13% of the runs leading to active disease, 41% of the runs showing bacterial clearance, and 46% of the runs resulting in persistence. This may be considered as a typical outcome of exposure to TB infection, where persistence is often the most dominant outcome. Of course, this is conditional on various parameters such as the growth rate, the efficiency of bacterial clearance and the delay in the onset of the various immune responses.

Variation of parameters

To simulate the outcomes that may result with different parameters, we have varied the parameters of the model, such as δ_{AI}, δ_{PH}, δ_{AP}, α, η and B_0. Table 3 highlights some of the

simulations with variations of one or more parameters, focussing on the variation of a single parameter, which gives an idea of how the outcome of infection is affected by an individual parameter. For each set of simulations, a new parameter set was generated, by uniformly sampling the 'range', for each of the parameters (see Table 2 for the parameters varied and the ranges). Following this, multiple runs of the simulations were performed. A table detailing the results of the simulations obtained with the different parameter sets has been provided as supplementary material (see Table S1).

Variation of α: Higher α correspond to higher growth rates. For α = 0.2, more than 90% of the runs led to persistence, and the remaining, to bacterial clearance. Active disease was not observed. For α = 0.4, 100% of the runs showed persistence.

Variation of bacteria load B_0: B_0 is a parameter that indicates initial bacterial load. Sensitivity of simulation results to this parameter was studied by varying B_0 from 1 to 100 bacilli. It has been reported earlier that typical number of bacteria in droplets of nasal exhalations from TB patients is in the range of 5 to 10, depending on the susceptibility of the individual. At the same time, there is also a study that reports possibility of an infection with a single bacterium; hence the choice of the range. Simulations indicate that higher B_0 values have high propensity of causing active disease, while low B_0 values have higher chances of resulting in persistence as an outcome.

Variation of η: η captures efficiencies of clearance of bacteria from the host cell. At lower values of η, the clearance of bacteria is much less. At η = 0.4, 0.6, no clearance was seen, with close to 95% of the runs showing persistence and the rest resulting in active disease. At η = 0.8, clearance was much improved (58%), while the rest resulted in persistence. This parameter represents the overall efficiency of the multitude of processes that act to clear bacteria from the cell. Higher the efficiency of such processes, it may be expected that the bacterial clearance improves. However, persistence is still seen to be a dominant outcome.

The administration of a drug, for example, would affect both the rate at which bacteria are cleared from the system, as well as laying a check on the rapid division of bacteria. It can thus be

interpreted as a tandem effect on both α and η. Such a model can therefore, also be used as a scaffold for integrating and analysing drug pharmacokinetics.

Variation of delays in phagocytosis and apoptosis: If the delay in phagocytosis/apoptosis is removed, i.e. $\delta_{PH} = \delta_{AP} = 0$, active disease is not observed as an outcome. Majority of the runs result in persistence, while in a few cases, bacteria are cleared.

Variation of δ_{AI}: For $\delta_{AI} = 0$, which can be taken to represent the scenario of infection of vaccinated individuals, only 5% active disease was seen, the remaining being persisters (see Figure 2D). For $\delta_{AI} > t_{max}$, which essentially represents a knock-out of the entire adaptive immune system, bacteria were never cleared, with majority of the runs leading to active disease (78%) and the rest resulting in persistence. These results underline the importance of the adaptive immune response to tubercular infection. It is conceivable that innate immunity by itself may not be sufficient to counter infection. It also highlights that while vaccination may be useful to avert disease, persistence may still be a critical problem. It is possible that the persistent bacteria may re-activate and progress to active disease at a later stage.

Simultaneous perturbation of multiple parameters

Although single parameter variations reflect the role of the parameters in pathogenesis, it does not capture the *in vivo* situation of TB infection, where the outcome is influenced by more than one parameter, like presence of drugs or vaccines or a faulty cell-mediated immune response. To study the effect of these parameters on the outcome of bacterial infection, simultaneous multiple parameter variations have been carried out. Table 3B shows the nature of some of the outcomes obtained by simultaneous multiple parameter variations. These simulations capture different combinations of initial bacterial load, growth and clearance rates, delay in onset of adaptive immunity and time for switching to persistence. A case of high occurrence of active disease is observed at $B_0 = 69$ (high bacterial load) and $\alpha = 0.71$ (high growth rate). This may be because of the low clearance efficiency of $\eta = 0.42$, indicating that not all the bacteria are cleared. Due to this low clearance efficiency, and the high growth rate, the bacteria may proliferate, resulting in active disease. A low bacterial load ($B_0 = 4$), and a relatively low growth rate ($\alpha = 0.54$) and low clearance efficiency ($\eta = 0.48$) leads to a persistent disease condition. The bacteria might enter

the dormant phase at this condition, and remain hidden from the immune system, thus leading to persistence. The simultaneous variation of the parameters, α, η, B_0, δ_{per} and δ_{AI} yields interesting results. For instance, even at a high clearance efficiency of η = 0.723, a low growth rate of α = 0.449, a low B_0 = 10, and a longer delay in adaptive immune response, the system moves towards Persistence. Clearly, η alone does not dictate the outcome in this case. The longer delay in the adaptive immune response, and the relatively lesser time taken by the bacteria to enter persistent state (δ_{per} = 11), and the low growth rate might play in a role in the observed disease outcome. High instances of clearance in the presence of high B_0, suggests a role for both α and η in parallel. The studies indicate the complex dependency of the disease outcome on the various factors.

Effect of deletion of immune system components

The effect of deletion of immune components *in silico* was also studied. Each node was removed from the model and the simulations were repeated. These simulations give an idea of the components critical to the immune response and virulence. We have performed single knock-outs for the network, by disabling each of the 75 nodes (excluding bacteria), individually. A systematic double knock-out study has also been carried out, disabling a pair of nodes in each run. A total of 74 single knock-outs and 2,701 double knock-outs were carried out.

Single node deletions

All cases where 'Phagocytosis' was disabled led to the onset of active disease (see Figure 2C). A similar observation holds for the deletion of 'Phagolysosome formation'. The knock-outs of PICs such as IFN-γ and TNF-α resulted in an impairment of bacterial clearance, with none of the cases reporting bacterial clearance. Such a phenomenon has in fact been observed in mice, where the gene for IFN-γ has been disrupted[32]. A normally sub-lethal inoculum of *Mtb* grew progressively to lethal levels, with widespread caseous necrosis throughout the major target organs. It has also been suggested that mice lacking a functional gene for IFN-γ are totally unable to contain and control a virulent *Mtb* infection[32]. The state maps obtained from our studies also indicate the above scenario, wherein the outcome seems to be related to the production of IFN-γ. Studies wherein infected mice deficient in the 55kDa TNF receptor and mice in which the TNF gene has

been disrupted, have shown defective granuloma formation and inability to control mycobacterial replication[37].

The knock-out of AICs such as TGF-β (see Figure 2E) or IL-10 favoured bacterial clearance, in 66% and 74% of the runs respectively. Where apoptosis was disabled, bacterial clearance was not seen in any of the cases with 68% of the cases leading to persistence. For the knock-out of inflammatory molecules, 52% of the cases led to active disease, while 48% of the cases resulted in persistence, again emphasising the criticality of these molecules in the host immune response. The *Mtb nuoG* gene is involved in inhibition of apoptosis of infected host cells[38]. This role of *nuoG* is highlighted in the virtual deletion of NuoG, where the major outcome is clearance, possibly indicating the uptake of *Mtb* by fresh and more efficient phagocytic cells and thus validating the role of nuoG in *Mtb* host–pathogen interactions. The knock-out of mycobacterial virulence factors such as urease and SodC also result in higher bacterial clearance, thus underscoring their role in the survival of *Mtb*. At higher growth rates, where 'active disease' was the predominant outcome, knock-outs of ManLAM significantly impaired the ability of bacteria to persist or result in active disease.

These knock-out simulations can be thought to mimic conditions similar to those upon the administration of antibodies, which may be targeted against a particular component in the model.

Double node deletions

Whenever a pair of nodes including either 'Phagocytosis' or 'Phagolysosome formation' was knocked out, bacteria were not cleared. The interesting results involve the deletion of 'Inflammatory molecules', which includes ROI, cytokines and granules like histamine released by mast cells, eosinophils and basophils. The double knock-out of inflammatory molecules alongside most other nodes, like pro-inflammatory cytokines, signalling molecules, T cells, TcRC, Th1RC, TNF-α leads to active disease, as can be seen in Figure 3A. Figures 3A-C indicate the results of the knock-out studies. The double knock-out of inflammatory molecules and TLR signalling led to active disease in all the cases.

TNF-α was observed to have a critical effect on the immune response, when it was knocked in conjunction with one of many other nodes. This can be observed from Figure 3C, which

indicates that on knocking out TNF-α, along with any of the nodes such as activated DCs, IL-10, IL-1, TNF-β, the major outcome is persistence. A similar outcome is observed for the deletion of nodes such as IFN-γ, KatG, apoptosis, DCs and macrophages, along with one of many other nodes. These nodes are such critical components of the immune response that disabling them seriously compromises the ability of the cell to clear bacteria. Understandably, the immune system appears to be generally robust to the failure of single components and is expectedly more sensitive to multi-component failure.

The knock-out of TGF-β, which is an AIC, appears to favour bacterial clearance. The knock-out of IL-10 alongside the bacterial virulence factor SapM also favours bacterial clearance. In the knock-out of apoptosis along with any of the nodes, no cases of clearance were reported. ManLAM, when disabled in conjunction with many other processes, significantly checks the onset of active disease, although the problem of persistence still remains. ManLAM significantly interferes with the host defence mechanisms, like phagosome maturation arrest, scavenging free oxygen radicals, and directly inhibiting macrophage response and TNF-α and IFN-γ production in macrophages[11, 31, 39]. Targeting ManLAM could prove to be a fruitful strategy to control TB infection.

Outcome of deletion of some of the interesting combinations are listed in Table 4. There are a number of cases where seemingly non-essential genes (nodes) when knocked out in specific combinations can lead to unexpected outcomes. For example, 19kDa lipoprotein and SapM themselves individually considered as non-essential by TrasH[40] and flux balance analysis[41]. However, the simultaneous deletion of these two molecules leads to clearance in 65% of the cases considered. The fact that their deletion leads to clearance suggests that these molecules are important for the virulence/survival of the *Mtb*. This is further supported by the fact that these virulence factors target processes which are important in clearing bacteria from the system, Antigen processing and Phagolysosome formation, respectively, the latter being a very critical process (Figure 3A). Such nodes, which result in high clearance upon deletion, can be considered as critical points in determining the eventual outcome of infection.

Discussion

Several methods that exist for the analysis of host–pathogen interactions have been reviewed by Forst[7]. Nearer to the realm of quantitative mechanistic models are qualitative models, using Boolean networks. The roots of Boolean network modelling may be traced to as early as 1969, when Kaufmann described the use of such models for studying cellular control processes[42]. Another insightful exposition of Boolean network theory for modelling genetic circuits was given later by Thomas[43]. Boolean network models have been used successfully, to predict the expression pattern of the segment polarity genes in *Drosophila melanogaster*[44]. Brahmachari and co-workers have applied Boolean network modelling to analyse a neurotransmitter pathway implicated in schizophrenia[45]. Albert and co-workers[28] have applied Boolean networks to model the host immune response to infection for two closely related bacteria of the Bordetella species. A similar effort has been directed towards the simulation of hepatocyte growth factor and *Helicobacter pylori* induced c-Met signal transduction, by Naumann and co-workers[46]. They represent Boolean networks as logical interaction hypergraphs, a representation suited to formalise, visualise and analyse logical models of such signal transduction networks. Their model highlights differences and similarities of the network response to hepatocyte growth factor and *H. pylori* induced c-Met signalling. Given the scarcity of the mechanistic parameters describing the immune system and its interactions with *Mtb*, we seek to use Boolean network modelling to derive insights into *Mtb* pathogenesis.

The prolonged co-evolution of *Mtb* with its human hosts and specifically within macrophages has resulted in the bacterium evolving mechanisms to overcome the challenges posed by the host immune system. It contains various virulence factors, which help in its growth and survival in the hostile host environment. It has more than 200 genes that may influence the degree of virulence[33]. The molecular mechanisms involved in mycobacterial host–pathogen interactions are yet to be understood completely. The global resurgence of TB and the rapid emergence of multi-drug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB) underscore the importance of the development of new anti-tubercular drugs. Understanding the mechanisms through which the cells of the immune system recognise *Mtb*, can be an important step in

designing new therapeutic approaches, as well as improving the limited and variable success of current vaccination strategies[47].

Disease is the outcome of the complex interplay between the various pathogenic virulence factors, as well as the host innate and adaptive immune systems, each combating the other in a battle for supremacy. The network model of the host–pathogen interactions developed here is a first step towards making sense of this complex interplay. Although the model is predominantly qualitative, it can still give valuable insights into the importance of the components from the perspective of controlling and combating disease. Quantitative parameters have also been integrated into the model, enhancing it to better account for some of more complex processes. The insights obtained from *in silico* deletions, particularly the double knock-outs are quite informative and further illustrate the robustness of the immune machinery, as well as the key processes. The influence of the various bacterial factors such as ManLAM and the complex network of cytokine regulation can be better understood in the light of this model. The model also reiterates the importance of PICs such as IFN-γ, whose importance has also been illustrated through other experiments[32] . As with many computational models, this model reaffirms the importance of predictive modelling and simulation. Several questions that may be very difficult to address experimentally can be easily queried against the model. The model presented here represents a succinct way of organising information on host–pathogen interactions and strongly facilitates integration and evaluation of new hypotheses. This includes the single and multiple deletions of the various system components, particularly from the human system, which are obviously not practical to carry out experimentally. In some sense, these simulations present the possible responses of immuno-compromised individuals to tubercular infection. The model re-emphasises the importance of persistent *Mtb* infection, which must be tackled in any programme to counter TB. The model also provides a ready framework for the incorporation of qualitative data as well as integration at various scales, when such data and models become available.

The persistence of bacteria following single or double gene knock-outs indicates that the bacteria have developed alternate mechanisms for survival, owing to its highly redundant genome. Though persistence is the major outcome, high instances of clearance are seen when the nodes corresponding to bacterial virulence factors such as SapM, SodA, LprG, LAM, NuoG, Ag85

complex (Ag85 A, B, C), PIM and FAP are knocked out. This reiterates the importance of cell wall components in the virulence and survival of bacteria. Targeting such nodes may be helpful in controlling TB. In fact, some of the nodes, like Ag85 complex (Ag85 A, B), and some of the proteins involved in the biosynthesis of PIM, LAM, and ManLAM (PimA, PimB and EmbC) and urease are present in the list of high-confidence targets predicted by targetTB, a target identification pipeline for *Mtb*[48].

The work presented here does have its limitations. Some of these limitations stem from our limited understanding of the various mechanisms involved, as well as the finer mechanistic details of the various immune processes. It is ultimately desirable to understand all these processes at a molecular level, but given that the individual molecular events in a particular process have not yet been adequately characterised, the only practical way of modelling, at present, is to use a model that spans different levels of biological hierarchy. The model presents an approximation of many immune processes; most of the elements in the model have only two states (on or off; active or inactive). Although lacking in molecular detail at all steps, a Boolean transfer function at the cellular level captures the outcome of a number of molecular events as a single cellular event. Even for processes where quantitative parameters have been defined, there is uncertainty as to what might be the most realistic set of parameters. While some parameters such as those involving growth rate and delayed onset of adaptive immunity have varying degrees of support from literature, others, such as the description of persistence have been included in the model, mainly to provide a scaffold for integrating knowledge from future experiments. Despite these limitations, the model provides a first glimpse into the complex web of interactions between the host and pathogen, laying the ground for combating TB, as it becomes more pervasive and threatening than ever before.

In the recent years, there has been an enormous interest in modelling whole cells to understand address a variety of issues and to use it in applications such as drug discovery[49, 50]. This study uses systems-level modelling and simulation to predict the outcome of infection, such as active disease, persistence or clearance. This complements several other parallel advances in systems biology approaches to understand TB, such as the modelling of pathways using flux balance analysis[51], reactome modelling through genome-scale flux balance analysis[41, 52] and network

analysis[53, 54], analysis of resistance pathways[55], as well as population-based approaches to model epidemiology and immune system dynamics[56, 57]. These studies, besides providing significant insights into systems-level understanding of TB, are also expected to aid in drug discovery significantly[48, 58]. The work reported here demonstrates that it would be possible, in the near future, to develop host–pathogen models as 'virtual patients' incorporating comprehensive quantitative information of the host–pathogen interactome and even individual genotypic variations. Experimentation and computational modelling must be used in complement, each deriving benefits from the other. Computational modelling can be used to generate novel hypotheses, which can then be used to guide experimentation. Experimental verification or validation of a model can render it much more useful, as more reliable predictions can be made, on the strength of its proven validity. Insights that such models can provide could become useful for predicting cases where an infection can lead to disease, ultimately translating into more rational and personalised therapeutic intervention strategies in clinical practice. The model presented here is thus an important step towards a holistic understanding of host–pathogen interactions and may further be used to design new experiments that can lead to a better understanding of this complex system.

Methods

Network assembly and simulation

The steps involved in the construction of the interactome model and simulation of host–pathogen interactions in TB are indicated in Figure 4. The network was manually assembled using extensive information on *Mtb*-host interactions available in literature. Published data were evaluated carefully and only approved results and data that were consistent were incorporated into the model. All information found relevant regarding *Mtb* host pathogen interactions was collected in a database specifying source node, target node, their respective functions, edges, and the type of interaction (activation/inhibition) (Supplementary Table S2). This information was then converted into an interaction network, with the orientation of the edges reflecting the direction of information propagation following the recognition of pathogen (Figure 1). The Boolean transfer functions used in the simulations are based on this network. The network figure was drawn using Microsoft Visio. The simulations were performed using a modified version of

the Boolean Net software[59] (http://code.google.com/p/booleannet/), developed by Istvan Albert. Boolean Net has been used by Albert and co-workers[28] for the analysis of the pathogenesis of *Bordetella pertussis* and *Bordetella bronchiseptica*. Boolean Net is based on Python and supports asynchronous updates using Boolean transfer functions[28].

