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Discovering differential activation machinery of the Toll-like receptor 4 signaling pathways in MyD88 knockouts

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Abstract **To understand differential time activation of nuclear factor κB (NF-κB) and the temporal features of the downstream pro-inflammatory cytokines' [tumour-necrosis-factor-α (TNF-α) and IP-10] mRNA levels in myeloid differentiation primary-response protein 88 (MyD88) knockouts (KOs), I developed a computational model of the TLR4 pathway. The result suggests that the late phase expression of NF-κB activity observed in MyD88 KOs is possibly due to a number of novel intermediates acting along the MyD88-independent pathway. I also simulate that the TNF-α levels will increase at a longer time in MyD88 KOs, not previously mentioned.**
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Keywords: Toll-like receptor 4 pathway; MyD88 knockouts; Computational modeling; Delayed signaling kinetics; NF-κB activity

1. Introduction

The Toll-like receptor (TLR) signaling pathways form an integral part of the innate immunity. These receptors, with 13 known members, function to recognize conserved pathogen-associated molecular patterns related to microorganisms (such as lipopolysaccharide and double-stranded RNA) and triggers not only microbial clearance, but also induces the production of immunoregulatory chemokines and cytokines. This is performed predominantly through the activation of the transcription factors nuclear factor κB (NF-κB), interferon (IFN) regulatory factor 3 (IRF-3) and AP-1 [1,2]. As these transcription factors' activity is also aberrated in many human pathology (especially NF-κB), the understanding of the differential time activation of this transcription factor is important as it could allow us to devise strategies to specifically modulate its activity to develop therapeutic treatment.

The cytoplasmic region of TLR consists of Toll-interleukin receptor (TIR) domain that mediates the association of adaptor molecules of which the best characterized is the myeloid differentiation primary-response protein 88 (MyD88). The recruitment of MyD88 results in the binding of IRAK-4 to MyD88 at the receptor and induces the association of IL-1R-associated kinase 1 (IRAK-1). IRAK-1 becomes phosphorylated and recruits tumour-necrosis-factor-receptor-associated factor 6 (TRAF-6). Phosphorylated IRAK-1 and TRAF-6 dissociates from the receptor and forms a complex with transforming-growth-factor-β-activated kinase (TAK)1, TAK-binding protein (TAB)1 and TAB2. This results in (i) the activation of AP-1 through mitogen-activated protein (MAP) kinases and, (ii) the activation of NF-κB through IκB kinases (IKK α, β and γ). The AP-1 and NF-κB translocate into the nucleus and induce the expression of many proinflammatory genes. More recently, the stimulation of TLRs on MyD88 deficient mice have revealed the induction of NF-κB activity through the active signal received from TIR-domain-containing adaptor protein inducing IFN-β (TRIF) and IRF-3. This other cascade of signal has been termed the 'MyD88-independent' pathway [1,3].

The TLR signaling field is evolving fairly rapidly over the last few years [4–7]. Timecourse experiments have been performed on these pathways to understand the regulatory roles of different adaptors and signaling molecules [8–12]. Despite this, the detailed mechanism of the signal propagation through both the MyD88-dependent and MyD88-independently pathway is still poorly understood. Also, as we obtain more information regarding these pathways, it becomes increasingly daunting to analyse the data without the aid of appropriate analytical tools. In this report, I discuss a computational model built to represent the TLR4 MyD88-dependent and MyD88-independent pathways. My work is designed not primarily for the quantitative simulation of the TLR4 pathway phenotype, but rather, for the qualitative understanding of the features of the signaling cascades. Specifically, I would like to address why there is a time delay response of NF-κB activity to lipopolysaccharide (LPS) stimulus under MyD88 knock-out conditions [3,10,13].

2. Methods

2.1. Creation of reference model

The modeling strategy consists of the following steps (Fig. 1). Step 1: A basic model is first developed using the pathways obtained from published source, the KEGG database [14]. In this step, mathematical

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Abbreviations: IKK, inhibitor of nuclear factor-κB-kinase; IRAK-1, IL-1R associated kinase 1; IRF-3, interferon (IFN) regulatory factor 3; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAP, mitogen-activated protein; MKK, map kinase kinase; MyD88, myeloid differentiation primary-response protein 88; NF-κB, nuclear factor κB; TAB1, TAK1-binding protein 1; TAK1, transforming-growth-factor-β-activated kinase; TNF-α, tumour-necrosis-factor α; TRAF-6, tumour-necrosis-factor-receptor-associated factor 6; TRAM, TRIF-related adaptor molecule; TRIF, TIR-domain-containing adaptor protein inducing IFN-β

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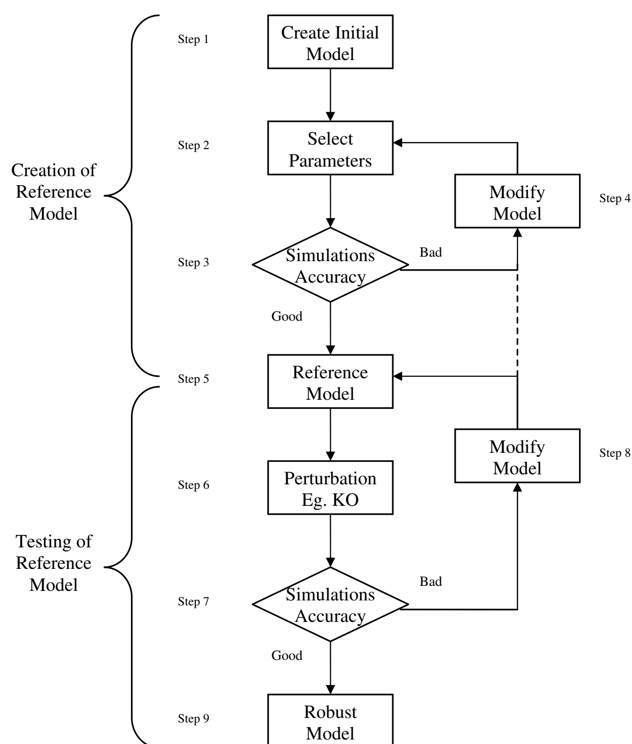

