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Forum

Questions of Stochasticity and Control in Immune Repertoires

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Four Scales of Interactions Underlying Immune Function

How does the adaptive immune system generate robust immune responses despite stochasticity at the genetic, molecular, and cellular levels? To address this multidisciplinary challenge, perspectives from systems biology, mathematical modeling, evolution, and experimental immunology are required. With new single cell high-throughput technology, the time is ripe to discuss it.

Predicting immune function is critical for public health. How does a vaccine actually 'work'? Taking genetic and demographic factors into account, who will suffer from influenza, autoimmunity, or cancer? High-throughput experiments open a new window into single-cell data from wide populations of cells. Along with great promises that these technologies bring with them, they also introduce 'noise' and are hard to interpret. Last summer, in Jerusalem, we had a unique multidisciplinary meeting that discussed these very issues for 1 week. During this meeting, we discussed 'what shall we ask next in immunology?'. We wish to share with the immunology community the questions that were raised at this meeting, as we feel that these are questions all immunologists must bear in mind. In particular, what novel theories, and combinations of conventional and emerging technologies should be applied to address questions of immune function? How can the integration of big data from multiple scales of biological interactions be performed to help us answer the questions specified above? What types of high-throughput experimental data are most informative in different experimental and descriptive circumstances? What mathematical and analytical tools do we need to interpret the data? In the end, how can we quantify our certainty in the biological inferences we can make from experimental observations, in order to make relevant clinical decisions?

The advent of high-throughput, single-cell experimental techniques [1] and their associated computational and analytical methods drive us to re-examine the basic questions and relationships that underlie the process of immune selection and response [2]. Roughly, interactions occur among four physical scales: responses of single clones of lymphocytes from the primary repertoire; competition among B cell clones during a response after somatic mutation; interactions among different cell types in the selection of expanded clones; and evolutionary selection by the integration of the immune system, epigenome, microbiome, and other physiological systems (Figure 1).

The functioning immune system is distributed among a huge number of independently acting cells arrayed in multiple related populations. Through cooperative interactions of adaptive and innate signals, and in particular, T and B cell synapses with antigen-presenting cells and with each other, these cell populations collectively engender an adaptive immune response. We can observe the behavior of individual cells, and indeed infer the entire innate and adaptive immune system at many scales of interactions. All lymphocytes must have a functional receptor. T cell receptor (TCR) or B cell receptor (BCR) activation initiates gene expression, epigenetic structural changes, translation, and post-translational modifications of cellular proteins. In collecting information, immune cells compete for antigens and respond to paracrine and autocrine cytokine signals, as well as a myriad of non-BCR/non-TCR cell surface receptor cognate interactions that enable responses to vaccines and later a recall response to confront disease. To understand the signals that drive immunity we must identify and understand all these types of interactions and their different temporal scales (Figure 1). Fully articulating all of these scales is beyond our scope, so here we focus on the scale of cellular interactions and try to identify key relationships to explore.

Exploring at the Individual Cell Level

One first question that emerges is how does BCR affinity influence the survival and downstream signaling of B cells? How are cytokine and co-stimulatory molecule signals integrated with the signals derived from the antigen receptor? The component parts of B cell and T cell internal signaling have been characterized, including kinase cascades [3], threshold dynamics of the NFκB transcription factor family [4], and MYC [3], each with different specific components of calcium dependence [5]. These signaling cascades can be divided into pathways downstream of the BCR (determined by the specificity of interactions with antigens), and pathways dependent on direct inflammatory signals (TNF, IFN, etc.) as well as on activation via innate signals such as Toll-like receptors and other pattern recognition receptors (PRRs) [6]. We can thus ask more specifically how the dynamics of cell signaling are based on the dynamics of cell binding. What, for instance, is the impact of on-rates or off-rates on binding, the density of presented antigens, or the timing of co-stimulation by other innate pathways?

A second question is how the activation links to changes in cell behavior; the different pathways of BCR or TCR signaling, acting in tandem with innate signaling