The states of the various nodes in the system were initialised and the system was then evolved for t_{max} time-steps. At the end of the simulation, a decision was made on the outcome of infection, based on the count of bacteria present. The model considers three major outcomes of infection, viz. active disease, bacterial clearance and persistence. Another possible outcome is the reactivation of persistent bacteria, culminating in active disease, which is influenced by the presence of cytokines such as TNF-α and IFN-γ. It has been shown that IFN-γ and TNF-α depletion during persistent stages lead to rapid disease reactivation in mouse model[60]. However, since this is a very complex process, dependent on several other unknown factors, as well as having a much longer and uncertain time-frame, we have chosen to factor it out of our model.

Boolean transfer functions

While using Boolean network formalism for modelling, the nodes of the network are related to one another through Boolean transfer functions, describing their inter-dependence. The major cells, molecules and processes involved in the immune response have been encoded as Boolean transfer functions, some of which have been illustrated in Table 5. A complete list of all transfer functions, with detailed explanations is available as supplementary material (see Text S2).

Initialisation: The critical immune components that are always present, such as the macrophage, DCs, neutrophils, endothelial cells and mast cells are set to 'True'. The activated forms of these immune components, as well as the various cytokines and chemokines that are produced conditionally upon infection are all initialised to 'False'. Bacteria and components of virulence such as ManLAM, PknE, SapM, NuoG and LAM are also initialised to 'True', while some virulence factors such as KatG, SodA and SecA2, which are expressed only after the onset of infection or in a macrophage, are set to 'False'.

Ranking of rules: The asynchronous update method involves assignment of ranks to indicate the sequence in which nodes are updated. The ranking scheme we have used has been based on the logical interdependence of nodes. Where the precedence of nodes was unclear, we assigned the same rank to all those nodes. Within a rank, the order of state updates is random. The ranks assigned are listed in Supplementary material (See Supplementary Text S3). In brief, the critical innate immune system factors are updated first, followed by processes involving the main immune system components such as the cytokines and adaptive immune system. This is followed by fibroblasts and B cell signalling, finally followed by updating of bacterial count and subsequent updating of bacterial virulence factors. To reduce any unintended bias due to randomness in the updating order of nodes of the same rank, simulation was repeated several times ranging from $t = 0$ to $t = t_{max}$., and a distribution of various outcomes was obtained.

Quantitative Model Parameters

A count of bacteria is maintained, beginning with an initial load, B_0. Thresholds of B_{min} and B_{max} are used to demarcate clearance of bacteria and the onset of active disease condition, respectively. The total length of the simulation was t_{max}. Thresholds of P_{min} and P_{max} span the interval, in which bacteria can turn to persistence. Given the poor understanding of the exact mechanisms of persistence and the factors triggering it, we assume that if the bacterial count remains in this interval for longer than a time interval δ_{per}, it leads to persistent infection. It has been reported that a delay of a few weeks is often observed, in the onset of adaptive immunity in infected patients[27]. This factor is encapsulated as δ_{AI}. Delays in the onset of phagocytosis and apoptosis were also incorporated, represented as δ_{PH} and δ_{AP}, respectively. These delays essentially represent the lag between the onset of the process, and their operation to clear bacteria. The efficiency of clearance, for the processes operating to clear bacteria was also considered (η). This is to account for the fact that in a step where bacterial clearance is to happen (*Bacteria* are computed as 'False'), it seldom means that the entire population of bacteria is cleared. Rather, it leads to the clearance of a fraction of the existing bacterial load. This fraction is represented as η. The other important factor related to the bacterium, represented by α, relates to its growth rate. If the growth rate of the bacterium is taken as λ, for exponential growth, we would have

$$B_t = B_0 e^{\lambda t}$$

In our model, updates of bacterial population are effected at the end of each time step, as

$$B_{t+1} = (1 + \alpha)\, B_t$$

Solving these, α can be seen to be e^{λ} - 1. α thus represents the incremental change in the bacterial population, in each time-step. At each time step, where bacteria is not cleared, it grows to

$$B_{t+1} = (1 + \alpha)\, B_t$$

If cleared,

$$B_{t+1} = (1 - \eta)\, B_t$$

Abbreviations

Ag85CX - Antigen 85 Complex (Ag85A, B, C) (Rv3804c, Rv1886c, Rv0129c); AIC - Anti-inflammatory Cytokines; APC - Activated Phagocytic Cells; BpoB - Possible peroxidase (Rv1123c); CR - Complement Receptors; DC-SIGN - DC-specific intracellular adhesion molecule-3 grabbing non-integrin; DC - Dendritic Cell; FAP - Fibronectin Attachment Protein (Rv1860); GM-CSF - Granulocyte Monocyte - Colony Stimulating Factor; IFN - Interferon; IL - Interleukin; KatG - Catalase-Peroxidase-Peroxynitritase; LAM - Lipoarabinomannan; LP 19kDa - 19kDa Lipoprotein (Rv3763); LprG - 24kDa Lipoprotein (Rv1411); ManLAM - Mannose capping of LAM; MDR-TB - Multi-drug Resistant Tuberculosis; MHC – Major histocompatibility Complex; *Mtb* - Mycobacterium tuberculosis; NK Cells - Natural Killer Cells; NuoG - Probable NADH Dehydrogenase I (Rv3151); PIC - Pro-inflammatory Cytokines; PIM - Phosphatidyl-myo-inositol mannoside; PknE - Probable transmembrane serine/threonine protein kinase (Rv1743); PknG - Serine/Threonine-Protein Kinase (Rv0410c); RNI - Reactive Nitrogen Intermediates; ROI - Reactive Oxygen Intermediates; SapM - PI3P phosphatase (Rv3310); SecA2 - Possible preprotein translocase ATPase (Rv1821); SodA - Superoxide Dismutase [Fe] (Rv3846); SodC - Probable Periplasmic Superoxide dismutase [Cu-Zn] (Rv0432); TB -

Tuberculosis ; TcRC - Tc related cytokines; TGF - Transforming Growth Factor; Th1RC - Th1 related cytokine; Th2RC - Th2 related cytokine; TLR - Toll-like Receptors; TNF - Tumour Necrosis Factor; Urease - Urea Amidohydrolase (Rv1848, Rv1849, Rv1850); XDR-TB - Extensively Drug Resistant Tuberculosis

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Figure Legends

Figure 1 – Representation of the host–pathogen network in *Mtb*

The connections between the various nodes are indicated by coloured arrows. Black arrows indicate connections between processes that follow sequentially. Green arrows indicate activation, while red arrows are representative of inhibitory action. Blue arrows indicate signalling for proliferation or recruitment. The various nodes have also been colour-coded: Bacteria is indicated in darker colour, while all immune cells are coloured pink. Processes such as phagocytosis are coloured gray, while molecules such as cathelicidin are all coloured cream. Cells such as NK, Th17 and γ/δ cells, which have only been implicated *in vitro*, have not been considered in the Boolean network, and the boxes have been hashed.

Figure 2 – State map of important nodes and bacterial count during a single simulation run

(A) State map, for the default set of parameters, coloured to indicate node states; blue indicates 'on', while white indicates 'off'. A delay in the onset of the activation of the adaptive immune machinery can be seen. (B) Variation in bacterial load in the system, for default parameter set. Note that *Bacteria* enter persistence in this scenario. (C) Phagocytosis disabled. (D) No delay in adaptive immunity ($\delta_{AI} = 0$). (E) TGF-β disabled.
While (B) indicates the most common outcome of persistence, (C) corresponds to the outcome of active disease due to disabling phagocytosis, (D) shows persistence due to disabling delay in adaptive immune response and (E) illustrates bacterial clearance due to deletion of TGF-β.

Figure 3 – Map of the double-knock out studies resulting in various infection outcomes

Black squares indicate that all the twenty simulation runs for the double knock-out ended in the particular outcome. Shades of gray indicate the fraction of runs that produced the outcome; higher the intensity, higher the fraction, as indicated by the colour bar. White indicates that none of the runs ended in active disease. The results of the single knock-outs (based on 100 simulation

runs) are represented along the diagonal. (A) Active Disease. (B) Persistence. (C) Clearance of bacteria.

Figure 4 – Flowchart describing the modelling and simulation of host—pathogen interactions in this study

Tables

Table 1: Critical events in the immune response

Entry into the host	*Mtb* enters the host through the respiratory route in the form of droplet nuclei and resides in the lungs, where macrophages form the preferred habitat
Binding	*Mtb* can bind to various receptors on the host cell surface, like TLR, CR, MR, Sp-A receptor, to mediate its entry into the host cell
Phagocytosis	*Mtb* sinks in or is engulfed into the host cell, depending on the receptor involved in binding
Receptor-mediated signalling	The signalling cascades triggered in the host cell upon infection, like the TLR signalling, CR signalling and MR signalling, result in the synthesis and release of potent anti-mycobacterial molecules like cytokines and reactive intermediates
Phagolysosome formation	The phagosome containing *Mtb* fuse with the lysosome to form the phagolysosome, where the antigen processing is enabled
Antigen presentation	The processed mycobacterial antigens (protein and non-protein) are loaded onto suitable presentation molecules, like MHC and CD1, and transported to the surface of the cell, which are then recognised by the TCRs, co-stimulatory molecules and adhesion molecules on the T cells
T cell differentiation	Naive T cells upon suitable stimulation can differentiate into helper T cells (Th cells), cytotoxic T cells (Tc), CD1-restricted T cells
Immune cells' influx	Upon infection, various cells of the immune system are recruited to the site of action, mainly through signalling molecules, like chemokines
Fas-FasL mediated killing	Binding of target cell to the cytotoxic T cell triggers the Fas-FasL pathway which induce target cell apoptosis
Perforin-Granulysin - mediated killing	The perforin and granulysin released by cytotoxic T cells are involved in target cell apoptosis
Apoptosis	Apoptosis is an effective mechanism of killing infected host cells, and is brought about by many factors
Granuloma	Various cells of the immune system aggregate at the infection site to form a granuloma (maintained by cytokines, chemokines and adhesion molecules), to contain the infection and prevent the dissemination of the bacteria

Table 2: Important parameters in the Boolean model

Description	Parameter	Default value	Possible Range
Initial bacterial load	B_0	5	0-25
Threshold for active disease	B_{max}	10^5	-
Threshold for clearance	B_{min}	1	-
Persistence interval	P_{min}, P_{max}	[5,100]	
Persistence time	δ_{per}	14	7-21
Delay in onset of adaptive immunity	δ_{AI}	14	7-21
Incremental bacterial growth	α	0.7	0.4-0.8
Bacterial clearance efficiency	η	0.4	0.4-0.8
Delay in phagocytosis	δ_{PH}	7	7-14
Delay in apoptosis	δ_{AP}	7	7-14
Simulation time	t_{max}	105	-

Table 3: Simulations using multiple sets of model parameters

Effect of perturbations of parameters in terms of average disease outcomes. Representative examples are shown in each case. (A) Results for variation of a single parameter at a time. (B) Effect of simultaneous variation of multiple parameters is shown. The three outcomes A, P, and C refer to Active Disease, Persistence and Clearance respectively. The parameters varied are α – Growth rate, η – Clearance efficiency, B_0 – Initial bacterial count, δ_{per} – Interval for bacteria in $[P_{min},P_{max}]$ range to turn persistent, δ_{AI} – Delay in onset of adaptive immunity, P_{min} – Minimum bacterial population that can turn persistent, A – Active Disease, P – Persistence, C – Clearance.

(A)

Description	Parameter varied	Outcome		
		A (%)	P (%)	C (%)
Default		15	43	42
High growth rate	$\alpha = 0.8$	27	18	55
Low growth rate	$\alpha = 0.4$	0	100	0
High initial bacterial count	$B_0 = 100$	62	9	29
	$B_0 = 50$	40	14	36
Low initial bacterial count	$B_0 = 25$	26	20	54
	$B_0 = 1$	3	84	13
High bacterial clearance	$\eta = 0.8$	0	51	49
Low bacterial clearance	$\eta = 0.2$	28	72	0
No delay in phagocytosis/apoptosis	$\delta_{PH} = \delta_{AP} = 0$	0	51	49
Adaptive immunity disabled	$\delta_{AI} > t_{max}$	78	22	0
No delay in adaptive immunity	$\delta_{AI} = 0$	5	95	0

The simulation was repeated 100 times for each scenario and the average percentages of each outcome are reported. Only the parameter varied in each run is indicated. The base set of parameters is as described earlier.

(B)

Variation of α, η and B_0						
Range Varied: α: 0.4 - 0.8; η: 0.4 - 0.8; B_0: 1 -74						
	α	**η**	***B_0***	**A (%)**	**P (%)**	**C (%)**
Outcome Summary	0.41	0.48	1	0	61	39
	0.71	0.42	69	52	15	33
	0.52	0.41	41	10	47	43
	0.54	0.48	4	0	87	13
	0.63	0.74	27	1	21	78
	0.72	0.44	52	34	18	48
	0.73	0.68	55	16	15	69

Variation of α, η, B_0, δ_{per}, δ_{AI}								
Range Varied: α: 0.2 - 0.8; η: 0.4 - 0.8; B_0: 1 -24; δ_{per}: 7 -28; δ_{AI}: 7 -21								
	α	**η**	***B_0***	**δ_{per}**	**δ_{AI}**	**A (%)**	**P (%)**	**C (%)**
Outcome Summary	0.601	0.549	1	7	17	0	100	0
	0.449	0.723	10	11	20	0	88	12
	0.581	0.696	24	21	9	0	14	86
	0.796	0.639	7	28	19	18	7	75

Variation of P_{min} and B_0					
Range Varied: P_{min}: 1- 100; B_0: 1- 100					
	P_{min}	***B_0***	**A (%)**	**P (%)**	**C (%)**
Outcome Summary	1	5	15	47	38
	1	25	30	25	45
	5	5	14	48	38
	5	25	30	15	55
	20	5	11	45	44
	20	25	22	21	57

Each row refers to an average of 100 runs carried out for that condition. The parameters varied and their ranges are indicated. The base set of parameters is as described earlier.

Table 4: Top double knock-outs of bacterial nodes leading to clearance

Clearance (%)	Nodes	Nodes inhibited by respective virulence factors
65	LP_19kDa + SapM	Antigen processing + Phagolysosome formation
60	LAM + NuoG	Phagolysosome formation + Apoptosis
60	LP_19kDa + Ag85CX	Antigen processing + Antigen processing
55	BpoB + LP_19kDa	ROI_RNI + Antigen processing
50	SapM + SodA	Phagolysosome formation + ROI_RNI
50	LprG + Urease	Antigen processing + Antigen processing
50	BpoB + SodC	ROI_RNI + ROI_RNI

Table 5: Important Boolean transfer functions in the model

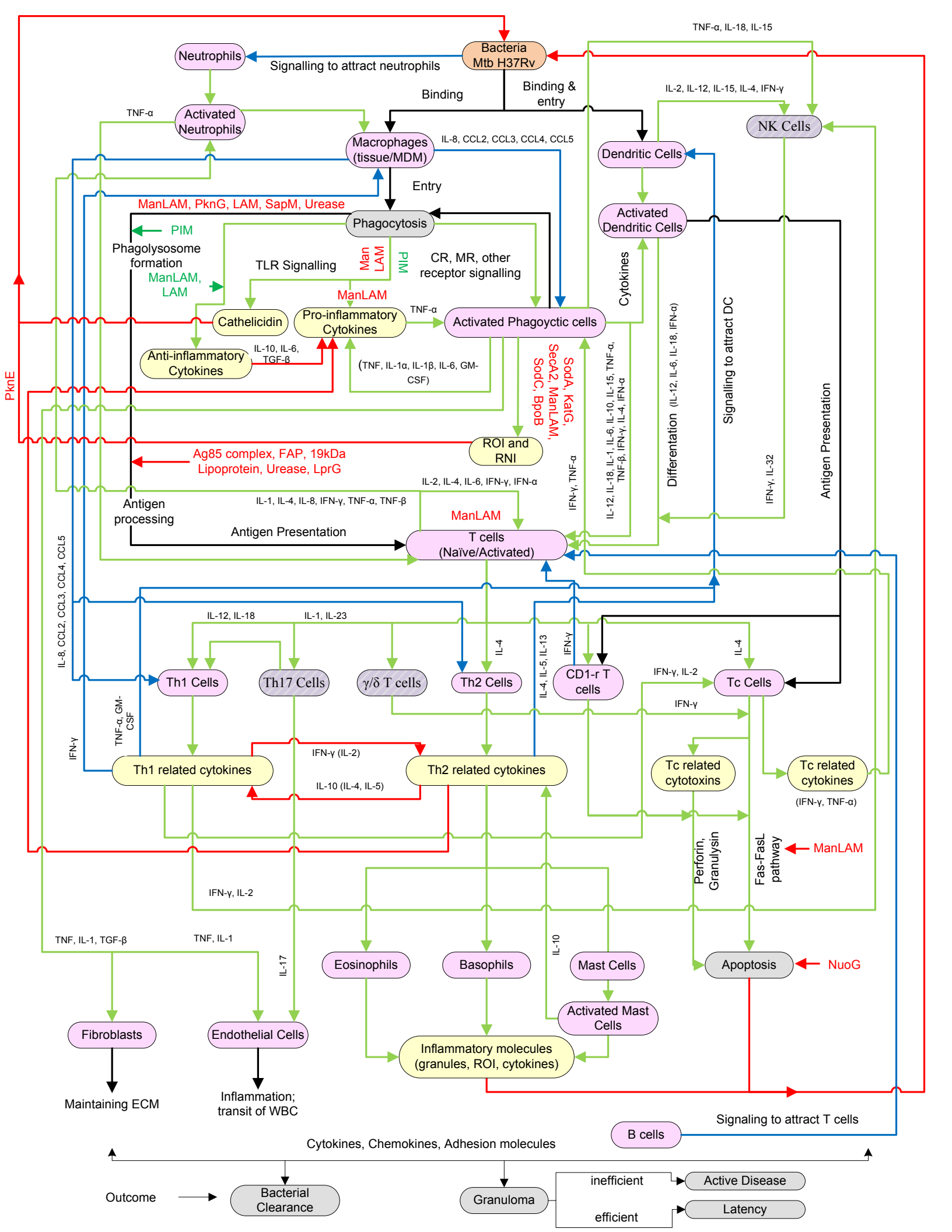
INNATE IMMUNITY	
Antigen presentation	Bacteria and Antigen_processing and Random
TLR signalling	(Macrophage or Activated_phagocytic_cells or Dendritic_cells or Mast_cells) and ((Bacteria or PIM) and not (ManLAM and Random))
Activated Dendritic cells	(Dendritic_cells and Bacteria) or Activated_phagocytic_cells or (Dendritic_cells and Bacteria and (Th1RC or Th2RC))
Phagocytosis	Bacteria and (Macrophage or Activated_phagocytic_cells or Dendritic_cells)
Activated phagocytic cells	Bacteria and ((Phagocytosis and CR_MR_other_signalling) or Pro_inflammatory_cytokines or TcRC or (CD1r_T_cells and IFN_gamma) or (Macrophage and Chemokine_signalling) or (T_cells and (IL_1 or IL_4 or (IFN_gamma and TNF_alpha) or IL_10 or IFN_alpha or TNF_beta)))
ROI	Activated_phagocytic_cells and Pro_inflammatory_cytokines and not (SodA or SodC or BpoB or KatG or SecA2 or ManLAM)
RNI	Activated_phagocytic_cells and Pro_inflammatory_cytokines and not KatG
Phagolysosome formation	(Bacteria or PIM) and Phagocytosis and (not (ManLAM or PknG or LAM or SapM or Urease) or Random)

