How Can Systems Biology Test Principles and Tools Using Immune Cells as a Model?

The field of systems immunology has grown extensively over the last few years, spurred by the generation of large datasets, new analytical tools, and modeling approaches. In this piece and its counterpart in *Trends in Immunology* [http://www.cell.com/trends/immunology/fulltext/S1471-4906(18)30013-9], eight authors discuss what immunologists can learn from systems biology and, conversely, how systems biologists can use immune cells as a model and outline the many directions this interdisciplinary field can expand in.

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A key advantage of using immune cells as a platform for systems biology applications is in the ease of isolating, modifying, and examining functionally how these cells behave not only *in vitro* but *in vivo*. Unlike other cell types, immune cells can be easily studied *ex vivo*, or modified in culture then easily placed back in their tissue environment. This enables fine-grained analysis of how changes in molecular composition, amount, activity, or structure influence the functional properties of cells and the extent to which these outcomes can be predicted from quantitative models of signaling or gene-response events. For the same reasons, immune cells are ideal models for probing larger-scale system behavior at the cell rather than molecular level.

Circulating immune cells react to removal from their normal tissue setting less dramatically than cells residing in dense tissues with many intercellular contacts, which when removed, rapidly lead to dysfunctional effects or even death. Immune cells can be employed in systems perturbation studies in a way that highly differentiated components of other organ systems cannot. Finally, lymphocytes exhibit cell-state changes critically dependent on balances between co-expressed factors that can be easily monitored, and differentiation can be followed in primary cells rather than transformed cells or cell lines. This provides an excellent environment for modeling at a systems level with real relevance to biology.

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The immune system is an ideal place to test systems biology approaches. As organisms are often under strong selective pressure from pathogens, the immune system is open to modeling that combines physical mechanisms of molecular discrimination, evolutionary dynamics, and information transmission and storage. Many features of the immune systems have also recently become better understood quantitatively. The molecular features that allow the innate immune system to sense non-self on evolutionary timescales have become far better characterized, along with the pressures they exert on pathogen evolution. Next-generation sequencing has opened windows into pathogen evolution so that the great diversity of quasi-species can be observed. The ability to sequence T cell and B cell receptors has changed the way we look at the capacity of the adaptive immune system to respond to challenges from pathogens over the course of one's lifetime.

Moreover, it has become clear that understanding the immune system is fundamental to new therapies. A fundamental breakthrough in understanding negative regulation of T cell recognition, and how that is co-opted by tumors, has led to immunotherapies that have fundamentally changed cancer care. In turn, these breakthroughs are generating datasets that have the size and depth to test quantitative principles in systems biology, using many of the technologies described above. These considerations have made the immune system a model for applying principles from systems biology.

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The promise of Systems Biology is that system-wide or systematic measurements lead to an understanding of the systems-level properties, i.e., in terms of the underlying, often complex regulatory mechanisms.

As such, systems biology has developed theoretical underpinnings or principles that are useful when characterizing e.g., dose-response behavior, temporal patterning, parameter sensitivity, timescale separation, predator-prey relationships, and many more. And complementarily, the drive for systems-wide measurements has produced phenomenal innovations that take advantage of the most powerful high-throughput technologies, next-generation sequencing, and mass spectrometry, as well as imaging to provide the spatial and temporal information often critical for system-level understanding.

I would argue that the immune system provides the finest applications of these new tools and theoretical underpinnings. That is because the immune organ largely consists of dissociable cells readily accessible, and immune responses involve highly dynamic intra-cellular molecular and inter-cellular population regulation.

Just think of how single-cell sequencing is enabling the identification of novel cell types, inference of developmental pathways, and characterization of the antibody repertoire; how CyTOF provides for the first time a quantitation of all known immune cells in a given tissue sample, transitioning immunology to the systems era the way Pat Brown's cDNA microarrays transformed gene-regulation studies.

In addition, the immune system provides amazingly rich models of systems principles and theory—for example, for the fine-tuning of the decision-making process during homeostatic hematopoiesis or the dynamic regulation involved in immune and inflammatory responses.

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Immune cells behave dynamically, adopting phenotypic states that can change on different timescales and that have varying degrees of reversibility. This dynamic behavior is critical for immune system function. For example, T cells activate to fight an infection within days, but activated T cells irreversibly differentiate into memory T cells to protect the host from recurrent infection for years. Interpreting experimental measurements of the intracellular and extracellular signals that regulate these transitions is challenging because the measurements are multivariate and often influenced by unknown inputs, especially *in vivo*. Computational systems models are useful for data interpretation because they can account for the multivariate network of signals that regulate cell states and can incorporate both aggregate and single-cell measurements. Incorporating single-cell measurements is particularly important because immune cells display cell-to-cell heterogeneity: while hundreds of T cells activate, only a small fraction of them become memory T cells. And "noise" measured at a single-cell level can help systems biologists learn network structure using appropriate computational analysis. The widespread use of single-cell profiling in immunology thus provides an opportunity to advance the utility of computational models not just to interpret single-cell datasets but also to integrate these data with cell-population-averaged data to propose how heterogeneous cell states contribute to immune system function.

