304g Identification of Important Signaling Proteins and Stimulants for the Production of Cytokines in Raw 264.7 Macrophages

Sylvain Pradervand, Mano R. Maurya, and Shankar Subramaniam

Abstract:

A main component of the inflammatory response is the production of immuno-regulatory cytokines and chemokines by macrophages. Upon macrophage activation, cytokine synthesis and release is triggered (Ma et al., 2003). This process is mainly regulated transcriptionally although posttranscriptional and translational mechanisms may also take place. Several pathways transmit the signals that trigger cytokines production. Among these signaling pathways, nuclear factor kappa B (NF-κB) pathway plays an essential role in activating genes encoding cytokines. Other signaling pathways such as mitogen-activated protein kinases (MAPKs) or signal transducer and activator of transcription (STATs) are also necessary. All these pathways are not distinct entities, but are part of a general network whose different signals are produced by multiple stimuli and generate different cytokine responses. Characterization of these pathways in great detail is difficult. However, using specific markers of signaling pathways, one can develop a coarse-grained model that will allow elucidating (1) what are the common and different signaling modules required for the production of different cytokines (2) what will be the quantitative estimate of the cytokine responses to given signaling pathways activations.

Towards that goal, a novel analysis strategy is developed that integrates statistical analysis approaches (ANOVA, correlation analysis and hierarchical clustering) with principal component regression (PCR) and a combined sequential- and combinatorial-test-based model-reduction. PCR and related modeling techniques are an appropriate choice for analyzing biological data that are highly variable in nature (Janes et al., 2004). They do not require detailed mechanistic knowledge. Rather, following a more inductive approach, they aid discovery of patterns in the data. The PCR based model predicts cytokine release based on the levels of ligands and phosphoproteins (predictors). Significant predictors are identified by comparing the predictor coefficients in the PCR model with the standard deviation in the coefficients corresponding to a PCR model with random outputs.

Using the systematic profiling of signaling responses and cytokines release in RAW 264.7 macrophages made available by the Alliance for Cellular Signaling (AfCS), network maps are developed that identify important phosphoproteins and ligands to model the response of seven different cytokines based upon the measurement of 22 phosphoproteins for single- and double-ligand stimulation from a total of 22 ligands.

In our study, we first identify the significant phosphoproteins associated with cytokine release and then, to reduce the number of false-positives, compute a minimal set of phosphoproteins able to predict cytokines release. Since the 22 phosphoproteins that are measured do not cover the entire spectrum of phosphoproteins responsible for cytokine production, our model includes an alternative branch going directly from the ligands to the response. This branch accounts for ligand-specific pathways that are not measured (e.g., STAT6 for IL-4). A ligand is included as a predictor in the model if it substantially improves the prediction of cytokine release. Our models predict extracellular cytokine concentrations and identify a total of 10 signaling components involved in cytokine production. We show that this data-driven approach is able to identify most of the known signaling pathways involved in cytokine release and predict potentially important novel signaling components. Both false positive and false negative rates are about 10%.

Key words: cytokine, macrophage, hierarchical clustering, ANOVA, principal component regression, model-reduction, signaling, statistical analysis.

References:

Janes, K. A., J. R. Kelly, S. Gaudet, J. G. Albeck, P. K. Sorger and D. A. Lauffenburger, "Cue-signal-response analysis of TNF-induced apoptosis by partial least squares regression of dynamic multivariate data", *J Comput Biol*, **11**(4), 544-61, 2004.

Ma, J., T. Chen, J. Mandelin, A. Ceponis, N. E. Miller, M. Hukkanen, G. F. Ma and Y. T. Konttinen, "Regulation of macrophage activation", *Cell Mol Life Sci*, **60**(11), 2334-46, 2003.

[1] Corresponding author: E-mail: shankar@sdsc.edu, Phone: (858) 822 0986, Fax: (858) 822 3752.