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Review article

Systems-synthetic biology in understanding the complexities and simple devices in immunology

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ABSTRACT

Systems and synthetic biology in the coming era has the ability to manipulate, stimulate and engineer cells to counteract the pathogenic immune response. The inherent biological complexities associated with the creation of a device allow capitalizing the biotechnological resources either by simply administering a recombinant cytokine or just reprogramming the immune cells. The strategy outlined, adopted and discussed may mark the beginning with promising therapeutics based on the principles of synthetic immunology.

1. Introduction

Large sets of differential "omics" data have revealed the complexities associated with the signaling mechanism in an immune response. The complexities arises due to many factors like the interconnection of signaling components and the simultaneous stimulation of multiple receptors; may be by a single ligand or multiple ligands. Layers of complexity are added by the feedback and feed-forward loops followed by diverse mechanisms of regulation mediated by small RNAs and post-translational modifications like proteolysis, ubiquitination, phosphorylation or acetylation. Further, a milieu of transcription factors (TFs) downstream of the specific pathways are activated for appropriate gene expression to a given stimulus. Micro environment of the immune effector cells, like the presence of cytokines, hormones, etc. also decide the response of these cells to a particular pathogenic stimulus. At an individual level, single nucleotide polymorphisms (SNPs) can alter signaling interactions or regulatory architecture, adding another layer of complexity and opening avenue for personalized therapeutics [32]. This complexity does not stop here; it is introduced in the way the mechanism of immune response is dissected in experimental setups, by using different cell-types and experimental animal models. Like, for example TLR2 receptor is expressed at low levels in human endothelial cells (ECs) which is sequestered intracellularly, while in murine, it is expressed at high levels in ECs and found on the cell membrane [16]. Though, the mouse immune system differ from human immune system with respect to each other at several crucial points, often mouse models are used as proxies for investigating human disease and infection [47].

These empirical observations, suggest that the level of complexity are far beyond the current models that are used, which limits our understanding of the immune response. Furthermore, these models also make it difficult for appreciating the mode of action of immunomodulators and pathogenesis, hampering the effective design of therapeutics. Therefore, it is important that an analytical approach is considered to decipher immune complexity, by integrating the systems approach.

Research in systems biology has resulted in an off shoot branch known as synthetic biology, which empowers the biologist to design, improve and rewire biological functions using well characterised biological parts (promoters, ribosome binding sites, coding sequences for chimeric proteins, RNA switches, etc.) for better system performance [23]. Synthetic biology has made progressive advancement with rapid development in various cellular biology fields including immunology, molecular biology, oncology, etc.

Though systems biology is a bottom-up approach, while synthetic biology a top-down approach, they complement each other very well in understanding the system and at the same time facilitating novel designs to modulate the system for a desired behaviour or output.

2. Synergy between systems and synthetic immunology

Systems biology approach assists the detailed level of exploration of a system that is as intricate as an immune system. Systems biology is a powerful and comprehensive archetype for deciphering the dynamics associated with biology, in contrast to the static reductionist approach. The reductionist approach breaks down a complex system like the cell or disease into small units such as genes, proteins and pathways. Breaking a system into smaller units makes the system more tractable and fits into the concept that a system is the sum of small units that

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simplifies and helps in predicting the system's behaviour. However, as stated earlier, the omics data leaves many gaps in the understanding of the biological system and opens a plethora of questions that needs to be answered. The analytical systems biology approach could come to the rescue, which deciphers the system emergent behaviour arising through the complex interaction among the small units. This requires comprehension of the big data which is the listing of genes, proteins, RNA, DNA; their interactions among themselves and with each other; regulatory behaviour; active concentration; kinetic rates of interaction and their behaviour under different environmental conditions. Such data is generated from a variety of high-throughput technologies, which is integrated into mathematical models that generate predictions about the system dynamics. The predictions are tested experimentally and validated, which helps in refining the model and hence the system predictions [32]. The high-throughput techniques like genomics, proteomics, transcriptomics, metabolomics and pharmacogenomics are resources to analyse biological systems. They have been used for genome-wide association studies to identify novel vulnerable genes or identify accurate biomarkers for disease progression by using the interaction network data. Such kinds of study enable better insights into the complex nature of immune response, revealing new components of the system, regulatory mechanisms and biomarkers in immune disorders. Experimental approaches and computational resources like transcriptomics are reliable sources to measure gene expression on a genome wide scale. It helps in the identification of novel genes differentially regulated in a given condition of interest. It also can be systematically used for generating hypothesis about co-regulation of a specific group of genes. Microarrays, next-generation sequencing (NGS), exon and microRNA arrays represent the future of transcriptomics. The outputs generated on these platforms are often noisy, with false positives and false negatives data points. Therefore, a meta-analysis approach is used, where multiple datasets are integrated from diverse sources and genes behaving similarly across several independent experiments are considered as true positives. Such meta-analysis is possible due to the deposition of relevant datasets in repositories which can be readily retrieved for use. Repositories like Reference Database of Immune Cells (RefDIC) (metadb.riken/jp) and the Immune Response *In Silico* database (IRIS) [1], store transcriptomic data from different cell-types, species and different environmental conditions. The Immunological Genome Project is a network of laboratories that rigorously generates standardised genome-wide gene expression datasets, profiling over 200 different mouse immune cell populations under a variety of conditions, like gene knockdowns or knockouts and drug treatments [37].