CYTOKINES	
IFN-γ	(Activated_T_cells or Th1_cells or Tc_cells or (CD1r_T_cells and Antigen_presentation) or Activated_Dendritic_cells or Activated_phagocytic_cells or ((Macrophage and Bacteria) and

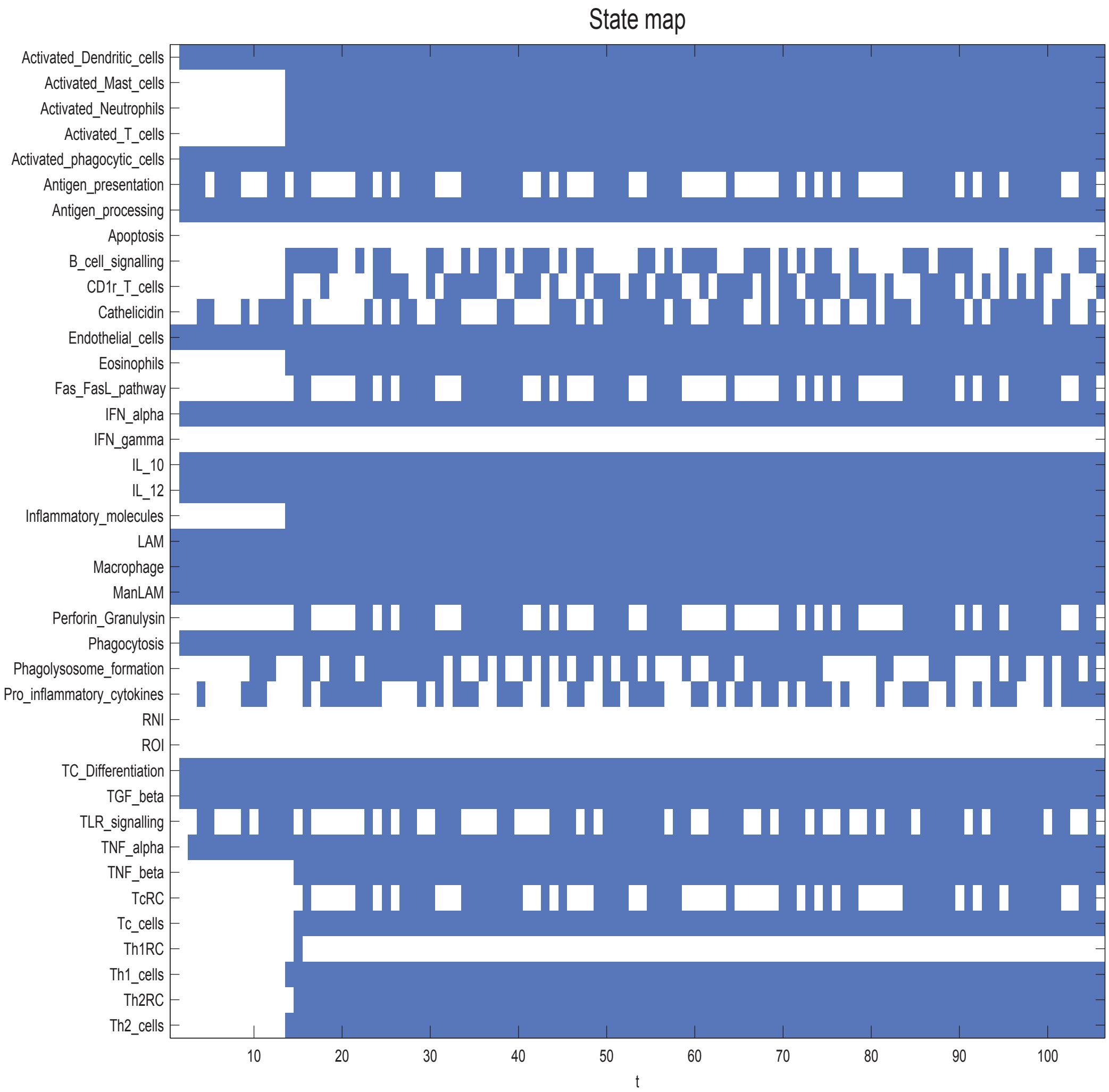
	not ManLAM)) and not (TGF_beta and IL_10)
TNF-α	Activated_Neutrophils or Th1_cells or Tc_cells or Eosinophils or Basophils or Activated_Mast_cells or Activated_T_cells or Activated_phagocytic_cells or (((Macrophage and Bacteria) or LAM or LP_19kDa) and not ManLAM)
Pro-inflammatory cytokines	((Activated_phagocytic_cells and (TNF_alpha or IL_1 or GM_CSF)) or (Phagocytosis and TLR_signalling and not ManLAM)) and (not (Th2RC or IL_6 or IL_10 or TGF_beta) or Random)

BACTERIAL FACTORS	
Bacteria	((Bacteria and (Macrophage or Activated_phagocytic_cells)) and not (ROI or RNI or Cathelicidin or Inflammatory_molecules)) or (Bacteria and not (Phagolysosome_formation or Apoptosis))
ManLAM	Bacteria and ManLAM
Urease	Bacteria and Phagocytosis
SodC	Bacteria and Activated_phagocytic_cells

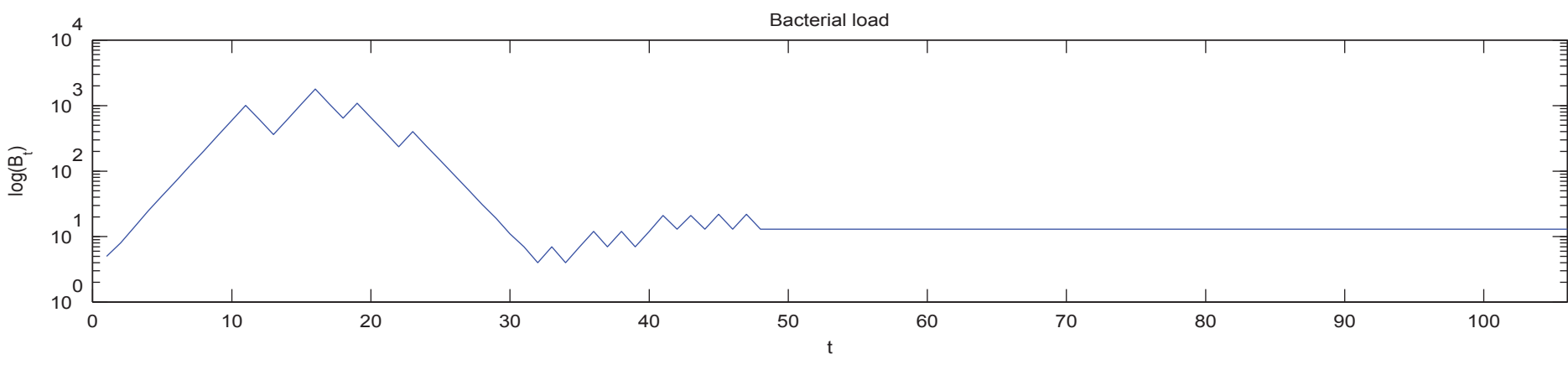
ADAPTIVE IMMUNE RESPONSE	
Activated T cells	Bacteria and ((Antigen_presentation and Phagocytosis) or Activated_phagocytic_cells or (Activated_Dendritic_cells and TC_Differentiation) or (B_cells and B_cell_signalling) or (Neutrophils and TNF_alpha) or (T_cells and (IL_2 or IL_4 or IL_6 or IFN_gamma or IFN_alpha)))
Th1 cells	(Bacteria and (T_cells and (IL_12 or IL_18))) or (Macrophage and Chemokine_signalling)
Th2 cells	(Bacteria and T_cells and IL_4) or (Macrophage and Chemokine_signalling)
Tc cells	Activated_T_cells or Th1RC
CD1-restricted T cells	T_cells and Bacteria and Random
Activated Neutrophils	Neutrophils and Bacteria and (signalling_molecules or (T_cells and (IL_4 or IL_8 or IFN_gamma or ((TNF_alpha or TNF_beta) and IL_1))))
Activated Mast cells	Mast_cells and (Bacteria or (IL_4 or IL_5 or IL_13) or TLR_signalling)
Apoptosis	((Bacteria and Macrophage and TNF_alpha) and not (IL_10 or (RNI and PknE))) or ((Fas_FasL_pathway or Perforin_Granulysin) and not (NuoG or ManLAM))


Neutrophils
Signalling to attract neutrophils
Bacteria Mtb H37Rv
TNF-α, IL-18, IL-15
Binding
Binding & entry
IL-2, IL-12, IL-15, IL-4, IFN-γ
TNF-α
Activated Neutrophils
NK Cells
Macrophages (tissue/MDM)
IL-8, CCL2, CCL3, CCL4, CCL5
Dendritic Cells
Entry
ManLAM, PknG, LAM, SapM, Urease
Activated Dendritic Cells
PIM
Phagocytosis
Phagolysosome formation
Man LAM
PIM
TLR Signalling
CR, MR, other receptor signalling
Cytokines
ManLAM, LAM
ManLAM
Cathelicidin
Pro-inflammatory Cytokines
TNF-α
Activated Phagocytic cells
Differentiation (IL-12, IL-6, IL-18, IFN-α)
Signalling to attract DC
Antigen Presentation
Anti-inflammatory Cytokines
IL-10, IL-6, TGF-β
(TNF, IL-1α, IL-1β, IL-6, GM-CSF)
SodA, KatG, SecA2, ManLAM, SodC, BpoB
IL-12, IL-18, IL-1, IL-6, IL-10, IL-15, TNF-α, TNF-β, IFN-γ, IL-4, IFN-α
PknE
ROI and RNI
Ag85 complex, FAP, 19kDa Lipoprotein, Urease, LprG
IFN-γ, TNF-α
IFN-γ, IL-32
IL-2, IL-4, IL-6, IFN-γ, IFN-α
Antigen processing
IL-1, IL-4, IL-8, IFN-γ, TNF-α, TNF-β
ManLAM
Antigen Presentation
T cells (Naïve/Activated)
IL-8, CCL2, CCL3, CCL4, CCL5
IL-12, IL-18
IL-1, IL-23
IL-4
IL-4, IL-5, IL-13
IFN-γ
IL-4
IFN-γ, IL-2
Th1 Cells
Th17 Cells
γ/δ T cells
Th2 Cells
CD1-r T cells
Tc Cells
IFN-γ
TNF-α, GM-CSF
IFN-γ
IFN-γ (IL-2)
Th1 related cytokines
Th2 related cytokines
Tc related cytotoxins
Tc related cytokines
(IFN-γ, TNF-α)
IL-10 (IL-4, IL-5)
Perforin, Granulysin
Fas-FasL pathway
ManLAM
IFN-γ, IL-2
TNF, IL-1, TGF-β
TNF, IL-1
IL-17
IL-10
Eosinophils
Basophils
Mast Cells
Apoptosis
NuoG
Activated Mast Cells
Fibroblasts
Endothelial Cells
Inflammatory molecules (granules, ROI, cytokines)
Maintaining ECM
Inflammation; transit of WBC
B cells
Signaling to attract T cells
Cytokines, Chemokines, Adhesion molecules
Outcome
Bacterial Clearance
Granuloma
inefficient
efficient
Active Disease
Latency

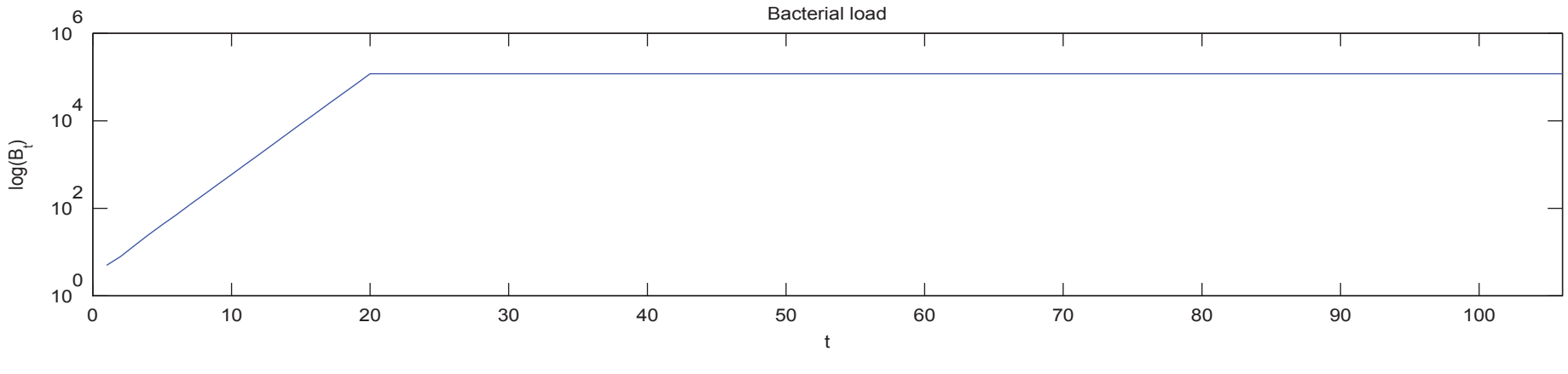
(A)


State map
Activated_Dendritic_cells
Activated_Mast_cells
Activated_Neutrophils
Activated_T_cells
Activated_phagocytic_cells
Antigen_presentation
Antigen_processing
Apoptosis
B_cell_signalling
CD1r_T_cells
Cathelicidin
Endothelial_cells
Eosinophils
Fas_FasL_pathway
IFN_alpha
IFN_gamma
IL_10
IL_12
Inflammatory_molecules
LAM
Macrophage
ManLAM
Perforin_Granulysin
Phagocytosis
Phagolysosome_formation
Pro_inflammatory_cytokines
RNI
ROI
TC_Differentiation
TGF_beta
TLR_signalling
TNF_alpha
TNF_beta
TcRC
Tc_cells
Th1RC
Th1_cells
Th2RC
Th2_cells
10 20 30 40 50 60 70 80 90 100
t

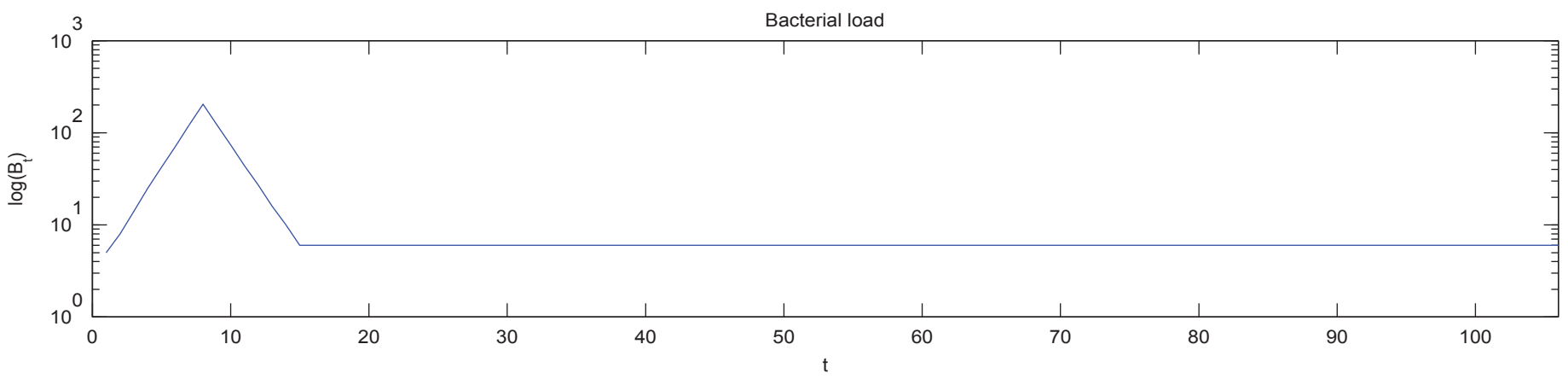
(B)


Bacterial load
log(B_t)
10^4
10^3
10^2
10^1
10^0
0 10 20 30 40 50 60 70 80 90 100
t