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The adaptive immune system is a wonderful laboratory for testing our quantitative understanding of biological processes at all scales: from signaling to cell-cell communication to evolution. For instance, affinity maturation of B cells can be viewed as a very fast version of Darwinian evolution, with similar results as observed in laboratory evolution experiments: protein stability is important, not all evolutionary paths are accessible, epistasis plays a major role, and diversity is preserved by retaining not only the strongest binders. More detailed quantitative analysis of affinity maturation, and the evolution of immune repertoires in general, can provide insights into evolutionary processes, evolvability, diversity, and the statistical reproducibility of evolution while of course keeping in mind the differences between somatic evolution and species evolution.

Antigen receptors such as antibodies are also very interesting systems in which to study the genotype-phenotype map. Receptor sequences can now be profiled by high-throughput sequencing, and massively parallel measurements of affinity against a given antigen are also being developed. Binding between receptors and antigens is not a one-to-one mapping, and both receptors and antigens (derived from viruses and cancers) can evolve, turning immune recognition as a co-evolution problem. This requires a probabilistic, systems-level description. In summary, repertoires of the adaptive immune system require combining many ideas from systems biology and constantly inspire us to consider new solutions and design options.

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Systems biologists routinely analyze large datasets generated by high-throughput functional assays to gain insights into biological questions. Such MPRAs (massively parallel reporter assays) frequently rely on synthetic biology approaches, for example using artificially generated libraries to learn about natural systems.

In immunology, the advent of next-generation sequencing has enabled studies previously thought to be impossible. Less than 2 decades ago, obtaining even a handful of TCR or BCR (T/B cell receptor) sequences had been cumbersome and laborious. Today, immune repertoire sequencing allows us to conveniently obtain millions of T/BCR sequences in parallel. These comprehensive datasets can provide profound insights into the orchestration of the human immune response.

However, while immune repertoire sequences have become abundant, knowledge on which antigens these diverse receptors and antibodies recognize remains scarce. Identifying the actual antigens recognized, and not only the cumulative T/BCR repertoires mediating their recognition, is imperative for understanding disease mechanisms and for drug development.

Adapting systems biology principles of high-throughput functional assays to immunology will help to tackle this major challenge. MPRAs using synthetic libraries, inspired by systems biology methodologies of studying gene regulation, have the potential to bridge the gap between receptor sequences and their specificity.

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For more than 3 decades, the immune system has served as an invaluable model for studies of development, disease, and cellular responses to external stimuli. Studies of immune cells led to the discovery of the first non-viral transcriptional enhancers, the discovery of the first post-translationally activated transcription factor, and the elucidation of multiple common signaling pathways.

The fact that many fundamental discoveries emerged from studies of the immune system was not a coincidence. A few of the reasons the immune system was advantageous at the dawn of the molecular biology revolution are no longer relevant (e.g., the availability of transformed cell lines representative of multiple developmental stages). However, those early studies led to today's advanced knowledge of the factors and pathways involved in cell-fate decisions, developmental transitions, and responses to extracellular cues. This advanced knowledge, when combined with the relative ease and precision with which immune cell types can be isolated, makes the immune system highly attractive for systems biology studies. When studying responses to extracellular stimuli, an added benefit is that the dynamic ranges of the responses observed is often far greater than with other systems. Finally, the depth of current knowledge of immunoregulatory mechanisms is highly beneficial for testing the predictive value of systems biology tools, as a tool's accuracy can only be rigorously assessed when detailed knowledge obtained using other approaches is available for validation.

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Immune system processes operate over a wide range of time and spatial scales and often involve cellular decision-making, e.g., what type of cell to become, what "activation" response to mount after integrating signals from the environment, and where to migrate amidst spatial cues. Immune cells are thus versatile models for systems investigations of how cellular behaviors emerge from information processing by signaling and transcriptional circuits. Beyond intracellular processes, immunologists have long studied immune cell-cell interactions and how they orchestrate immune responses. For example, multiple populations of immune cells can be co-cultured *in vitro* under different environmental, spatial, and genetic perturbations followed by population dynamic measurements and iterative modeling to understand how phenotypes emerge from cellular interactions. 3D organoids, or transfer into model animals for *in vivo* analysis, can also be used to extend the relevance of these *in vitro* models.

How single-cell phenotypic diversity (or "noise"), which is prevalent even within well-defined cell populations, is regulated and what functions it serves remain fundamental questions in systems biology. Given their heterogeneity and diversity, immune cells are excellent models for studying these questions, both *ex vivo* and within tissues and under both homeostatic and "activating" conditions, such as vaccination and infection. Indeed, immune system activation across the whole organism in these contexts, followed by a time- and space-resolved assessment of immune cells in individual tissues, offers an attractive organismal model for systems biology.