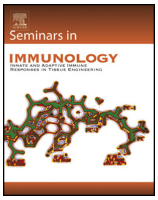
Seminars in Immunology 25 (2013) 201–208

Contents lists available at ScienceDirect

Seminars in Immunology

journal homepage: www.elsevier.com/locate/ysmim



Review

Recent progress using systems biology approaches to better understand molecular mechanisms of immunity

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ARTICLE INFO

Keywords:
Global analysis
High-throughput
Computational modeling
Signaling networks
Transcriptional networks
Single-cell analysis
Heterogeneity

ABSTRACT

The immune system is composed of multiple dynamic molecular and cellular networks, the complexity of which has been revealed by decades of exacting reductionist research. However, understanding of the immune system sufficient to anticipate its response to novel perturbations requires a more integrative or systems approach to immunology. While methods for unbiased high-throughput data acquisition and computational integration of the resulting datasets are still relatively new, they have begun to substantially enhance our understanding of immunological phenomena. Such approaches have expanded our view of interconnected signaling and transcriptional networks and have highlighted the function of non-linear processes such as spatial regulation and feedback loops. In addition, advances in single cell measurement technology have demonstrated potential sources and functions of response heterogeneity in system behavior. The success of the studies reviewed here often depended upon integration of one or more systems biology approaches with more traditional methods. We hope these examples will inspire a broader range of immunologists to probe questions in a quantitative and integrated manner, advancing collective efforts to understand the immune "system".

1. Introduction

The enormous complexity of the vertebrate immune system, both with respect to its many components and the elaborate interconnections that control its operation, has created a tension in the field of immunology. On the one hand, the success of traditional experimental approaches in identifying cellular and molecular players, ascribing them major functions, and developing a rich set of 'working models' describing major intercellular and intracellular activities, have provided strong impetus to continue using the same tools and techniques in dissecting the system. On the other hand, some have come to appreciate that we are far from a 'predictive' understanding of immune function, and that the very complexity that traditional investigation has uncovered speaks to a need for additional methodologies to organize, expand, and fully utilize the mass of information in modern data sets, so that important but non-canonical aspects of immune operation can be discerned.

The result is the development of two schools of thought about the future. One is wedded to the experimental methods and fine-grained studies that have succeeded in the field for decades; the other seeks to attack the complexity problem using new computational and global-scale methods, taking a systems biology approach to immunology. We propose that this is a false dichotomy. The promise of "systems immunology" will not be realized until immunologists see systems biology not as a distinct field of study, but rather as a complementary means of approaching immunological questions from another angle. Just as reductionist approaches fail to elucidate the complex workings of integrated systems, systems biology methods have limited value without coordination with traditional scientific methods. Systems biology approaches can range from using high-throughput techniques to measure large numbers of changes in the system, to using quantitative methods to assess the relationship between a few interconnected components. Importantly, it is not the methods themselves, such as proteomics and computational modeling, which should be considered systems biology, but rather the use of these tools to understand how integration of multiple non-linear processes result in emergent biological behaviors of a system.

Signals downstream of varying combinations of immune receptors are integrated to induce activation and differentiation of cells into successors with unique phenotypes and functions. In the context of complex intercellular interactions, quantitative differences in ligand stimulation, such as density and duration, have significant biological consequences, with stimulation of select receptor combinations yielding results not predicted by responses downstream of

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1044-5323/$ – see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.smim.2012.11.002