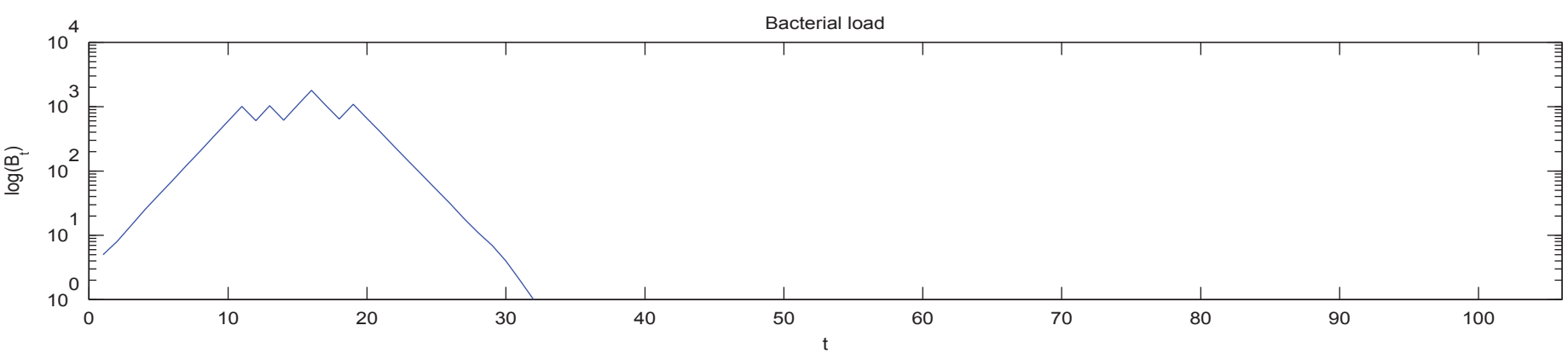
(C)

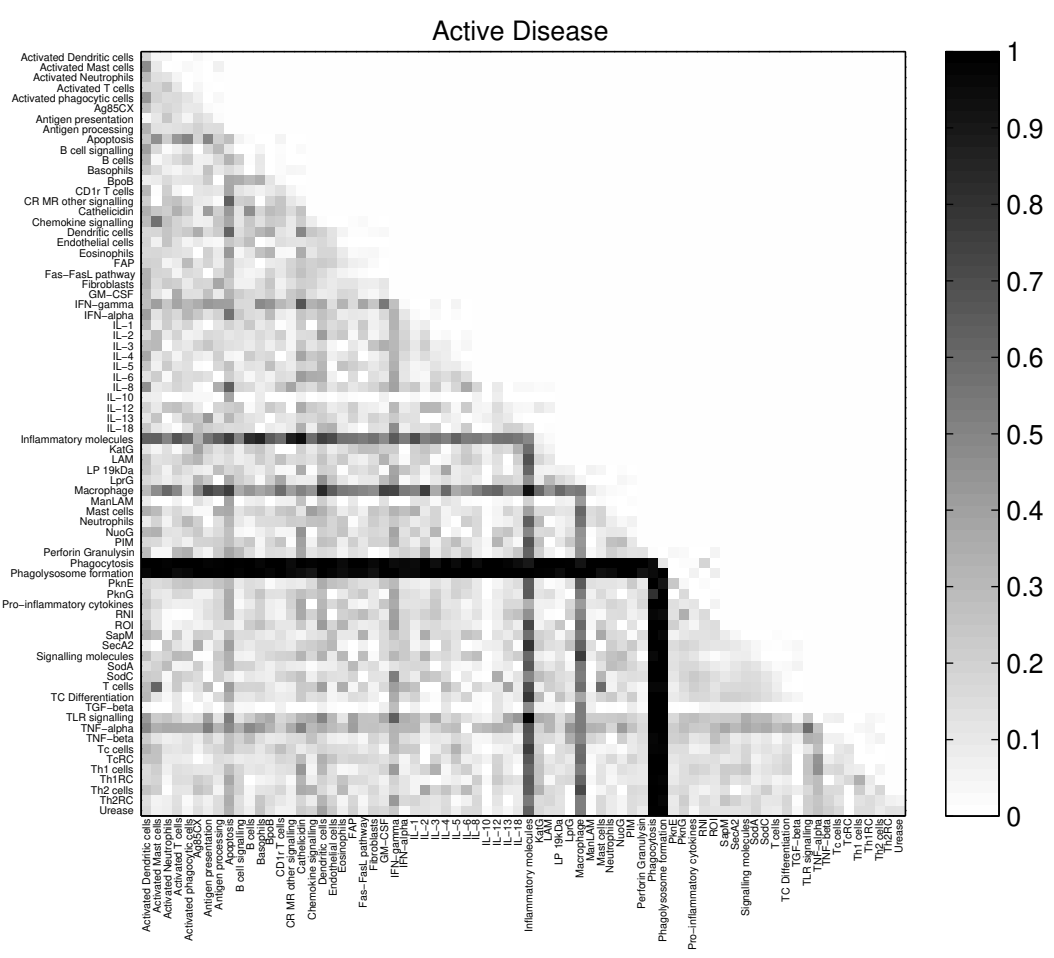

Bacterial load
log(B_t)
10^6
10^4
10^2
10^0
0 10 20 30 40 50 60 70 80 90 100
t

(D)


Bacterial load
log(B_t)
10^3
10^2
10^1
10^0
0 10 20 30 40 50 60 70 80 90 100
t

(E)


Bacterial load
log(B_t)
10^4
10^3
10^2
10^1
10^0
0 10 20 30 40 50 60 70 80 90 100
t


Active Disease
Activated Dendritic cells
Activated Mast cells
Activated Neutrophils
Activated T cells
Activated phagocytic cells
Ag85CX
Antigen presentation
Antigen processing
Apoptosis
B cell signalling
B cells
Basophils
BpoB
CD1r T cells
CR MR other signalling
Cathelicidin
Chemokine signalling
Dendritic cells
Endothelial cells
Eosinophils
FAP
Fas−FasL pathway
Fibroblasts
GM−CSF
IFN−gamma
IFN−alpha
IL−1
IL−2
IL−3
IL−4
IL−5
IL−6
IL−8
IL−10
IL−12
IL−13
IL−18
Inflammatory molecules
KatG
LAM
LP 19kDa
LprG
Macrophage
ManLAM
Mast cells
Neutrophils
NuoG
PIM
Perforin Granulysin
Phagocytosis
Phagolysosome formation
PknE
PknG
Pro−inflammatory cytokines
RNI
ROI
SapM
SecA2
Signalling molecules
SodA
SodC
T cells
TC Differentiation
TGF−beta
TLR signalling
TNF−alpha
TNF−beta
Tc cells
TcRC
Th1 cells
Th1RC
Th2 cells
Th2RC
Urease
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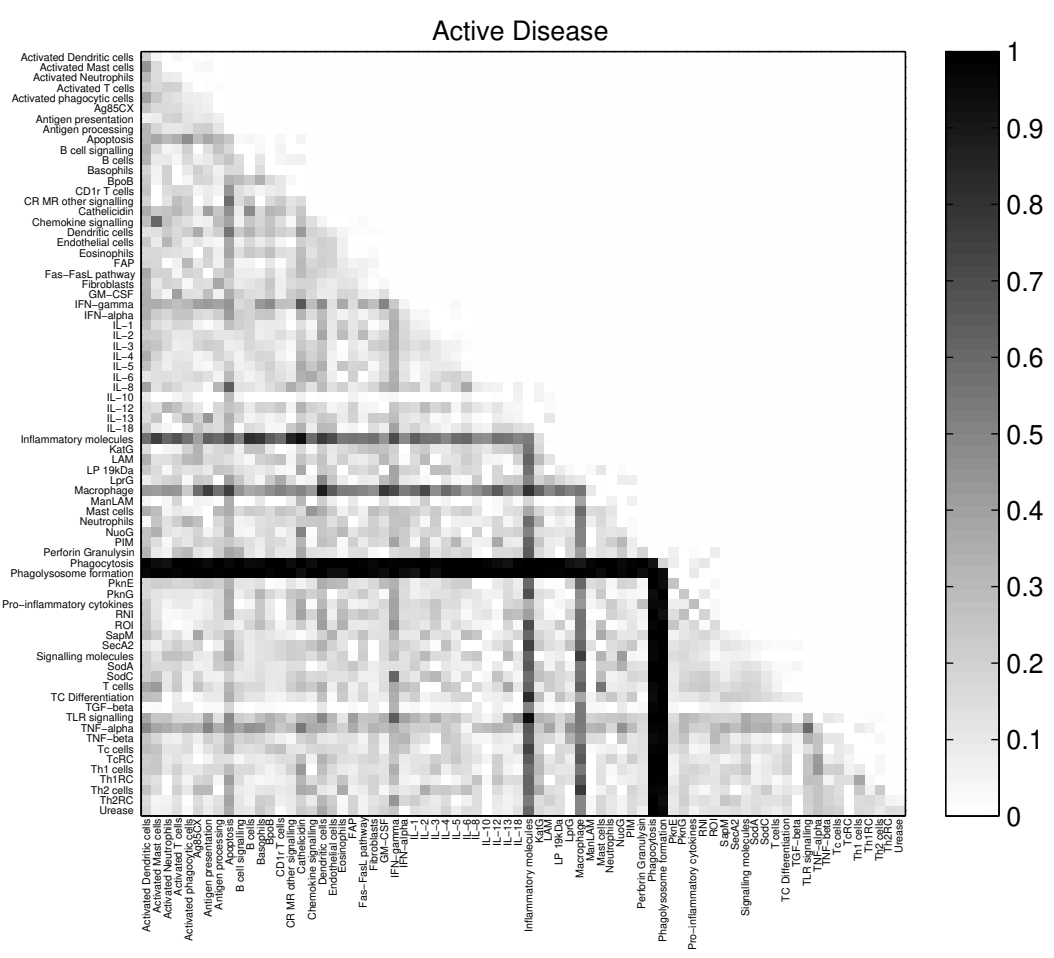
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0


Active Disease
Activated Dendritic cells
Activated Mast cells
Activated Neutrophils
Activated T cells
Activated phagocytic cells
Ag85CX
Antigen presentation
Antigen processing
Apoptosis
B cell signalling
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Macrophage
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Mast cells
Neutrophils
NuoG
PIM
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Phagocytosis
Phagolysosome formation
PknF
PknG
Pro−inflammatory cytokines
RNI
ROI
SapM
SecA2
Signalling molecules
SodA
SodC
T cells
TC Differentiation
TGF−beta
TLR signalling
TNF−alpha
TNF−beta
Tc cells
TcRC
Th1 cells
Th1RC
Th2 cells
Th2RC
Urease
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0.9
0.8
0.7
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0.5
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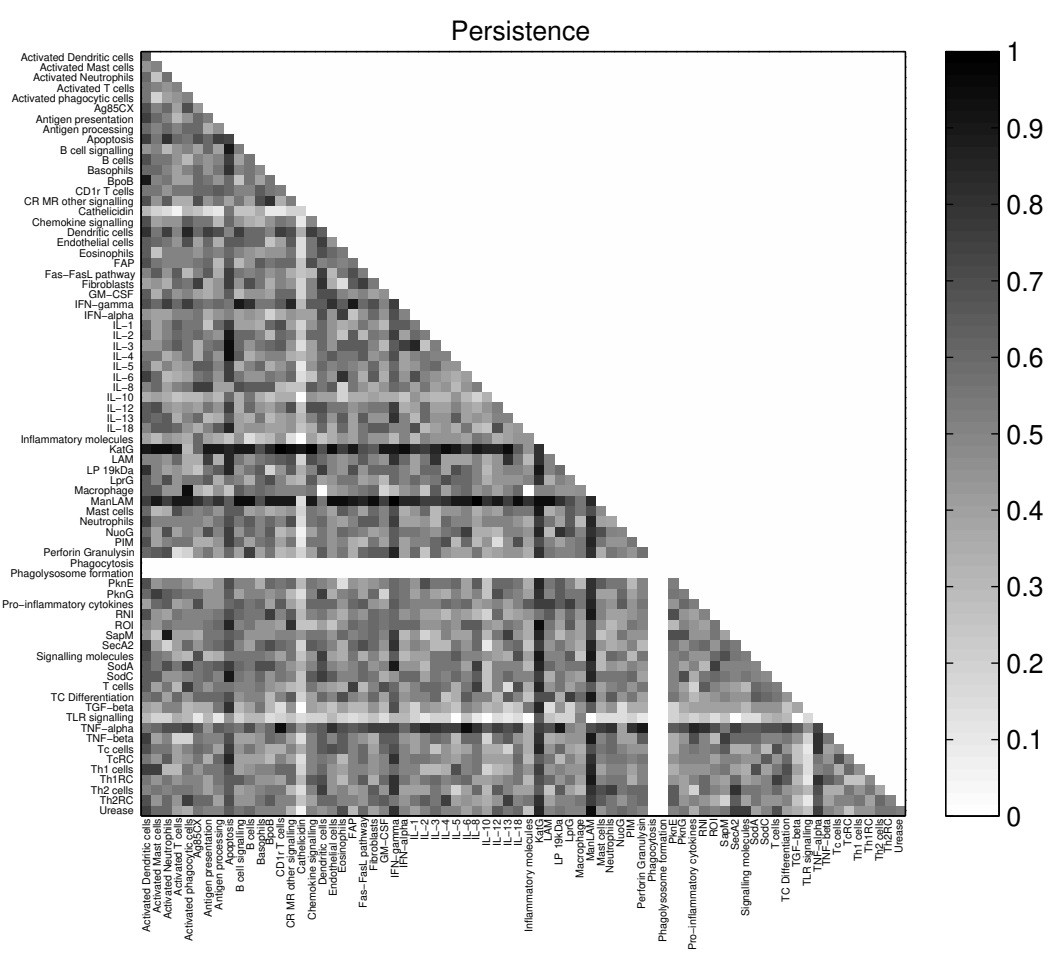
0.3
0.2
0.1
0


