Mathematical Modeling Reveals a Synergistic Mechanism of Toll-like Receptor Pathways

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***Short Abstract* — Toll-like receptor (TLR) pathways play a key role in innate immune response. We observed an intriguing synergistic cytokine response to time-dependent combinatory stimulations of TLR3 and TLR7 pathways in macrophages. To reveal its underlying mechanism, we built and calibrated a mathematical model involving TLR3-TLR7 pathways and potential signaling crosstalk. The model predicted a STAT1-mediated synergistic mechanism: TLR3 activation leads to type 1 interferon production, which in turn, triggers JAK-STAT pathway, inducing gene expression that primes the cell for cytokine production. Experiments confirmed the importance of STAT1 and further implicated a STAT1-mediated incoherent feed-forward loop, modulating the final immune response.**

***Keywords* — Toll-like receptor, signaling crosstalk, STAT1, ODE dynamics, statistical model checking.**

I. Introduction

TOLL-LIKE receptors (TLRs) act as the first line of host defense against a broad range of infectious agents. They recognize specific pathogen-associated molecular patterns (PAMPs) on viruses and bacteria and trigger inflammatory responses to produce cytokines and interferons (INFs). Ten TLR pathways have been identified and characterized in human recently [1]. Recently, systems biology efforts were being made to understand TLR-mediated immune responses [2]. However, an accurate and validate mathematical model of TLR signaling pathways, taking into account of their dynamic behavior has not yet been derived. Early work in this direction only focused on NFκB, which constitutes a core component downstream TLR pathways [3].

Here we developed the first calibrated kinetic model for multiple TLR pathways and their crosstalks. Through a combined experimental and computational study, we found a STAT1-mediated crosstalk mechanism between TLR3 and TLR7 pathways in macrophages, which could lead to a stimulation-time-dependent synergistic production of inflammatory cytokines such as interleukin (IL)-6 and IL-12p70. Our study revealed how macrophages create 'innate immune memory' by coordinating the activation of TLR3 and TLR7 pathways. The insights we gained could contribute to the development of immunotherapy strategies.

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II. Results & Discussion

We constructed an ODE based model for TLR3 and TLR7 signaling cascades as well as four hypothetical crosstalk mechanisms mediated by (i) IRF3-ISRE pathway (ii) JAK-STAT pathway (iii) NIK-TRAF2 pathway and (iv) PI3K-AKT pathway, respectively. The model consists of 91 differential equations and 139 kinetic parameters. An SMC-based method [4] was employed to estimate 112 unknown parameters in the model by fitting the training dataset comprising 192 protein concentration/gene expression data points. An independent test dataset, consisting of 20 data points, was used to further validate the model. As a result, the model reproduced both training and test data.

We carried out property-based global sensitivity analysis [4] to identify crucial species and reactions for cytokine synergy. The results show that immune response is governed by coordination of NFκB and AP-1, which is mainly regulated by p38 pathway. *In silico* results highlighted the superior role of p38 over other MAPKs including ERK and JNK. Empirical verification of the role of p38 is in progress.

Further, we also found that, among the four hypothetical crosstalk mechanisms, JAK-STAT pathway predominately mediates the cytokine synergy we observed. Specifically, type 1 INFs resulted from TLR3 activation trigger JAK-STAT pathway via autocrine signaling. This initial response changes gene expression profile and primes the host cell for a more effective immune response to subsequent TLR7-specific PAMP challenge. A maximal immune response requires a subtle synchronization of the timing of TLR3 and TLR7 signaling. The significant role of STAT1 in regulating cytokine synergy was confirmed by experiments. The experimental and simulation results suggested complex kinetics of STAT1, which implicated that target genes of STAT1 involve both pro- and anti-cytokine production factors, which form an incoherent type 1 feed-forward loop (I1-FFL) modulating final immune responses.

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