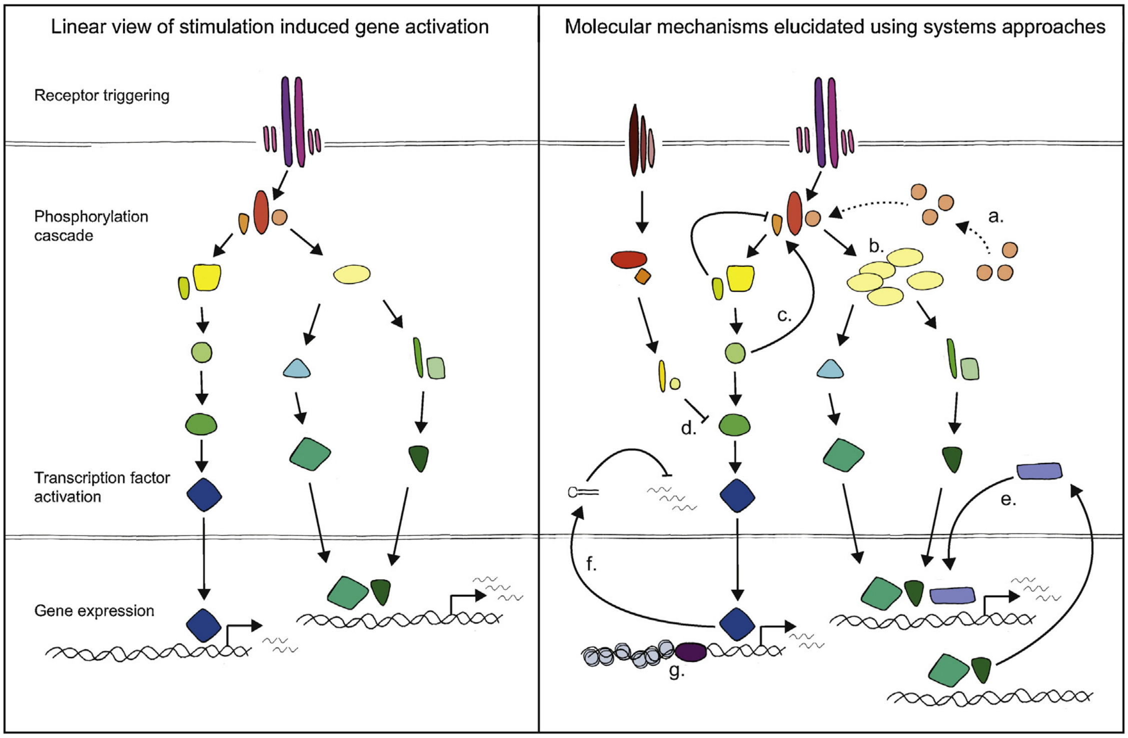

Fig. 1. Contributions of systems biology approaches to our current understanding of immune activation. Global, quantitative and computational methods have been successfully used to elucidate complex non-linear processes involved in the regulation of responses downstream of immune receptor triggering. Some examples include (a) spatial regulation of signaling intermediates [33], (b) impact of component concentration variation [74], (c) signaling feedback loops [76], (d) pathway cross-talk [25], (e) transcriptional regulatory circuits [37], (f) network interference by miRNAs [53], and (g) global regulation of gene expression through chromatin modifications [46].

individual receptors in isolation. These processes are further complicated by the movement of cells and soluble ligands through the microenvironment and by dynamic changes in cell number as a result of cell proliferation and death during the response. Thus, the need for quantitative analysis and consideration of network interactions is clear, leading to increased use of these high-throughput methods and computational approaches in immunological studies. However, is systems immunology living up to its potential?

What has the use of systems biology approaches taught us about the immune response that other immunological methods could not? We have previously reviewed a spectrum of systems biology methods of immunological interest and discussed insights potentially gained from these techniques [1]. Here we focus on the current state of systems immunology, reviewing recent contributions of these approaches to our knowledge of signal transduction and gene regulation in particular. Examples provided in this review demonstrate that with proper experimental design, integrated and quantitative studies of immune receptor signaling highlight biological phenomena that would not be readily identified by purely reductionist approaches (Fig. 1).

2. Signaling

Living organisms adapt their cellular processes in response to a variety of stimuli. For immune cells, receptor triggering allows sensing of pathogens and foreign antigens. Subsequent responses depend on transmission of these signals to the nucleus, resulting in changes in gene expression. This signal transduction relies on a relatively fast cascade of reversible covalent modifications [2] or *via* the reversible binding of allosteric regulators [3]. Among the covalent post-translational modifications, phosphorylation is arguably the most studied [4]. Thus, the combinatorial action of over 500 kinases and 100 phosphatases in the human genome determines the response of cells to particular stimuli [5]. Elucidating signaling cascades triggered by immune receptors is not only critical for our understanding the basic biology of signal transduction, but also has clear therapeutic implications. For example, defects in signaling components downstream of Toll like receptors (TLRs) have been implicated in susceptibility to infection and multiple inflammatory disorders, resulting in increased efforts to target these pathways using novel drug therapies [6].

2.1. Stimulation induced phosphorylation

In the last few years, the development of several mass spectrometry instruments with shorter cycle times and higher resolving power [7,8], as well as software tools allowing reliable identification and quantification of the data sets, have led to an explosion in the number of protein phosphorylation sites described [9–11]. Phosphoproteomics can give quantitative information on thousands of proteins, including those for which phospho-antibodies are not currently available. The predominant use of existing antibody reagents specific for canonical phosphoproteins limits investigation to the same small set of molecules in the majority of experimental studies. In contrast, phosphoproteomics allows for the unbiased analysis of signaling networks and has led to the identification of novel phosphorylation events downstream of diverse immune receptors including the T cell receptor (TCR), Toll like receptors (TLRs), cytokine receptors, and chemokine receptors [12–15]. Complementary experimentation has been used to validate the biological relevance of such findings. For example, protein pulldown and siRNA knockdown assays were used to reveal protein interactions and a functional role for a novel TCR signalosome component [12], THEMIS, whose TCR-dependent phosphorylation was detected by a phosphoproteomic approach. This protein associates with LAT and positively modulates NFAT/AP-1 and ERK activity, elevating IL-2 production. As another example, in a proteomic study identifying TLR-induced phosphorylation of the B-cell