Persistence
Activated Dendritic cells
Activated Mast cells
Activated Neutrophils
Activated T cells
Activated phagocytic cells
Ag85CX
Antigen presentation
Antigen processing
Apoptosis
B cell signalling
B cells
Basophils
BpoB
CD1r T cells
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Th2 cells
Th2RC
Urease
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0.8
0.7
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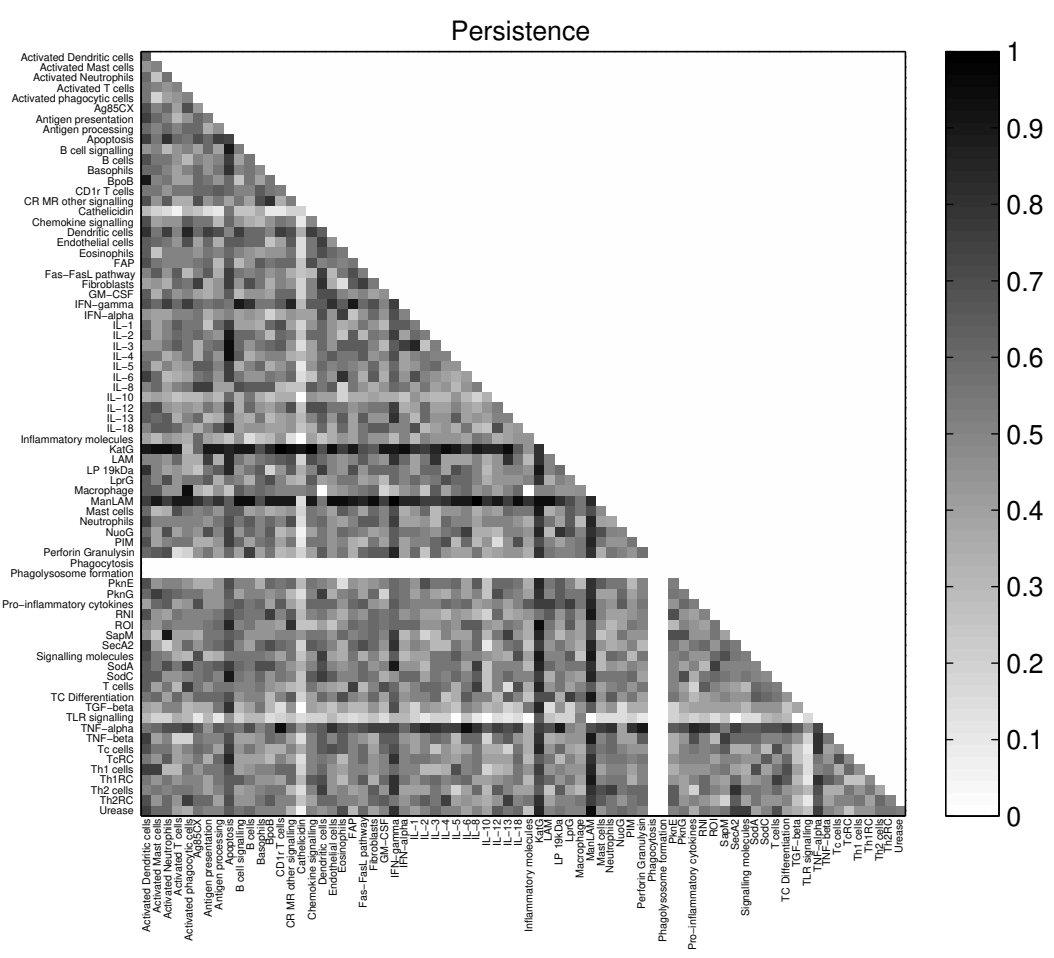
Persistence
Activated Dendritic cells
Activated Mast cells
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Activated phagocytic cells
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Antigen processing
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PknE
PknG
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TGF−beta
TLR signalling
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TcRC
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Th1RC
Th2 cells
Th2RC
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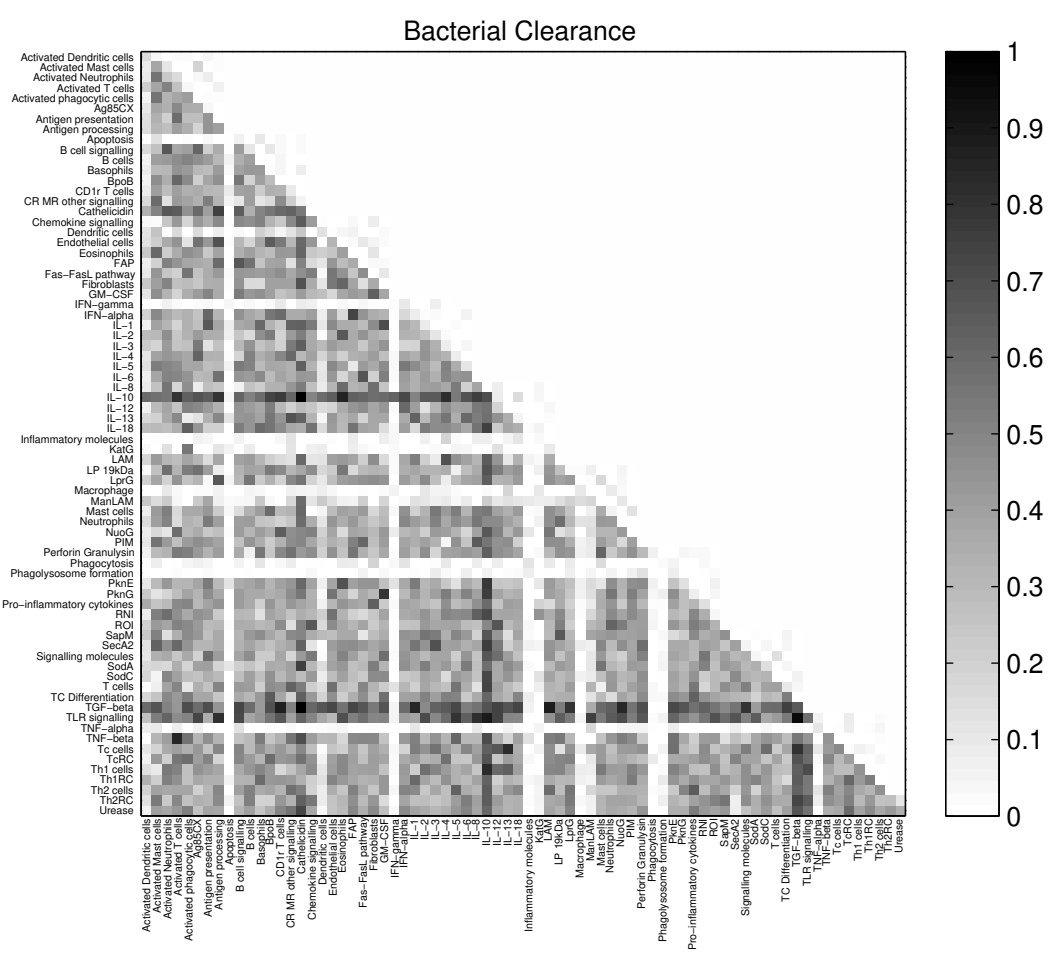
Bacterial Clearance
Activated Dendritic cells
Activated Mast cells
Activated Neutrophils
Activated T cells
Activated phagocytic cells
Ag85CX
Antigen presentation
Antigen processing
Apoptosis
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B cells
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Th1RC
Th2 cells
Th2RC
Urease
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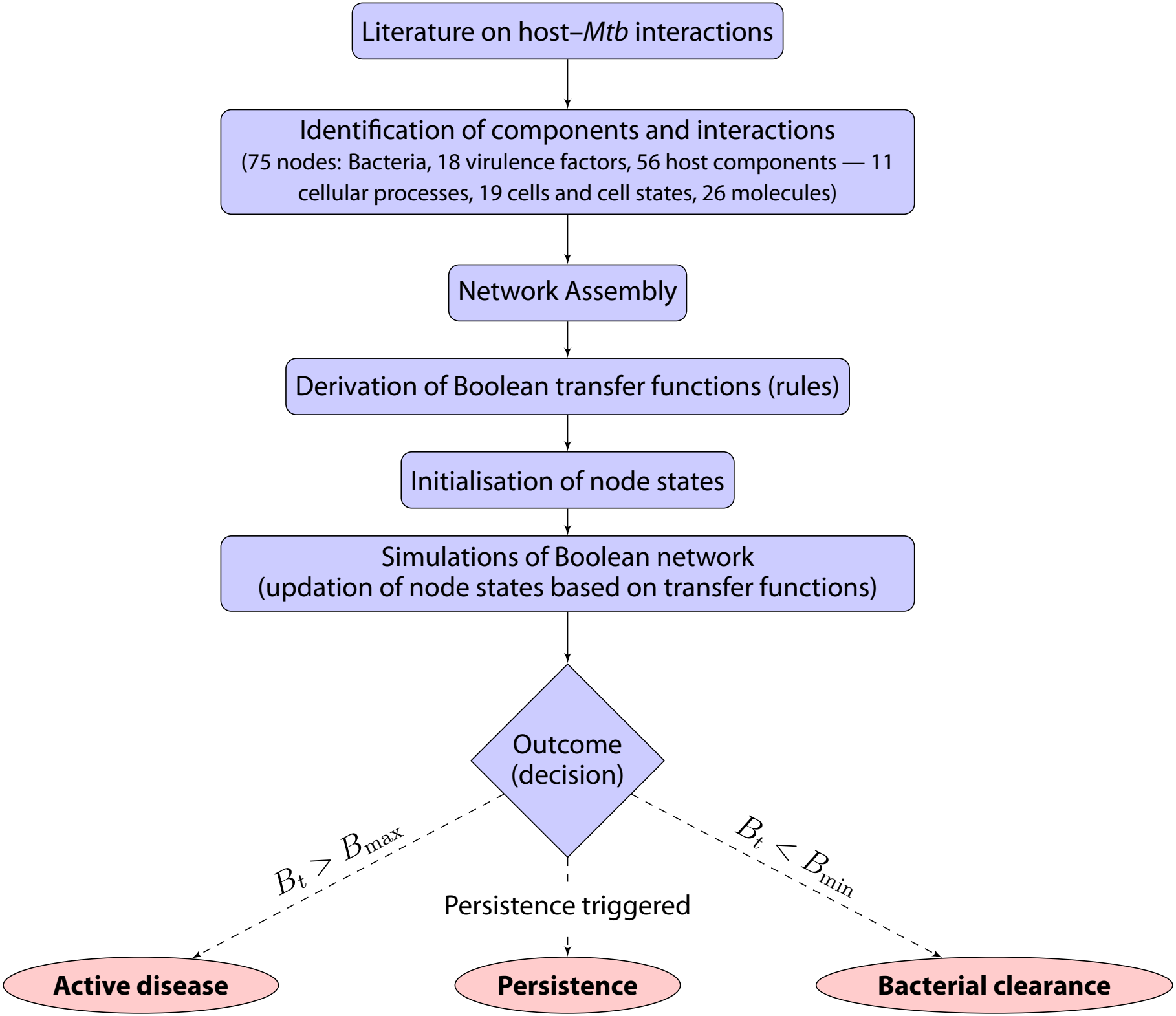
Literature on host–*Mtb* interactions
Identification of components and interactions
(75 nodes: Bacteria, 18 virulence factors, 56 host components — 11 cellular processes, 19 cells and cell states, 26 molecules)
Network Assembly
Derivation of Boolean transfer functions (rules)
Initialisation of node states
Simulations of Boolean network
(updation of node states based on transfer functions)
Outcome
(decision)
$B_t > B_{max}$
Persistence triggered
$B_t < B_{min}$
Active disease
Persistence
Bacterial clearance

#	B0	delta_per	delta_AI	alpha	eta	Active Disease	Persistence
1	4	28	12	0.684	0.591	0	21
2	3	14	14	0.437	0.427	0	120
3	13	14	8	0.299	0.522	0	119
4	23	28	16	0.738	0.769	7	3
5	9	28	13	0.735	0.674	3	11
6	23	7	13	0.707	0.515	0	120
7	8	28	17	0.558	0.574	1	35
8	6	21	8	0.451	0.547	0	32
9	3	7	16	0.534	0.600	0	120
10	15	14	7	0.526	0.463	0	110
11	14	14	15	0.631	0.579	2	67
12	15	14	12	0.424	0.766	0	93
13	20	14	12	0.344	0.436	0	118
14	1	7	17	0.601	0.549	0	120
15	24	28	16	0.222	0.421	0	80
16	3	28	18	0.431	0.729	0	7
17	22	28	15	0.670	0.722	11	4
18	6	28	13	0.406	0.737	0	5
19	20	28	9	0.540	0.665	0	12
20	15	7	9	0.391	0.784	0	120
21	7	28	19	0.796	0.639	22	8
22	16	28	12	0.216	0.751	0	1
23	22	7	12	0.364	0.421	0	120
24	10	7	14	0.501	0.409	0	120
25	21	28	14	0.422	0.482	0	36
26	22	14	12	0.333	0.631	0	115
27	22	14	18	0.432	0.709	0	94
28	22	7	12	0.683	0.749	0	120
29	6	21	10	0.671	0.432	7	84
30	1	14	16	0.593	0.740	0	108
31	3	14	20	0.414	0.772	0	108
32	18	7	9	0.403	0.615	0	120
33	8	28	18	0.232	0.421	0	74
34	6	28	12	0.718	0.755	1	3
35	3	14	14	0.244	0.464	0	118
36	9	14	19	0.611	0.718	1	72
37	8	28	12	0.544	0.523	1	36
38	15	7	15	0.459	0.661	0	120
39	1	28	12	0.492	0.750	0	4
40	3	21	9	0.607	0.458	1	86
41	14	14	19	0.322	0.748	0	112
42	24	14	19	0.421	0.745	0	88
43	19	21	14	0.656	0.699	3	18
44	9	7	10	0.263	0.667	0	120
45	23	21	19	0.583	0.773	2	28
46	23	21	9	0.653	0.557	0	36
47	8	7	16	0.588	0.578	0	120
48	7	28	13	0.601	0.487	3	30
49	11	7	18	0.555	0.719	0	120
50	18	28	7	0.644	0.748	0	1
51	3	7	13	0.478	0.690	0	120
52	19	14	12	0.286	0.452	0	120
53	7	28	7	0.391	0.438	0	36
54	4	21	19	0.709	0.437	30	33
55	23	7	20	0.595	0.478	0	120

56	20	14	10	0.433	0.577	0	108
57	4	14	12	0.750	0.527	7	78
58	18	7	16	0.517	0.658	0	120
59	6	21	11	0.295	0.697	0	8
60	3	21	20	0.616	0.533	1	74
61	6	7	9	0.670	0.727	0	120
62	20	28	16	0.708	0.501	29	26
63	5	7	8	0.474	0.645	0	120
64	22	21	11	0.253	0.743	0	4
65	19	7	18	0.281	0.472	0	120
66	22	14	9	0.543	0.602	0	83
67	1	14	11	0.574	0.685	0	90
68	6	7	17	0.618	0.492	0	120
69	10	14	11	0.449	0.723	0	106
70	10	28	15	0.283	0.632	0	13
71	6	21	17	0.386	0.791	0	44
72	22	14	16	0.627	0.755	1	33
73	3	14	16	0.327	0.706	0	107
74	16	21	11	0.640	0.753	0	12
75	6	14	17	0.434	0.530	0	120
76	3	7	17	0.213	0.689	0	120
77	15	7	16	0.590	0.585	0	120
78	24	21	9	0.581	0.696	0	17
79	18	14	11	0.631	0.625	0	70
80	3	7	11	0.681	0.565	0	120
81	5	14	20	0.609	0.424	7	84
82	8	21	18	0.366	0.600	0	103
83	3	21	16	0.593	0.606	0	91
84	19	28	18	0.659	0.607	21	19
85	13	28	12	0.375	0.603	0	15
86	5	28	18	0.691	0.569	8	34
87	14	21	17	0.247	0.662	0	77
88	6	7	16	0.745	0.688	0	120
89	4	28	7	0.799	0.666	0	10
90	15	28	20	0.451	0.597	0	39
91	7	7	17	0.360	0.438	0	120
92	12	21	11	0.356	0.724	0	18
93	5	7	7	0.404	0.558	0	120
94	3	28	13	0.673	0.549	0	25
95	8	21	15	0.582	0.526	0	74
96	5	14	14	0.648	0.564	1	92
97	21	7	10	0.742	0.606	1	119
98	17	7	18	0.800	0.513	55	65
99	9	28	17	0.786	0.590	17	12
100	16	7	16	0.486	0.411	0	120

Clearance
99
0
1
110
106
0
84
88
0
10
51
27
2
0
40
113
105
115
108
0
90
119
0
0
84
5
26
0
29
12
12
0
46
116
2
47
83
0
116
33
8
32
99
0
90
84
0
87
0
119
0
0
84
57
0

12
35
0
112
45
0
65
0
116
0
37
30
0
14
107
76
86
13
108
0
0
0
103
50
0
29
17
29
80
105
78
43
0
110
81
0
102
0
95
46
27
0
0
91
0

