Supplementary Material (ESI) for Molecular BioSystems

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.

References

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