This integrated approach avoids the difficulties associated with merger of data generated on different array platforms. A systematic meta-analysis of such immunologic data has shed new light on the conserved host innate immune response to a range of pathogenic conditions, like, data from 32 *in vitro* human studies were combined and analysed. This revealed a cluster of 511 genes that represent a common host response to bacteria, viruses and other selected pathogens. These were genes that were understood poorly or not previously known to be involved in immunity [38].

A similar approach to examine lung inflammation was carried out using rodent and primate models. Core set of up regulated and down regulated genes were identified as contributing factors to lung inflammation, regardless of whether the cause was an infection, asthma or an airborne pollutant. Such studies when taken a step further for understanding the transcriptional regulation may help in shaping our understanding of the genetic regulatory network functioning prudently within living cells. Transcriptional regulation is a result of transcription factors (TFs) binding to short, degenerate sequence motifs that occur throughout the genome sequence. High-throughput approaches for identification of TF binding sites include chromatin-immunoprecipitation (ChIP) array platforms (ChIP-chip) and ChIP-seq technology [52].

Data from such platforms help identify genetic regulatory networks on a genome-wide scale. ChIP-chip has been used to validate predicted associations between nuclear factor-kB-1 (NFKB1), interferon response factor 1 (IRF1) and the promoters of co-expressed genes in TLR-stimulated murine macrophages [46], identification of signal transducer and activator of transcription 3 (STAT3) downstream regulator of the cytokine leukaemia inhibitory factor (LIF) [62] and its binding sites in interferon-responsive human genes. A combination of comprehensive computational and high-throughput experimental approaches to transcription factor binding site (TFBS) identification is a promising approach to analyse gene regulatory networks, which will have a profound effect on our understanding of the immune response regulation.

On similar lines as that of transcriptomics, proteomics has opened up investigations at the protein level, both quantitatively and qualitatively to generate large-scale protein–protein interactome. Similar to transcriptomic repositories, the publicly available protein interaction databases like Pathguide and Reactome, is an endeavour that is crucial for systems-level analyses. The InnateDB project have collated, reviewed, annotated and made available more than 7000 innate immunity-relevant interactions involving 2000 human and mouse genes [9]. Such interactions help us identify novel host interactome to that of the pathogen and the host–pathogen interface, revealing many pathogen-encoded proteins that directly interact with components of the immune system (Fig. 1). A proteome-wide map of the interactions between hepatitis C virus-encoded proteins and human proteins using a yeast two-hybrid approach and literature mining has revealed interactions found in infection only. The VirusMINT [14] and Pathogen Interaction Gateway (PIG) [19] databases provide online resources that describe the known host-pathogen interactions. Proteomics work flows can also reveal the effect of post-translational modifications and protein dynamics influencing the immune response. For example, 'phosphoproteomics' assists in unravelling the dynamics of signalling cascades, which is an integral bio-process to immune response. The ultimate goal of such studies is the identification of the complete 'kinome' of a cell. The analysis of the Jun terminal kinase (JNK) signaling in *Drosophila melanogaster* using phosphoproteomics and RNAi screens, yielded a comprehensive regulatory (activators and repressors) map of this important signaling cascade.

Phenotypic insights can be revealed using the genome-wide RNAi

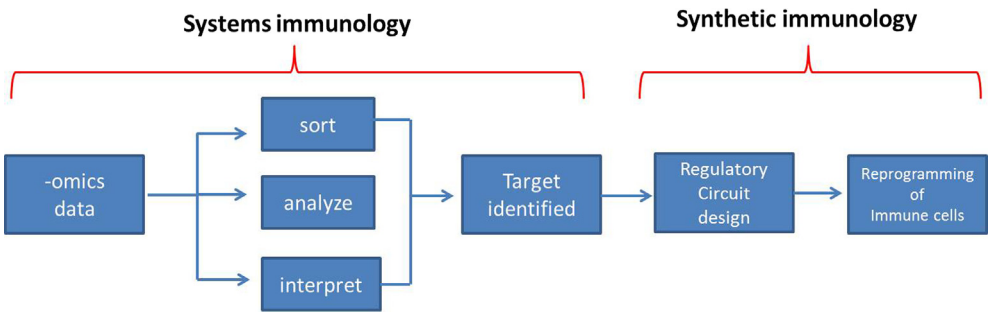

Fig. 1. Schematic diagram showing synergy between systems and synthetic immunology.