Source Node	Target Node	Edge	Regulation	Explanation	References
Mtb	Neutrophils	Mtb attracts neutrophils to the site	activation	At the site of multiplication of bacilli	(Raja, 2004)
Mtb	Macrophages	TLR, CR, MR and other receptors	activation	Bacilli take up residence in lung pa	(Algood *et al*, 2003; Kaufmann, 2001; Schluger and Rom, 1998)
Mtb	Dendritic Cells	TLR, CR, MR and other receptors	activation	Mtb enters human monocyte-derive	(Basu, 2004; Bhatt and Salgame, 2007; Herrmann and Lagrange, 2005)
Neutrophils	Activated Neutrophils	signaling molecules	activation	Neutrophils become activated upor	(Goldsby RA, 2004)
Activated Neutrophils	Macrophages	TNF-α	activation	Neutrophils are thought to contribut	(Algood *et al*, 2003; Hernandez-Pando R, 2007; Velasco-Velázquez *et al*, 2003)
Macrophages	Phagocytosis	entry via sinking (CR) or formatio	activation	Polysaccharide-mediated binding t	(Ehlers and Daffé, 1998; van Crevel *et al*, 2002)
Phagocytosis	Pro-inflammatory cytokines	TLR1/TLR2, TLR2/TLR6, TLR4,	activation	Phagocytosis of the pathogen is the	(Goldsby RA, 2004)
Phagocytosis	Activated phagocytic cells	CR, MR and other receptor signa	activation	The macrophage mannose recepto	(Goldsby RA, 2004; Le Cabec *et al*, 2005; May and Machesky, 2001)
Phagocytosis	Cathelicidin	TLR1/TLR2	activation	TLR1/2 activation results in two diff	(Liu *et al*, 2007)
Cathelicidin	Mtb	inhibit growth	inhibition (of grow	Antimicrobial peptide, Cathelicidin,	(Liu *et al*, 2007)
Activated phagocytic cells	Pro-inflammatory cytokines	TNF, IL-1α, IL-1β, IL-6, GM-CSF	activation	Macrophages are the first line of de	(Moreno and Rees, 1993; Thakar *et al*, 2007)
Pro-inflammatory cytokines	Activated phagocytic cells	TNF-α	activation	Cytokines and chemokines attract	(Raja, 2004; Schluger *et al*, 1998)
Activated phagocytic cells	ROI and RNI	IFN-γ, TNF-α	activation	Phagocytes, upon activation by IFN	(Andersen, 1997; Raja, 2004)
ROI and RNI	Mtb	H2O2, nitric oxide	inhibition (of grow	Phagosome acidification, lysosoma	(Andersen, 1997; Ehlers *et al*, 1998; Kaufmann, 2001; Raja, 2004; Schluger *et al*, 1998)
Phagocytosis	T cells	TCRs, MHCs, adhesion molecule	activation (T cell	MHC-II molecules present mycoba	(Kaufmann, 2001; van Crevel *et al*, 2002)
T cells	T cells	IL-2, IL-4, IL-6, IFN-γ, IFN-α	activation	The cytokines released by T cells c	Textbook
Activated phagocytic cells	Activated Dendritic Cells	IL-1, TNF	activation	The cytokines released by activate	(DeFranco *et al*, 2007)
Activated phagocytic cells	NK cells	TNF-α, IL-15, IL-18	activation	IL-15 stimulates NK cell proliferatio	Textbook
Dendritic Cells	Activated Dendritic Cells	signaling	activation	Dendritic cells are activated upon s	(Goldsby RA, 2004)
Activated Dendritic Cells	T cells	IL-12, IL-6, IL-18, IFN-α	activation	Dendritic cells, by assimilating sign	(DeFranco *et al*, 2007; Marino and Kirschner, 2004)
T cells	Th1 cells	IL-12, IL-18 (IFN-γ, IL-23, IL-27,	activation	Currently, there are three well-defir	(Bhatt *et al*, 2007; DeFranco *et al*, 2007; Goldsby RA, 2004; Kaufmann, 2001)
T cells	Th2 cells	IL-4 (IL-5, IL-10, TGF-β)	activation	The most potent determinant of po	(Bhatt *et al*, 2007; DeFranco *et al*, 2007; Reiner, 2001)
T cells	γ/δ T cells	cytokines	activation	T cells are differentiated into γ/δ T	(Goldsby RA, 2004)
T cells	Tc cells	IL-4	activation	T cells in the presence of IL-4 can	(DeFranco *et al*, 2007)
Activated Dendritic Cells	Tc cells	TCRs, MHCs, adhesion molecule	activation	Dendritic cells – take up pathogens	(Fortsch *et al*, 2000; Herrmann *et al*, 2005)
Th1 cells	Th1 related cytokines	IFN-γ, TNF- α, IL-2, TNF-β, GM-C	activation	Th1 cells are important in the contr	(Andersen, 1997; Goldsby RA, 2004; Raja, 2004)
Th2 cells	Th2 related cytokines	IL-4, IL-5, IL-10, IL-13, IL-3, GM-C	activation	Th2 cells produce IL-4, IL-5, IL-10	(Goldsby RA, 2004; Raja, 2004; Reiner, 2001)
Th1 related cytokines	Th2 related cytokines	IFN-γ (IL-2)	inhibition	Key cytokines produced by each su	(Goldsby RA, 2004; Thakar *et al*, 2007)
Th2 related cytokines	Th1 related cytokines	IL-10, IL-4 (IL-5)	inhibition	Key cytokines produced by each su	(Goldsby RA, 2004; Thakar *et al*, 2007)
Th2 related cytokines	Pro-inflammatory cytokines	IL-10, IL-4	inhibition	Some Th2 related cytokines can in	(Thakar *et al*, 2007)
Tc cells	Tc related cytokines	IFN-γ, TNF- α, TNF-β	activation	Cytokines (Th1 like) are synthesize	Textbook
Tc cells	Tc related cytotoxins	perforin, granulysin	activation	Tc cells have preformed cytotoxic c	Textbook
Th1 related cytokines	Dendritic Cells	TNF-α, GM-CSF	activation	TNF-α acts as an important mediat	(Lambrecht *et al*, 2001; Thakar *et al*, 2007)
Th2 related cytokines	Dendritic Cells	IL-4, IL-5, IL-13	activation	The Th2 related cytokines attract th	(Thakar *et al*, 2007)
Th1 related cytokines	Macrophages	IFN-γ	activation	Macrophage activation is an import	Textbook
Th1 related cytokines	Tc cells	IFN-γ, IL-2	activation	Cytokines produced by Th1 cells ac	Textbook
γ/δ T cells	Apoptosis	IFN-γ	activation	Human γ/δ T cells are stimulated b	(Algood *et al*, 2003; Kaufmann, 2001)
Tc related cytokines	Activated phagocytic cells	IFN-γ, TNF- α	activation	IFN-γ and TNF- α are very importa	Textbook
Tc cells	Apoptosis	Fas-FasL interactions	activation	Human T cells can induce target ce	(Vergne *et al*, 2004)
Tc related cytotoxins	Apoptosis	Perforin, Granulysin	activation	Upon release from Tc cell vesicles,	(Goldsby RA, 2004)
Apoptosis	Mtb	TNF- α	inhibition (of grow	Macrophage apoptosis represents	(Basu, 2004; Canaday *et al*, 2001; van Crevel *et al*, 2002; Velmurugan *et al*, 2007)
Th2 related cytokines	Eosinophils	IL-5, GM-CSF, IL-3, IL-4, IL-13	activation	At the site of infection, eosinophils	(DeFranco *et al*, 2007; Lambrecht *et al*, 2001)
Th2 related cytokines	Basophils	IL-4, IL-13, IL-5	activation	Th2 cells, identified by the release	(DeFranco *et al*, 2007; Medzhitov, 2007)
Th2 related cytokines	Mast Cells	IL-3, IL-4, IL-10, IL-5, IL-13	activation	Th2 related cytokines act on mast c	(DeFranco *et al*, 2007; Medzhitov, 2007)
Activated Mast Cells	Th2 related cytokines	IL-10	activation	Mast cells are critical for the develo	(Hernandez-Pando R, 2007)
Mast Cells	Activated mast cells	TLR signalling, IL-4, IL-5, IL-13	activation	Mast cells upon stimulation becom	Textbook
Eosinophils	Inflammatory molecules	ROI, IL-1, IL-2, IL-4, IL-5, IL-6, IL-	activation	Following activation, eosinophil effe	(DeFranco *et al*, 2007)
Basophils	Inflammatory molecules	TNF-α, IL-4, ROI, granules	activation	Release potent inflammatory medi	(DeFranco *et al*, 2007)
Activated Mast Cells	Inflammatory molecules	TNF-α, IL-4, IL-5, IL-6, ROI, gran	activation	Release potent inflammatory medi	(DeFranco *et al*, 2007)
Inflammatory molecules	Mtb	ROI, IL-1, IL-2, IL-4, IL-5, IL-6, IL-	inhibition (of grow	Inflammatory molecules released b	(DeFranco *et al*, 2007)
Th1 related cytokines	NK cells	IFN-γ, IL-2	activation	Cytokines produced by activated T	(DeFranco *et al*, 2007)
NK cells	T cells	IFN-γ, IL-32	activation	Before adaptive T cell immunity ha	(van Crevel *et al*, 2002)
Dendritic Cells	NK cells	IL-12, IL-2, IL-15, IL-4, IFN-γ	activation	Microbially activated myeloid DCs p	(Marino *et al*, 2004; Moretta, 2005)
Activated Neutrophils	T cells	TNF-α	activation	The cytokines released by activate	Textbook
T cells	Neutrophils	IL-1, IL-4, IFN-γ, TNF-α, IL-8 (che	activation	Recruits neutrophils; IL-8 activates	(Dinarello, 2000; Raja, 2004)
Activated phagocytic cells	Fibroblasts	TNF, IL-1, TGF-β	activation	The tuberculous granuloma is mad	Textbook
Activated phagocytic cells	Endothelial Cells	TNF, IL-1	activation	(IL-1 and TNF are inducers of endo	(Dinarello, 2000; Raja, 2004)
Macrophages	Th1 cells	CCL2, CCL5, CCL3, CCL4, IL-8	activation	Macrophages, when infected with M	(Algood *et al*, 2003; Basu, 2004; van Crevel *et al*, 2002)
Macrophages	Th2 cells	CCL2, CCL5, CCL3, CCL4, IL-8	activation	Macrophages, when infected with M	(Algood *et al*, 2003; Basu, 2004; van Crevel *et al*, 2002)
Macrophages	Activated phagocytic cells	CCL2, CCL5, CCL3, CCL4, IL-8	activation	Macrophages, when infected with M	(Algood *et al*, 2003; Basu, 2004; van Crevel *et al*, 2002)
B cells	T cells	cytokines and chemokines	activation	B lymphocytes and Ab are not gen	(Algood *et al*, 2003)
Activated phagocytic cells	T cells	IL-1, IL-6, IL-10, IL-12, IL-15, IL-1	activation	The cytokines released by activate	(Goldsby RA, 2004)
T cells	Activated phagocytic cells	IL-1, IL-4, IFN-γ, TNF-α, IL-10, IF	activation	Activated macrophages are the eff	Textbook
Activated phagocytic cells	Phagocytosis	increased phagocytic activity	activation	Increased phagocytic activity, incre	(Goldsby RA, 2004)
T cells	Th17 cells	IL-1, IL-6, IL-23, TGF-β (?)	activation	IL-6 and TGF-β (for mice and not h	(DeFranco *et al*, 2007; Korn *et al*, 2007; Laurence and O'Shea)
Th17 cells	Th1 cells	CXCL9, CXCL10, CXCL11	activation	In mycobacterial infection, an early	(Korn *et al*, 2007)
Th17 cells	Endothelial Cells	IL-17	activation	Th17 cells produce IL-17, which ind	(Medzhitov, 2007)
Phagocytosis	Anti-inflammatory cytokines	production of cytokines	activation	Macrophages release downregulat	(Goldsby RA, 2004)
Anti-inflammatory cytokines	Pro-inflammatory cytokines	TGF-β, IL-10, IL-6	inhibition	Anti-inflammatory cytokines have th	(Flynn, 2004; Opal and DePalo, 2000)
T cells	CD1-r T cells	differentiation	activation	The lipid antigen presenting molecu	(Brigl and Brenner, 2004)
CD1-r T cells	Apoptosis	perforin, granulysin	activation	The lysis of targets by CD8+ subse	(Jullien *et al*, 1997)
CD1-r T cells	Apoptosis	Fas-FasL interactions	activation	CD4- CD8- CD1-restricted T cells l	(Jullien *et al*, 1997)
CD1-r T cells	T cells	IFN-γ	activation	This Th1 cytokine pattern can direc	(Jullien *et al*, 1997)
CD1-r T cells	Activated phagocytic cells	IFN-γ	activation	This Th1 cytokine pattern can direc	(Jullien *et al*, 1997)
Activated Dendritic Cells	CD1-r T cells	TCRs, MHCs, adhesion molecule	activation	The special role of DC in Ag presel	(Fortsch *et al*, 2000)

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Text S1 – Network Assembly Summary

Critical components of immune system

Several components of the immune system are always present, viz. T cells, B cells, mast cells, macrophages, neutrophils, endothelial cells, etc. Macrophages are versatile cells found in practically every tissue in the body, where they participate in an overwhelming array of biological processes. They are the sentinels of the immune system[1]. Lymphocytes (B lymphocytes and T lymphocytes) are produced in the bone marrow, and they circulate in the blood and lymphatic systems, and reside in various lymphoid organs[2]. T cells contribute significantly to the anti-mycobacterial adaptive immune response[3]. Neutrophils, a type of granulocyte, are produced in the bone marrow and released into the peripheral blood and circulate for a few hours before migrating into the tissues[2]

Abstraction of innate immune response

The control of *Mtb* infection is mainly through cell-mediated immunity; hence, the humoral immunity has limited role in controlling the infection [4, 5]. The control of infection requires the co-ordinated interaction of macrophages, DCs and T cells. *Mtb* follows the respiratory route for entering into the host. Once inside the host, they take up residence in the lungs, where they grow and multiply [4]. Entry of *Mtb* activates the host immune response and attracts various components of the immune system to the site of infection. Macrophages and DCs are the major antigen presenting cells involved [6, 7]. Macrophages are the preferred habitats of *Mtb* [8]. Fig. 1 illustrates that macrophages and DCs occupy a prominent role in the model, right from the initiation of infection.

The TLRs on the macrophages recognise the pathogen associated molecular patterns of *Mtb*, which help in binding and entry of the bacilli into the host macrophage. CRs act as a preferred route of entry of Mtb into the macrophages [9]. Entry of the pathogen is either by engulfment (via TLRs and other receptors) or sinking of the bacilli into the cell (via CRs) [10, 11]. The role of TLRs is captured in the TLR signaling boolean transfer function, while the role of CRs is incorporated into the CR MR other signalling transfer function. While the signalling events of TLRs are well understood, the signalling events of CR, MR and other receptors are not well characterised [12, 13]; 34]. Cholesterol acts as the docking site for the binding of Mtb to the surface receptors on the host macrophages [8]. Phagocytosis and subsequent signalling, depending on the type of receptors involved, leads to the production of cytokines and chemokines (IL-8, CCL2, CCL3, CCL5), which are the major signalling molecules in the host immune response. The signalling molecules play an important role in both innate immune response and adaptive immune response. The accumulation of inflammatory cells, along with their interactions, activation and specifc cell-tracking patterns at the site of disease, is mediated by cytokines and chemokines. Due to the redundancy of the chemokine system, the contribution of individual chemokines is difficult to

evaluate [14]. For this reason, the individual chemokines have not been encoded separately in the model.

The cytokines released are either pro-inflammatory (tumour necrosis factor (TNF), IL-1, IL-1, IL-6, GM-CSF (granulocyte monocyte colony stimulating factor)) or anti-inflammatory (transforming growth factor TGF-β, IL-10, IL-6), with the AICs having an inhibitory effect on the production of pro-inflammatory cytokines (PICs) [36, [15]]. A balance between the effects of PICs and AICs is thought to determine the outcome of disease, whether in the short term or long term [16]. Simultaneously, a phagosome is formed inside the macrophages and cytoskeleton rearrangement of the macrophage takes place [17]. These events are captured through nodes such as PICs, the individual cytokines, Phagolysosome formation and their interactions encoded in their respective Boolean transfer functions. Phagosomes acquire the early and late endosome markers and fuse with the lysosome to form the phagolysosome [40]. Once this organelle is formed, the next step is antigen processing, followed by antigen presentation. The order in which the various events are expected to occur are enforced through the ranks for the various nodes, for the asynchronous update during simulations, as discussed in the Methods section. Antigen presentation can be either through the classical MHC presentation pathway, which present the protein antigens to T cells or through the non classical CD1 presentation pathway, which present the non-protein antigens, like lipids to T cells. Non polymorphic MHC-I molecules such as CD1 (-a, -b, -c) molecules, expressed on macrophages and DCs, present mycobacterial lipid antigens to CD1-restricted T cells (which do not react with mycobacterial protein antigens) [14], while mycobacterial peptides, along with the MHC molecules are transported to the surface of the macrophage, where they are recognised by the T cells. Macrophages, upon phagocytosis, can become activated phagocytic cells (APCs), which have increased phagocytic activity, show increase in cytokine production and release the effector molecules, such as ROIs (hydrogen peroxide) and RNIs (nitric oxide) [18]. TLR signalling also leads to the upregulation of the antimicrobial peptide, cathelicidin, which inhibits the growth of Mtb [19].

DCs are the other major antigen presenting cells involved in the control of TB infection; they link the innate and adaptive immunity [20]. DC-SIGN (DC-specifc intracellular adhesion molecule-3 grabbing non-integrin), the major receptor on DCs, and other receptors like TLRs, CRs, MRs, are involved in the binding and entry of *Mtb* into the DCs [21]. DCs mature upon infection with *Mtb*, present the mycobacterial antigens to T cells in the secondary lymphoid organs and not at the site of action [7, 22]. DCs have a special role in antigen presentation due to their ability to present non-protein antigens to T cells via CD1 molecules [20]. The cytokines (TNF-α, IL-12, IL-6, IL-18, IFN- γ, IL-1, IL-10, IL-15, IFN-α, TNF-β) released by the antigen presenting cells play a role in the activation of T cells.

The other cells involved in the innate immunity against Mtb are the neutrophils and natural killer (NK) cells. Neutrophils are the first cells to arrive at the site of multiplication of the bacilli; and they can transfer their microbicidal granules to the infected macrophages [5, 6]. NK cells, upon stimulation by the cytokines released by APCs or DCs, produce cytokines like IFN- γ and IL-32.

The role of NK cells has not been definitively demonstrated in vivo [4], and hence they have not been included in the present implementation of the model. Nevertheless, the network and the model reported here provide a ready framework to incorporate such components when their biological roles get better understood.

Abstraction of adaptive immune response

The onset of adaptive immunity in infected patients occurs several weeks after initial infection [23], a factor accounted for by the parameter δ_{AI}. The innate immune machinery is only the first line of defence against the pathogen. The adaptive immune response is more specific and more potent, involving several complex mechanisms. T cells are the main components of the adaptive immune response. T cells can recognise the antigen presenting cells loaded with the peptides on the MHC molecules, through the T cell receptors and other co-stimulatory molecules (CD80/CD86) and adhesion molecules (intracellular adhesion molecule ICAM-1). T cells can differentiate into CD4+ cells (Th cells) or CD8+ cells (Tc cells) or γ/δ T cells, depending on the cytokines that stimulate the naive T cells. The Th cells can differentiate into Th1 cells, Th2 cells, or the newly characterised Th17 cells. This differentiation also depends on the cytokines involved, viz. IL-12 for the formation of Th1 cells [7], IL-4 for the formation of Th2 cells [24], IL-6 and TGF-β for formation of Th17 cells [25, 26]. These Th cells release cytokines, which have varying effects: the Th1 related cytokines (IFN-γ, TNF- β, IL-2) are pro-inflammatory in nature, while Th2 related cytokines (IL-4, IL-5, IL-10, IL-13) are anti-inflammatory in nature. The cytokines released by each subset negatively regulate the cytokines released by the other subset. The Th2 related cytokines can also inhibit the production of PICs produced by the macrophages [27]. In the model, the adaptive immune system is connected to the innate immune system through several complex processes and various regulatory molecules. For example, IL-12 is a regulatory cytokine, which connects the innate and adaptive host response to mycobacteria, by activating the naive T cells [14, 28]. The chemokines released by the macrophages attract these Th cells to the site of action. The Th1 cells and Th2 cells can attract DCs to the site of infection [27]. The Th2 related cytokines can activate the eosinophils, basophils and mast cells, which release potent inflammatory molecules like ROI and cytokines (IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-13, TNF-α), and express cell adhesion molecules on their surface, thus playing a role in the formation of granuloma to contain the infection [50]. The newly characterised Th17 cells produce the IL-17 family cytokines (IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, IL-17F), which can attract the Th1 cells to the site of infection [26] or stimulate the endothelial cells and other non-haematopoietic cells, to produce chemokines, which recruit neutrophils to the site of infection [29]. γ/δ T cells play a role in the apoptosis of the infected cells, but their role has not been definitively determined in vivo [4], and hence they have not been included in the present implementation of the model.

The Tc cells or the CD8+ cells are involved in the killing of the infected target cells, by releasing the Tc related cytotoxins and Tc related cytokines (IFN-γ, TNF- α). When the Tc cells interact with the MHC-peptide complex on the antigen presenting cells, it activates the Fas-FasL pathway, which leads to the apoptosis of the target cell. The Tc cells also release cytotoxins such as perforins and granulysin, which are involved in the apoptosis of the target cell [30]. Apoptosis is an effective mechanism of killing the infected cells containing Mtb [21].

CD1 restricted T cells can lyse heavily infected macrophages, which can contribute to host defence either by directly killing the bacteria or indirectly by disbursing the pathogen and allowing freshly recruited macrophages to take up and more effectively eliminate the bacteria [6, 31]. The Tc related cytokines are Th1-like cytokines, which can activate phagocytic cells. APCs recruit fibroblasts and activate endothelial cells [6, 16].

Fibroblasts play a role in maintaining the extra-cellular matrix during granuloma formation. Though humoral immunity is not prominent in Mtb infection, B cells do play a role in the granuloma formation. They release cytokines and chemokines, which attract T cells. B cells are found in large numbers in the granuloma [4]. The infected macrophages, multi-nucleated giant cells (fused macrophages), T cells, fibroblasts, other cells of the immune system, cytokines, chemokines and adhesion molecules are the important components of the granuloma, the characteristic feature of Mtb infection, where the bacilli become latent. The granuloma prevents the dissemination of Mtb and thus contains the infection.

Mtb virulence factors

The prolonged co-evolution of Mtb with its human hosts and specifically within macrophages has resulted in the bacterium evolving mechanisms to overcome the challenges posed by the host immune system. It contains various virulence factors, which help in its growth and survival in the hostile host environment. It has more than 200 genes that may influence the degree of virulence [32].

