Adaptation, Tolerance, and Bistability in Endotoxin Signaling

Robert P. Sheehan,[1] Arshi Arora,[1] Yoram Vodovotz,[2] and James R. Faeder[1]

We present a rule-based model of signaling downstream of Toll-like receptor 4 (TLR4), which initiates first responses to bacterial infection by activating the transcription factor NF-κB through a complex signaling cascade. In addition to the NF-κB activation mechanism, the model includes several feedback mechanisms, including negative and positive loops. A novel feature of the current model is the inclusion of positive feedback arising from secretion of tumor necrosis factor alpha (TNF-α), which is induced by NF-κB activation and enhances activation of NF-κB through the TNF-α receptor (TNFR). The model exhibits responses characteristic of both positive and negative feedback regulation: adaptation in response to low stimulation, bistability with sustained activation above an input threshold, and tolerance to repeated stimulation pulses.

I. Background

THE initial immune response to a bacterial infection involves the activation of TLR4, which recognizes lipopolysaccharide (LPS) [1]. TLR4 activates a signaling cascade that results in the activation of NF-κB, which influences a number of genes key to inflammation. One NF-κB target is the canonical pro-inflammatory cytokine TNF-α [2], which acts as a positive feedback control on NF-κB through the activation of the TNFR signaling cascade [3]. Additionally, NF-κB targets genes for negative regulators IκB and A20. IκB deactivates NF-κB [4], while A20 deactivates upstream elements [5]. This complex regulation gives rise to LPS tolerance, in which consecutive LPS doses yield decreasing inflammatory outcomes [6]. While some relevant factors have been identified, [7,8,9] the molecular mechanisms that give rise to tolerance remain unclear.

We model this complex signaling system using the rule-based modeling approach [10], in which signaling proteins are modeled as structured objects and rules describe their biochemical interactions. The model includes 37 molecule types and 82 reaction rules that produce a reaction network that has 1,750 species and 13,985 reactions, which we simulate using ODEs. Key features of the model include dynamic clearance of the LPS ligand, the dual mechanisms coupling TLR4 to the activation of IKK, negative feedback through IκB and A20, and positive feedback through TNFR.

II. Results

Our model allows for stimulation by either LPS or TNF-α. Signaling through TLR4 or TNFR is processed through numerous signaling intermediates, resulting in the activation of NF-κB. The inflammatory response is assessed by the level of TNF-α produced.

A. Inflammatory response to LPS

Stimulation with low doses of LPS produces TNF-α, indicating a rise in inflammation, but clearance of LPS is accompanied by a decline in TNF-α production to zero, illustrating adaptation in the system as it returns to baseline.

Stimulation with doses LPS above a threshold trigger a high level of TNF-α production that is not reversed even when LPS is removed, demonstrating that the system is bistable and capable of maintaining a sustained inflammatory state.

B. Endotoxin tolerance

Stimulation with consecutive doses of LPS results in a second TNF-α response that is lower than the initial response. Negative regulators produced during initial NF-κB activation cause a weaker secondary activation.

This tolerance allows the system to handle higher doses of LPS while remaining in a healthy state. Doses that would otherwise trigger a sustained state of inflammation result in levels of TNF-α that return to zero when given within 15 hours of an initial, tolerizing dose.

III. Conclusion

Our model shows in the presence of positive feedback NF-kB activation can exhibit bistability, with low doses resulting in a transient response and large doses producing sustained inflammation. Endotoxin tolerance results in a decreased inflammatory response following a second LPS dose. We are currently performing quantitative experiments to test these predictions as well as further calibrate model parameters and mechanisms.

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[1]Department of Computational and Systems Biology, University of Pittsburgh, E-mail: {rps32, faeder}@pitt.edu.

[2]Department of Surgery, University of Pittsburgh. E-mail: vodovotz@pitt.edu.

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