Fig. 1. Flowchart of the modeling procedure.

expressions are also chosen to represent each reaction kinetics (Appendix 1A). Step 2: The parameters of the model (e.g., rate constants) are selected such that the model predictions fits the experimental data obtained from published sources accurately. Step 3: If model simulations accurately predict the phenotype of a particular cell type to a given stimulus [e.g., wildtype (WT) tumour-necrosis-factor α (TNF-α) expression with LPS stimulus], I accept the model and called it the reference model (Step 5). Otherwise, I move to step 4. Step 4: This is an iterative procedure where I modify both the model expressions (e.g., adding an additional reaction or changing the reaction rule) and parameters until I improve the accuracy of the model predictions before accepting the model.

2.2. Testing of reference model (e.g., intracellular perturbation)

Once the reference model has been created, the next step is to 'perturb' the model at any step within the pathway (Step 6) and compare the model predictions with suitable experimental phenotype (Step 7). For example, in the case of MyD88 knock-out (KO) experiments, I 'knocked-out' the equivalent step of the reference model, in silico (i.e., removing the reaction at that step by setting the relevant rate constant zero), and observing the overall phenotype. If the simulation results are similar to the experimental phenotype, then the model is robust (Step 9). That is, the same model predicts the experimental phenotypes accurately under WT and KO conditions for a given stimulus. Otherwise, the model is modified (Step 8 or even move to Step 4) until favorable simulations are observed (Step 9) (Fig. 1).

In this paper, I performed the testing of our reference model to predict three phenotype (NF-κB, TNF-α and IP-10) under two experimental conditions (WT and knockout) simultaneously.

3. Results and discussion

The TLR4 signaling pathway constitutes of what we know today as the MyD88-dependent and MyD88-independent pathways [1,2]. It has been demonstrated in MyD88-deficient mice, the existence of delayed kinetics of NF-κB activity. Though this result has been reproduced on several occasions [3,10,13] and has been implicated as the role played by the MyD88-independent pathway, till today, there is no mechanistic reasoning for the delayed response. Furthermore, it is still a daunting question whether proinflammatory cytokines such as the TNF-α and IL-1, which requires the activation of the NF-κB transcriptional factors, would also become expressed at a later phase. Though there is no evidence to support this as yet from the experimental perspective, I approached this question in a systemic way.

In this report, I have developed a computational model that predicts the concentrations of TLR4 signaling molecules with respect to time. An initial reference model was created to predict the activity of NF-κB in WT cells. Fig. 3A shows the relative expression of NF-κB as a function of time for an active TLR4 state. The model parameters were chosen to simulate the expression of NF-κB as previously observed, Fig. A.3.1 and [3]. Next, I performed in silico KO of the equivalent step in the model that represents the MyD88 KO. The model simulation resulted in the expression of NF-κB that looks similar to the WT phenotype, that is, no noticeable delay in kinetics (Fig. 3B). This result does not match as what has been previously observed [3,10,13].

As mentioned earlier, the reference model was initially developed using the pathway obtained from the KEGG database (Fig. 2A). In my model, when I disrupt the activity of the MyD88-dependent pathway (i.e., MyD88 KO), the NF-κB expression is purely the result of the MyD88-independent pathway (Fig. 2A). One way that I could obtain the desired result is to manipulate this pathway (Step 8, Fig. 1). I next changed the rate constants of all the reactions with lower values to represent slower rate, the results did improve but the peak value decreased significantly (Fig. 3C). Then, I changed the kinetics of each reaction to different types of expressions (e.g., Michaelis–Menten) and result did not improve (data not shown). However, when I chose to increase the number of intermediates in the pathway, in combination with slower rate constants, the model simulations started to improve. After the incorporation of a few unknown intermediates, I was able to obtain the desired delayed time profile of NF-κB (Fig. 3D and Fig. A.3.1). This result suggests that in the MyD88-independent pathways, there may exist, 'unaccounted' intermediates that delays the signaling of NF-κB. (One other possible explanation for the delay in kinetics is to consider large spatial distance that the molecules in the independent pathways needs to travel before reaction could take place. This is quite unlikely as the spatial distance issue cannot be specifically long for independent pathway molecules only.)

A computational model is deemed satisfactory only if it is able to predict more than one experimental phenotype. I then decided to focus on the expression of the chemokine IP-10 and proinflammatory cytokine TNF-α. Using the latter model (with the newly included intermediates, Fig. 2B), I reselected the parameters such that it simulates the WT mRNA expression of IP-10 and TNF-α collectively (Fig. 4A and 5A) (new reference model). Next, I performed the in silico MyD88 KO on this reference model. Fig. 4B shows the simulation of the relative mRNA expression of IP- 10 for MyD88 KO. We can observe the model simulation qualitatively mimics the experimental phenotype (Fig. A.3.2, [10]). We notice that though the WT and MyD88 KO profiles looks similar, the relative expression of IP-10 mRNA is higher for the MyD88 KO case. The computational reasoning is that as the flux through

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