The mycobacterial cell envelope plays a role in protecting the bacteria from host immune response. Arabinogalactan, mycolic acid and other lipids form a hydrophobic barrier and provide resistance to certain drugs [33]. The extremely glycolipid-rich cell of Mtb may contain compounds involved in cholesterol-mediated entry into macrophages [34]. Cholesterol mediates the phagosomal association of TACO (tryptophan-aspartate containing coat), which prevents the maturation of phagosome into phagolysosome [35]. Mtb can specifically block a transportation pathway between trans-golgi network and endocytic pathway, resulting in the absence of proton-ATPase and certain lysosomal proteases on the phagosome [36]. The direct or indirect modification of cellubrevin (a SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) protein, existing on the phagosome) by Mtb prevents the phagolysosome formation [37]. Mtb blocks Ca2+ signalling and phagosome maturation by inhibiting sphingosine kinase [21]. The model contains 18 different bacterial virulence factors that that are important in bacterial defence

against host immune responses. All these virulence factors are indicated in red or green typeface in Fig. 1, depending on whether they promote or inhibit a particular process. ManLAM is an important virulence factor which has various functions such as inhibiting the production of PICs like TNF-α and IFN- γ, arresting the phagosome maturation and scavenging the ROIs [36]. Binding of Phosphatidyl-myo-inositol mannoside (PIM) to TLR2 leads to cellular activation. PIM stimulates phagosome and early endosome fusion by generating a bypass mechanism [7, 38]. The 19kDa lipoprotein is known to inhibit the MHC expression and antigen processing [37]. The FAP (fibronectin attachment protein) and Ag85 complex, which are released into the mycobacterial phagosome, interfere with antigenprocessing [39].

Urease is involved in the inhibition of phagosome-lysosome fusion [40] and alkalisation of MHC class II compartments, thus reducing the maturation of class II dimers (dependent on the removal of invariant chain and peptide loading) [36]. LprG, a 24kDa lipoprotein, inhibits MHC class II antigen processing [41]. The superoxide dismutase, catalase peroxidase and SecA2 of Mtb can deal with the ROI and RNI [17, 42]. ManLAM and LAM can increase the production of the AICs such as TGF-β.

LAM can inhibit the increase in intracellular calcium, destroying the activity of phosphoinositide-3-kinase (PI3K), resulting in a block in the sorting pathway between the trans-Golgi network and phagosomes. ManLAM also interferes with the PI3K signalling [36]. LAM prevents generation of Phosphatidylinositol-3-phosphate (PI3P) and SapM removes the PI3P that escaped the LAM block and thus, they ensure phagosome maturation block [43].

Sigma factors like SigC, SigD, SigF, SigH and SigE are essential for virulence; SigE is also required for the growth and survival of Mtb in the macrophages [44]. MmpL7, Pks10, Msl7, Pks7 and OtsB2 are required for the growth of Mtb [33, 45]. Mtb may avoid apoptosis by regulating the multimeric Death Inducing Signal Complex (DISC) [21]. The trehalose dimycolate or cord factor exerts a number of immuno-modifying effects [33].

PknG inhibits the maturation of mycobacterial phagosome, thus enabling Mtb to survive within the phagosomes [7, 46, 47]. The mycobacterial proteins SodA, SodC, KatG, BpoB play a role in detoxifying the ROI and RNI [42, 48]. NuoG is critical for inhibition of host cell death [49]. PknE is important for the survival of Mtb; it senses nitric oxide stress and prevents apoptosis by interfering with host signaling pathways [50]. These bacterial virulence factors have been captured in our model through 18 nodes and their corresponding transfer functions. Many of these factors, which are always present in the bacterial cell, are initialised to `True', while those which are expressed only during infection are initialised to `False'. During Mtb infection, the balance between the bacterial growth and survival and the magnitude of the host immune response determines the final outcome of the disease.

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Boolean Transfer Functions for the *Mtb*–host interactome

Supplementary Material

Karthik Raman
{karthik@rishi.serc.iisc.ernet.in}

Ashwini G Bhat
{ashwinigbhat@gmail.com}

Nagasuma Chandra*
{nchandra@serc.iisc.ernet.in}

Summary

In this text, we discuss all the Boolean transfer functions that have been employed in our model of the *Mtb*–host interactome. These Boolean transfer functions represent the inter-dependence of the various nodes in the model on one another. We first discuss the components of innate immunity, followed by the cytokines, the components of the adaptive immune response and finally bacteria, and the bacterial virulence factors.

Critical components of immune system Several components of the immune system are always present, viz. T cells, B cells, mast cells, macrophages, neutrophils, endothelial cells, etc. These are not updated at each step, i.e., they remain 'on', as initialised. Instead, there are activated forms of most of these components, which come into play.

Macrophages are versatile cells found in practically every tissue in the body, where they participate in an overwhelming array of biological processes. They are the sentinels of the immune system [1]. Lymphocytes (B lymphocytes and T lymphocytes) are produced in the bone marrow, and they circulate in the blood and lymphatic systems, and reside in various lymphoid organs [2]. T cells contribute significantly to the anti-mycobacterial adaptive immune response [3]. Neutrophils, a type of granulocytes are produced in the bone marrow and released into the peripheral blood and circulate for a few hours before migrating into the tissues [2].

1 Innate Immunity

Antigen presentation *Antigen_presentation* = Bacteria and Antigen_processing and Random*
Antigen presentation follows antigen processing. There is quite a bit of uncertainty in this process, particularly for *Mtb*, which is encapsulated as 'Random' [3–6]. When a 'Random' element is involved in a transfer function, it is taken as on or off during the simulations based on a uniform random distribution. It can be expected that the 'Random' element would evaluate to 'True', roughly 50% of the time, and 'False' otherwise.

TLR signalling *TLR_signalling* = (Macrophage or Activated_phagocytic_cells or Dendritic_cells or Mast_cells) and ((Bacteria or PIM) and not (ManLAM and Random))*
TLRs stimulate host-defence mechanisms [7]. TLR2 and TLR4 have been implicated in the activation of macrophage by mycobacteria [8]. TLR stimulation in macrophages up-regulates phagocytosis of bacteria and apoptotic cells. Mycobacterial components can activate cells through hetero-dimers of TLR1 and TLR2, as well as through TLR4 and TLR6 [9]. All TLRs except TLR3 signal through the MyD88 pathway, leading to activation of the NF-κB gene transcription program and production of pro-inflammatory cytokines [10]. TLR signalling may be actuated by macrophages, APCs, DCs or mast cells in the presence

of bacteria. PIM promotes this process. It is inhibited by ManLAM, although it is possible that at times, ManLAM may not bind to TLR, which is represented by the 'Random' component [5; 7–12].

CR MR other signalling *CR_MR_other_signalling* = (Macrophage or Activated_phagocytic_cells or Dendritic_cells) and (Bacteria and Random)*
Bacteria can bind to the complement receptors, mannose receptors and other receptors, like the DC-SIGN receptor in case of DCs, which triggers subsequent signalling, the mechanisms of which are not well-characterised [10; 13–17].

signalling molecules *signalling_molecules* = Bacteria and Random*
These signalling molecules are produced in the presence of bacteria, through mechanisms that are not very well-defined [4].

Macrophage *Macrophage* = Macrophage or Th1RC*
Macrophages are always present in the host. Th1RC promotes the influx of macrophages at the site of action [2].

Activated DCs *Activated_Dendritic_cells* = (Dendritic_cells and Bacteria) or Activated_phagocytic_cells or (Dendritic_cells and Bacteria and (Th1RC or Th2RC))*
Immature DCs, upon stimulation by bacteria, get activated and mature in the lymph nodes. APCs, Th1RC and Th2RC also aid in activating DCs [16; 18–20].

Phagocytosis *Phagocytosis* = Bacteria and (Macrophage or Activated_phagocytic_cells or Dendritic_cells)*
Phagocytosis is a type of endocytosis, the general term for the uptake of material from its environment by the cell. Phagocytosis involves the expansion of the cell's plasma membrane around the particulate material, which may include whole pathogenic microorganisms, to form large vesicles called phagosomes [2]. Phagocytosis initiates the innate immune response, which in turn orchestrates the adaptive immune response [21]. Phagocytosis is initiated when bacteria bind to the macrophage, APCs or DCs [2; 4; 5; 20; 22].

Activated phagocytic cells *Activated_phagocytic_cells* = Bacteria and ((Phagocytosis and CR_MR_other_signalling) or Pro_inflammatory_cytokines or TcRC or (CD1r_T_cells and IFN_gamma) or (Macrophage and Chemokine_signalling) or (T_cells and (IL_1 or IL_4 or (IFN_gamma and TNF_alpha) or IL_10 or IFN_alpha or TNF_beta)))*
Phagocytosis of bacteria and the subsequent signalling activates the phagocytic cells. Alternatively, PICs, TcRC, cytokines released by T cells, such as IL-1, IL-4, IL-10, IFN-α, TNF-β and IFN-γ and TNF-α in synergy can activate the phagocytic cells. Chemokine signalling stimulates the macrophage to recruit APCs [2; 4; 14; 15; 23; 24]. The cytokines (IFN-γ) released by the CD1-restricted T cells contribute to the cell-mediated immunity by activating phagocytic cells [25].

PICs *Pro_inflammatory_cytokines* = ((Activated_phagocytic_cells and (TNF_alpha or IL_1 or GM_CSF)) or (Phagocytosis and TLR_signalling and not ManLAM)) and (not (Th2RC or IL_6 or IL_10 or TGF_beta) or Random)*
PICs are secreted on phagocytosis followed by TLR signalling, which is inhibited by ManLAM. PICs may also be secreted by APCs on stimulation by cytokines such as TNF-α, IL-1 or GM-CSF. The production of PICs is inhibited by anti-inflammatory cytokines such as Th2RC, IL-6, IL-10 or TGF-β. A balance between the effects of PICs and anti-inflammatory cytokines is thought to determine the outcome of disease [26]. This is accounted for by the 'Random' factor, which permits the activation of PICs, even in the presence of anti-inflammatory cytokines [5; 10; 27].

ROI *ROI* = Activated_phagocytic_cells and Pro_inflammatory_cytokines and not (SodA or SodC or BpoB or KatG or SecA2 or ManLAM)*
ROIs are released by APCs, under the influence of PICs. The detrimental effect of ROIs on the bacteria is mitigated by the various bacterial defence components, such as SodA, SodC, BpoB, KatG, SecA2 and ManLAM [27–29].

RNI *RNI* = Activated_phagocytic_cells and Pro_inflammatory_cytokines and not KatG*
RNIs are released by APCs, under the influence of PICs. The detrimental effect of RNIs on the bacteria is mitigated by KatG [3; 4; 28; 30].

Cathelicidin *Cathelicidin* = Bacteria and Macrophage and TLR_signalling*
TLR2-mediated activation of macrophages upregulated the expression of Vitamin D receptor and Vitamin-D-1-hydroxlyase genes, leading to induction of antimicrobial peptide, cathelicidin, as well as its co-localisation to intracellular vacuoles containing mycobacterial cells. Cathelicidin significantly inhibits the growth of *Mtb* [20; 31]. Infection of macrophage with bacteria, followed by TLR signalling leads to the production of cathelicidin [32].

Phagolysosome formation *Phagolysosome_formation* = (Bacteria or PIM) and Phagocytosis and (not (ManLAM or PknG or LAM or SapM or Urease) or Random)*
Phagosomes containing viable, virulent mycobacteria show the presence of early endosomal markers such as transferrin receptor, MHC class II molecules, and the ganglioside GM1 and exclude late endosomal markers such as the proton ATPase, mannose-6-phosphate receptor and the lysosomal protease cathepsin D, Rab7, LAMP-1 and, LAMP-2 [33; 34]. Virulent mycobacteria maintain the phagolysosome as a habitable environment by preventing normal vacuole acidification through the exclusion of the vesicular proton-ATPase [28]. Some mycobacterial phagosomes can proceed to develop to the more mature stages of the phagolysosome [7]. The initial analyses of Rabs on mycobacterial phagosomes have indicated that *Mtb* phagolysosome biogenesis arrest occurs between the stages controlled by the early endosomal GTPase Rab5 and its late endosomal counterpart Rab7 [35].

Phagocytosis of bacteria leads to the formation of phagosome, which fuses with the lysosome to form the phagolysosome. PIM enhances this process, while the bacterial virulence factors such as ManLAM, PknG, LAM, SapM and urease inhibit this process. It has been stated that phagolysosome formation may take place despite the inhibitory action of the various factors listed above; this is accounted for by the 'Random' component [20; 33; 34; 36–39].

Antigen processing *Antigen_processing* = ((Macrophage and Phagolysosome_formation and not (Ag85CX or FAP or LP_19kDa or Urease or LprG)) and Random) or (Dendritic_cells and Bacteria)*
Phagolysosome formation in the macrophage leads to antigen processing. This is inhibited by various bacterial virulence factors such as Antigen-85 complex, FAP, 19kDa lipoprotein, urease or LprG. In some cases, even after phagolysosome formation, antigen processing may not happen, which is encoded by using 'and Random', in the transfer function [3; 4; 6; 20; 34; 40; 41]. DCs are also involved in antigen processing. Cross priming of T cells by apoptotic vesicles released from infected macrophages for subsequent uptake and presentation by DCs. This detour pathway includes not only a mechanism of antigen distribution, but describes infection-induced apoptosis as a key prerequisite for CD8+ T cell activation due to the nature of phagosomally enclosed pathogens [42].

2 Cytokines

IL-1 *IL_1* = (Activated_T_cells or Eosinophils or Activated_phagocytic_cells or (Macrophage and Bacteria))*

and not (IL_6 or IL_10 or TGF_beta)
IL-1 is inhibited by various cytokines such as IL-6, IL-10 and TGF-β. It is produced by T cells or macrophages in the presence of bacteria, eosinophils and APCs [2; 4; 5; 28; 43].

IL-2 *IL_2* = Activated_T_cells or Th1_cells or Eosinophils or Activated_Dendritic_cells*
IL-2 is produced by T cells and DCs in the presence of bacteria, as well as the Th 1 cells and eosinophils [2; 4].

IL-3 *IL_3* = Th1_cells or Th2_cells*
IL-3 is produced by both Th1 and Th2 cells [2; 28; 44].

IL-4 *IL_4* = (Activated_T_cells or Th2_cells or Eosinophils or Basophils or Activated_Mast_cells) and not IFN_gamma*
IL-4 is inhibited by IFN-γ. It is produced by T cells and mast cells when exposed to bacteria, as well as the other immune cells, such as eosinophils, basophils and Th2 cells [2; 4; 5].

IL-5 *IL_5* = (Th2_cells or Eosinophils or Activated_Mast_cells) and not IFN_gamma*
IL-5 is also inhibited by IFN-γ. It is produced by mast cells in the presence of bacteria, Th2 cells and eosinophils [2; 4].

IL-6 *IL_6* = Activated_phagocytic_cells or Activated_T_cells or Activated_Dendritic_cells or Eosinophils or Activated_Mast_cells or (Macrophage and Bacteria)*
IL-6 is produced by APCs and eosinophils, as well as macrophages, T cells, DCs and mast cells in the presence of bacteria [2; 4; 5; 28; 43; 45].

IL-8 *IL_8* = Macrophage and (Bacteria or LAM) and TLR_signalling*
IL-8 is produced by macrophages upon stimulation by bacteria or LAM, through TLR signalling [4; 5; 43].

IL-10 *IL_10* = (Th2_cells or Activated_T_cells or Activated_phagocytic_cells or (Activated_Dendritic_cells and ManLAM) or (Macrophage and (Bacteria or ManLAM or LAM))) and not IFN_gamma*
IL-10 is inhibited by IFN-γ. It is produced by Th2 cells and activated T cells and phagocytic cells. It is also produced by activated DCs, where it is actuated by ManLAM. It is also produced by macrophages containing bacteria, where it is actuated by both ManLAM and LAM [4; 5; 19; 43].

IL-12 *IL_12* = Activated_T_cells or (Activated_Dendritic_cells and not (ManLAM or LAM)) or Activated_phagocytic_cells or (Macrophage and (Bacteria or LP_19kDa))*
IL-12 is a regulatory cytokine which connects the innate and adaptive host response to mycobacteria, by activating the naïve T cells [5; 46]. IL-12 is produced by activated T cells, activated DCs, APCs as well as macrophages containing bacteria. The 19kDa lipoprotein enhances the production of IL-12 in macrophages containing bacteria. The production of IL-12 by activated DCs is inhibited by both ManLAM and LAM [4; 5; 11; 19; 20].

IL-13 *IL_13* = Th2_cells or Eosinophils*
IL-13 is produced by Th2 cells, as well as eosinophils [47].

IL-18 *IL_18* = Activated_Dendritic_cells or Activated_T_cells or Activated_phagocytic_cells*
IL-18 is produced by activated DCs, activated T cells and APCs [5].

GM-CSF *GM_CSF* = Activated_phagocytic_cells or Activated_T_cells or Th1_cells or Th2_cells*
GM-CSF is produced by APCs, activated T cells, as well as Th1 and Th2 cells [7; 20; 48].

IFN-α *IFN_alpha* = Activated_T_cells or Activated_Dendritic_cells*
IFN-α is produced by activated T cells or activated DCs [2; 19].

IFN-γ *IFN_gamma* = (Activated_T_cells or Th1_cells or Tc_cells or (CD1r_T_cells and Antigen_presentation) or Activated_Dendritic_cells or Activated_phagocytic_cells or ((Macrophage and Bacteria) and not ManLAM)) and not (TGF_beta and IL_10)*
IFN-γ is a central factor in the activation of anti-mycobacterial activities of macrophages, and thus crucial for protection against tuberculosis [7]. Production of IFN-γ is critical in the control of *Mtb* infection, whether produced early in infection as a by-product of the activation of immune defence mechanisms, or by Ag-specific T cells following the induction of specific immunity [8]. IFN-γ is produced by several cells [2; 7; 28; 49], viz. activated T cells, Th1 cells, Tc cells, activated DCs, APCs and macrophages (where it is inhibited by ManLAM [34]). It is inhibited by TGF-β and IL-10 [4; 5]. CD1-restricted T cells produce IFN-γ upon stimulation with mycobacterial antigens [25; 50].

TNF-α *TNF_alpha* = Activated_Neutrophils or Th1_cells or Tc_cells or Eosinophils or Basophils or Activated_Mast_cells or Activated_T_cells or Activated_phagocytic_cells or (((Macrophage and Bacteria) or LAM or LP_19kDa) and not ManLAM)*
TNF-α leads to the recruitment of monocytes and lymphocytes from the blood and the development of the inflammatory process. It also helps in granuloma formation [28]. TNF-α is required for the induction of apoptosis in response to infection with *Mtb* [5]. TNF-α is produced by various cells, viz. neutrophils, Th 1 and Tc cells, eosinophils, basophils and activated mast [45] and T cells. It is also produced by APCs and macrophages [19; 28]. ManLAM inhibits the production of TNF-α by both APCs and macrophages [11], while LAM and the 19kDa lipoprotein [20; 51] promote production of TNF-α by both APCs and macrophages [2; 4; 5; 7; 43; 49].

TNF-β *TNF_beta* = Th1_cells or Activated_T_cells or Tc_cells*
TNF-β is produced by Th1 cells, Tc cells and activated T cells [7].

TGF-β *TGF_beta* = ((Macrophage and Bacteria) or Activated_phagocytic_cells) and (ManLAM or LAM)*
TGF-β is produced by macrophages and APCs, in the presence of either ManLAM or LAM [2; 4; 5].

Chemokine signalling *Chemokine_signalling* = Bacteria and (Macrophage or Neutrophils or Activated_phagocytic_cells)*
Chemokines are small chemo-attractant cytokines that control a wide variety of biological and pathological processes, ranging from immuno-surveillance to inflammation and from viral infection to cancer [52]. Chemokine signalling here represents the complex signalling mechanisms initiated by chemokines, since the contribution of individual chemokines is difficult to evaluate. Various chemokines such as CCL2, CCL3 and CCL5 are produced, contributing to chemokine signalling, by cells such as macrophages, neutrophils and APCs, in the presence of bacteria [4; 5; 28; 53].

3 Adaptive Immunity

It must be noted that the nodes involved in adaptive immunity are all activated only after a delay of δ_{AI}.

T cells *T_cells* = T_cells or (CD1r_T_cells and IFN_gamma)*
IFN-γ released by CD-restricted T cells enhances T cell proliferation [25].

TC Differentiation *TC_Differentiation* = Bacteria and (Activated_Dendritic_cells and (IL_12 or IL_6 or IL_18 or IFN_gamma))*
This is a critical step in the immune response, promoted by DCs in the presence of cytokines such as IL-12, IL-6, IL-18 or IFN-γ [16; 19].

B cell signalling *B_cell_signalling* = B_cells and Bacteria and Random*
The cytokines released through this process play a role in the activation of T cells. The mechanism is again not very well understood [24].

Activated T cells *Activated_T_cells* = Bacteria and ((Antigen_presentation and Phagocytosis) or Activated_phagocytic_cells or (Activated_Dendritic_cells and TC_Differentiation) or (B_cells and B_cell_signalling) or (Neutrophils and TNF_alpha) or (T_cells and (IL_2 or IL_4 or IL_6 or IFN_gamma or IFN_alpha)))*
This is another critical step in the adaptive immune response. Naïve T cells are activated when they recognise an antigen-MHC complex on an appropriate antigen presenting cell or target cell. Activation depends on a signal induced by engagement of TCR complex and a co-stimulatory signal induced by the CD28-B7 interaction [2]. Upon antigen presentation, the naïve T cells get activated. DCs also play a major role in the activation and differentiation of T cells. The various cytokines release by the T cells, viz. IL-2, IL-4, IL-6, IFN-γ and IFN-α activate the T cells in an autocrine fashion. Neutrophils, in the presence of TNF-α, B cells, on signalling, and APCs also play a role in the activation of T cells [20; 54].

Th1 cells *Th1_cells* = (Bacteria and (T_cells and (IL_12 or IL_18))) or (Macrophage and Chemokine_signalling)*
Th1 cells are important in the control of tuberculosis infection as they produce the cytokines IFN-γ and TNF-α [28; 40]. T cells in the presence of bacteria, upon stimulation by IL-12 or IL-18 differentiate into Th1 cells. Macrophages, in the presence of chemokines increase the population of Th1 cells [7; 19; 52; 55].

Th2 cells *Th2_cells* = (Bacteria and T_cells and IL_4) or (Macrophage and Chemokine_signalling)*
Th2 cells are a type of effector T cells, which are usually characterised by less stringent activation requirements, increased expression of cell adhesion molecules and production of soluble effector molecules [2]. T cells in the presence of bacteria, upon stimulation by IL-4 differentiate into Th2 cells. Macrophages, in the presence of chemokines increase the population of Th2 cells [7; 19; 52; 55].

Tc cells *Tc_cells* = Activated_T_cells or Th1RC*
CD8+ T cells (Tc cells or cytotoxic T cells) have been suggested to play a special role in the human immune response to *Mtb* by injecting anti-mycobacterial effector molecules such as granulysin into the target cell [6].

T cells can either differentiate into Th or Tc cells; the differentiation into Tc cells is promoted by Th1RC, as well as DCs, on antigen presentation [16; 56].

CD1-restricted T cells *CD1r_T_cells* = T_cells and Bacteria and Random*
The lipid antigen presenting molecule, CD1 stimulates a repertoire of unique CD1-restricted T cells. These cells appear to go through processes of negative and positive selection in the thymus similar to MHC-restricted T cells. Some CD1-restricted T cells have been found to possess the co-receptors CD4 or CD8, while other CD1-restricted T cells have been found to be double negative for the CD4 and CD8 co-receptors [50].

Th1RC *Th1RC* = (Th1_cells and IL_12) and not Th2RC*
Th1RCs are produced by Th1 cells in the presence of IL-12. Th2RCs inhibit Th1RCs [2; 4; 7; 39].

Th2RC *Th2RC* = ((Th2_cells and IL_4) and not Th1RC) or Activated_Mast_cells*
Th1RCs are produced by Th2 cells in the presence of IL-4. Th1RCs inhibit Th2RCs. Activated mast cells also produce Th2RCs [2; 4].

TcRC *TcRC* = Tc_cells and (Th1RC or (Activated_Dendritic_cells and Antigen_presentation))*
TcRCs are produced by Tc cells on stimulation by Th1RCs. Antigen presentation by activated DCs also induces the production of TcRCs [2].

Eosinophils *Eosinophils* = T_cells and Chemokine_signalling and (IL_3 or GM_CSF)*
Eosinophils are recruited by T cells, on chemokine signalling and the cytokines IL-3 or GM-CSF [16; 57].

Basophils *Basophils* = T_cells and Chemokine_signalling and (IL_3 or IL_5 or GM_CSF)*
Basophils are recruited by T cells, on chemokine signalling and the cytokines IL-3, IL-5 or GM-CSF [55; 57].

Activated Neutrophils *Activated_Neutrophils* = Neutrophils and Bacteria and (signalling_molecules or (T_cells and (IL_4 or IL_8 or IFN_gamma or ((TNF_alpha or TNF_beta) and IL_1))))*
Neutrophils are always present in circulation; they are activated in the presence of bacteria, on stimulation by various signalling molecules, and by cytokines released by the T cell, such as IL-4, IL-8, IFN-γ, or the synergistic action of IL-1 and TNF-α or TNF-β [4; 24; 58].

Activated Mast cells *Activated_Mast_cells* = Mast_cells and (Bacteria or (IL_4 or IL_5 or IL_13) or TLR_signalling)*
Mast cells are inflammatory cells typically found in relatively large numbers in the mucosa of the respiratory, gastrointestinal and urinary tracts and near blood or lymphatic vessels [45]. Bacteria, TLR signalling and cytokines such as IL-4, IL-5 and IL-13, act as stimulants of mast cells [45].

Apoptosis *Apoptosis* = ((Bacteria and Macrophage and TNF_alpha) and not (IL_10 or (RNI and PknE))) or ((Fas_FasL_pathway or Perforin_Granulysin) and not (NuoG or ManLAM))*
Apoptosis is a type of programmed cell death involving a series of biochemical events leading to characteristic cell morphology and death. Apoptotic cell death is characterised by several cellular changes, including loss of membrane symmetry and mitochondrial potential, membrane blebbing, and rapid and profound nuclear damage resulting in chromatin condensation and nuclear fragmentation [59]. Apoptosis is controlled by a complex machinery comprising various cellular components. Macrophage infected with bacteria can undergo apoptosis on stimulation by TNF-α. Apoptosis is inhibited by IL-10 and PknE, which responds to the nitric oxide stress in macrophages. Apoptosis can also happen as a result of the Fas-FasL pathway or the production of Tc cell related cytotoxins, viz. perforin and granulysin. NuoG and ManLAM block the initiation of apoptosis through either of these mechanisms [5; 34; 49; 60–63].

Perforin Granulysin *Perforin_Granulysin* = ((Tc_cells and ((IL_2 and IL_6) or IL_1)) or CD1r_T_cells) and Antigen_presentation*
These are Tc cell related cytotoxins, that are produced by Tc cells on antigen presentation, followed by the stimulation through IL-1 or the cytokines IL-2 and IL-6, in synergy [49]. The lysis of target cells by CD1-restricted T cells depends on the release of granules like perforin and granulysin [25].

Fas-FasL pathway *Fas_FasL_pathway* = ((Tc_cells and (IFN_gamma or IL_2)) or CD1r_T_cells) and Antigen_presentation*
During the Fas-based cytotoxic response, the cytotoxic cell produces FasL upon recognition of the target cell. FasL on the cytotoxic cell cross-links the Fas receptor on the target cell and induces the intrinsic suicide program of the target cell. Each FasL trimer binds three Fas receptor molecules on the surface of the target cell. The complex of Fas receptor, FADD (cytosolic adapter protein) and caspase-8 is called the Death Inducing Signaling Complex (DISC). Self-activation of caspase-8 activates downstream caspases, committing the cell to apoptosis [64].

This pathway is initiated by antigen presentation to Tc cells, followed by the stimulation through either of the cytokines, IFN-γ or IL-2 [49]. CD1-restricted T cells lyse target cells through the Fas-FasL pathway [25].

Inflammatory molecules *Inflammatory_molecules* = (Eosinophils or Basophils or Activated_Mast_cells) and (Chemokine_signalling or (IL_8 or IL_3 or IL_1 or GM_CSF))*
Eosinophils, basophils and activated mast cells, produce on stimulation by chemokine signals or cytokines such as IL-8, IL-3, IL-1 and GM-CSF, various inflammatory molecules, which may include granules, ROIs and cytokines [45].

Endothelial cells *Endothelial_cells* = Endothelial_cells or (Activated_phagocytic_cells and (TNF_alpha and IL_1))*
Endothelial cells are always present; they are activated by the cytokines TNF-α and IL-1, released by APCs [4].

Fibroblasts *Fibroblasts* = Activated_phagocytic_cells and ((TNF_alpha and IL_1) or TGF_beta or Chemokine_signalling)*
Fibroblasts are recruited by APCs, on stimulation by cytokines such as TGF-β or TNF-α and IL-1 in synergy or chemokine signalling [4; 26].

4 Bacterial Virulence Factors

Bacteria *Bacteria* = ((Bacteria and (Macrophage or Activated_phagocytic_cells)) and not (ROI or RNI or Cathelicidin or Inflammatory_molecules)) or (Bacteria and not (Phagolysosome_formation or Apoptosis))*
Bacteria on the right hand side of the transfer function imply the need for bacteria in a previous run, if there are to be bacteria in the current run. Bacteria remain viable in the macrophage or APCs in the absence of phagolysosome formation, apoptosis, or molecules such as ROIs, RNIs, cathelicidin and inflammatory molecules [30; 32; 57; 62; 65; 66].

ManLAM *ManLAM* = Bacteria and ManLAM*
The abundance of ManLAM on the surface of *Mtb* would be a determinant for the outcome — survival versus intracellular killing of mycobacteria [34]. ManLAM significantly interferes with the host defence mechanisms, like phagosome maturation arrest, scavenging free oxygen radicals, and directly inhibiting macrophage response and TNF-α and IFN-γ production in macrophages [11; 20; 34]. It is an important virulence factor of *Mtb*, that plays a crucial role in defending against the various immune mechanisms of the host. It is always present in the pathogen.

LAM *LAM* = Bacteria and LAM*
LAM is a phosphatidylinositol-anchored lipoglycan composed of a mannan core with oligoarabinosyl-containing side chains with diverse biological activities [11].

PknG *PknG* = Bacteria and Phagocytosis*
PknG, a protein kinase affects the intracellular traffic of *Mtb* in macrophages. PknG is released by the bacteria within the macrophage cytosol by an unknown mechanism and can be efficiently inhibited by specific kinase inhibitors. Since the kinase activity of PknG is absolutely required for its activity in blocking lysosomal delivery, PknG presumably functions through the phosphorylation of a host factor, thereby preventing its normal function in phagosome-lysosome fusion [9; 37].

PknE *PknE* = Bacteria and PknE*
PknE, a serine/threonine kinase, is important for the survival of *Mtb*. It prevents apoptosis by interfering with the host signalling pathways [61].

SapM *SapM* = Bacteria and SapM*
SapM, a PI3P phosphatase, is involved in the PI3P depletion at the mycobacterial phagosome, thus blocking the association of FYVE proteins with phagosomes [9; 39].

19kDa Lipoprotein *LP_19kDa* = Bacteria and LP_19kDa*
19kDa lipoprotein, anchored in the cell wall of *Mtb*, has been implicated in various immunological responses [3; 11; 34]. 19kDa lipoprotein interacts with host APC via TLR1 and TLR2, leading to antigen processing and MHC II expression, turning what is normally regarded as a pro-inflammatory pathway into an anti-inflammatory one [12; 56].

Ag85CX *Ag85CX* = Bacteria and Ag85CX*
Ag85 complex (Ag85 A, B, C) demonstrate varying degrees of fibronectin binding and have been suggested to play an important role in macrophage uptake of the mycobacteria [28].

FAP *FAP* = Bacteria and FAP*
The attachment and internalisation of several mycobacterial species to their host cell is dependent on bacterial attachment to fibronectin, and FAP (Rv1860) has been proposed as the bacterial mediator of this process [40].

Urease *Urease* = Bacteria and Phagocytosis*
Mycobacterial urease, an enzyme that hydrolyses urea to carbon dioxide and ammonia, has the potential to be active within the host cell, thereby leading to inadequate acidification of the MHC class II compartment and processing of class II complexes [67]. Ammonia generated by the action of urease may be of importance in alkalinising the micro-environment of the organism and in preventing phagosome-lysosome fusion. Urease may provide a source of nitrogen for biosynthesis [33; 34]. Urease is expressed only after bacteria undergo phagocytosis [67].

PIM *PIM* = Bacteria and PIM*
PIM is present on the cell surface of *Mtb* [68]. Mycobacterial pro-inflammatory PIM induce the fusion of granuloma macrophage into multi-nucleated giant cells [69].

NuoG *NuoG* = Bacteria and NuoG*
nuoG of *Mtb*, which encodes a subunit of the type I NADH dehydrogenase complex, is a critical bacterial gene for inhibition of host cell death [60].

LprG *LprG* = Bacteria and LprG*
LprG, a 24kDa lipoprotein found in the *Mtb* cell wall, is a TLR2 agonist [20; 41].

BpoB *BpoB* = Bacteria and Activated_phagocytic_cells*
BpoB, a peroxidase enzyme, is involved in the neutralisation of reactive radicals [29]. BpoB is expressed only after the bacteria undergo phagocytosis [29].

SodA *SodA* = Bacteria and Activated_phagocytic_cells*
SodA (Fe), a superoxide dismutase enzyme, is among the major extracellular proteins released by *Mtb* during growth. It is exported in an active form via a signal peptide-independent pathway that has not been fully characterised [30].

SodC *SodC* = Bacteria and Activated_phagocytic_cells*
SodC (Cu-Zn), also a superoxide dismutase enzyme, is essential for survival of *Mtb* in macrophages [29; 30]. SodC is expressed only after bacteria undergo phagocytosis [29].

KatG *KatG* = Bacteria and Activated_phagocytic_cells*
KatG, catalase-peroxidase-peroxynitritase enzyme converts hydrogen peroxide to water and oxygen and can also break down peroxynitrate, which is a dangerous reaction product of superoxide and nitric oxide. KatG is expressed only after the bacteria undergo phagocytosis [27].

SecA2 *SecA2* = Bacteria and Activated_phagocytic_cells*
SecA2 protein of *Mtb* is an accessory secretion factor that promotes secretion of a subset of proteins that include superoxide dismutase (SodA) and catalase peroxidase (KatG). SecA2 is expressed only after the bacteria undergo phagocytosis [9; 27].

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1 : Chemokine_signalling , TLR_signalling , CR_MR_other_signalling , signalling_molecules , Macrophage , T_cells , Activated_Dendritic_cells , Phagocytosis
2 : Phagolysosome_formation
3 : Antigen_processing
4 : Antigen_presentation
5 : Activated_phagocytic_cells , Pro_inflammatory_cytokines , Endothelial_cells , ROI , RNI , Cathelicidin , TC_Differentiation , Activated_T_cells , Th1_cells , Th2_cells , Tc_cells , CD1r_T_cells , Th1RC , Th2RC , TcRC , Eosinophils , Basophils , Activated_Neutrophils , Activated_Mast_cells , Apoptosis , Perforin_Granulysin , Fas_FasL_pathway , Inflammatory_molecules , IL_1 , IL_2 , IL_3 , IL_4 , IL_5 , IL_6 , IL_10 , IL_12 , IL_13 , IL_18 , GM_CSF , IFN_alpha , IFN_gamma , TNF_alpha , TNF_beta , TGF_beta , IL_8
6 : B_cell_signalling , Fibroblasts
7 : Bacteria
8 : ManLAM , LAM , PknG , PknE , SapM , LP_19kDa , Ag85CX , FAP , Urease , PIM , NuoG , LprG , BpoB , SodC , SodA , KatG , SecA2