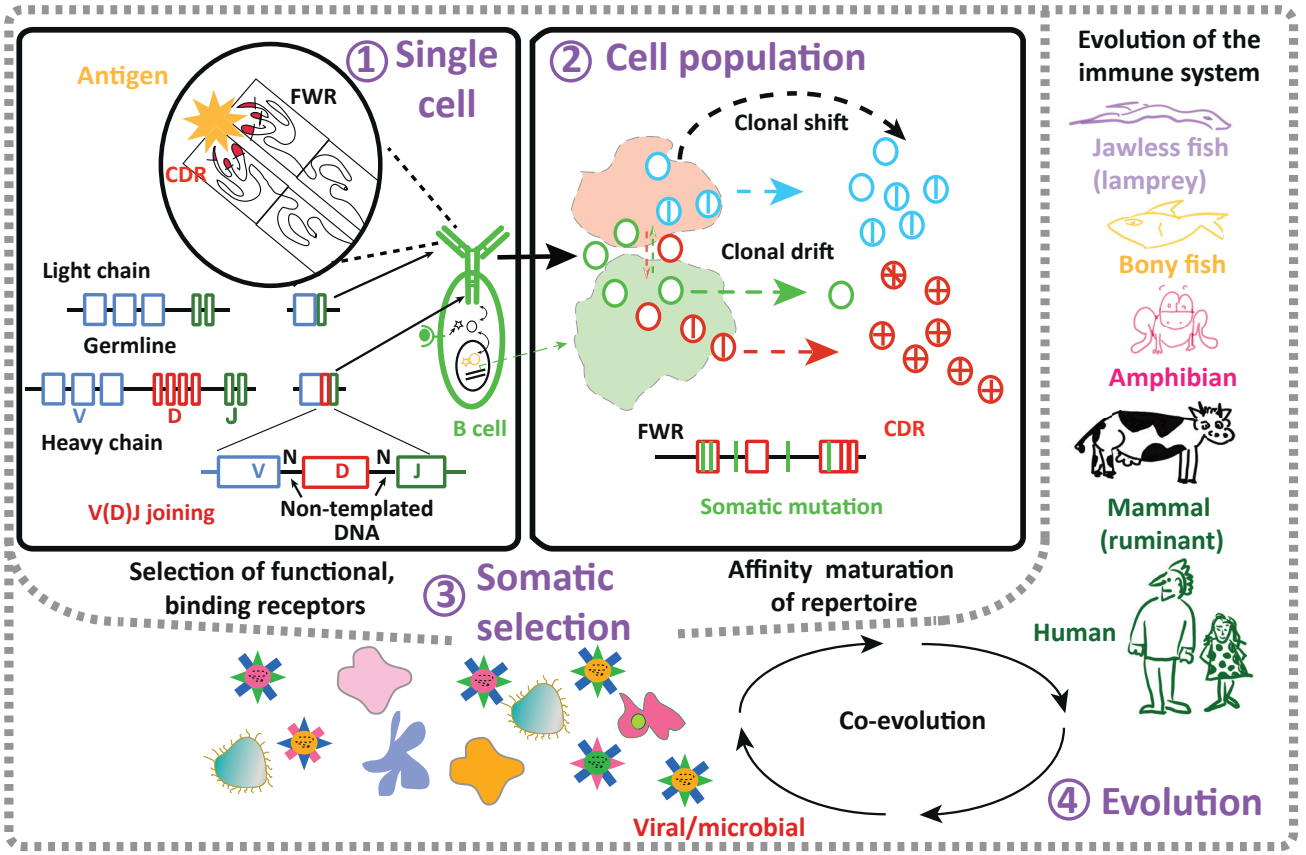

Figure 1. Four Scales of Interactions Underlying Immune Function. Immune cells undergo selection based on variable proliferation, death, and diversification rates, adapting to changing molecular environments. This adaptive immune behavior enables vertebrates to match the shorter timescale of microbial and viral evolution. Relationships between timescales of organismal evolution, microbial pathogenesis, and immune adaptation are complex, and changes cannot be simply explained by the Darwinian 'selection of the fittest'; studying the integration of these scales is daunting. Using novel high-throughput techniques, how individual cells relate to each other and how they cooperate over time to create efficient immune responses can now be studied. The B cell repertoire is used as an example, across all four scales. (1) Single cell diversity is generated through V(D)J joining and the insertion of non-templated N nucleotides to encode heavy chain–light chain pairs which can generate $\sim 10^{14}$ different receptors. From this diversity, all B cells are selected for B cell receptor (BCR) function: proper folding of their framework regions (FWR) and antigen binding of their complementarity determining regions (CDR). BCR activation generates and 'interacts' with proinflammatory (green) and anti-inflammatory (red) cytokines. (2) Every repertoire can be divided into a set of clones [cells derived from a common progenitor cell with a specific, unique BCR (groups of circles of a same color)]. Selection, and therefore all changes in B cell populations, can be divided into two related forms of competition: clonal shift (black arrow; competition between clones) and clonal drift (colored arrows; competition between mutant members of each clone) (mutations: extra lines on circles). Together, these two scales of cell interactions underlie the process of somatic selection in adaptive immunity (3). The final scale is the evolutionary scale of epigenomes, microbiomes, and immune systems (4) including the co-evolution of our immune system, the microbes/virons that infect us, and the evolution of adaptive immunity across species, from first appearance in lampreys, through amphibians and ruminants, to humans.

pathways, create a pattern of competitive proliferation and death [5]. Improved affinity has been shown to decrease death rates [7]. Other evidence shows that a cell's division and apoptotic dynamics occur along parallel competitive time lines [8]. From these observations and using single cell analysis, we can now ask, how do different signaling pathways, triggered either through BCR-specific activation or by innate pathways, influence proliferation, death, and differentiation?

Exploring at the Cell Population Level

From these new ways of studying the dynamics of individual cells, a more complex view arises for the modes of cooperation between cells: first, what are the roles of the different cell types in controlling immune responses and how do these roles change as cells adapt [9]? For instance, the balance of regulation for activation and inhibition depends on cell activity and its context. Thus, ergotypes (indicators of activity such as HSP60) may upregulate regulatory T cell (Treg) activity

when presented by professional antigen presenting cells and downregulate it when presented by T effector cells. This is a relationship, which when dysregulated, can lead to autoimmunity or cancer and if controlled, could underlie future putative treatments for these pathologies [10]. This leads us to further ask if we should consider immune cells as a continuum of context-specific responses, instead of dividing cells into distinct types and irreversible differentiation stages.

Second, what is the effect of the somatic environment in determining cellular cooperation and behavior? Motion through specialized tissue environments in the lymph nodes and bone marrow that is mediated by different chemokines (such as Cxcl 12 and Cxcl 13) is key for the development of germinal centers [11] and the development of memory and effector cells [12,13]. So, how do cells communicate their roles and responses in such situations? What are the minimal sets of cells and factors that balance positive and negative signals to help maintain homeostasis? [14]

The questions related to the two scales of cellular interactions discussed above describe a process of somatic change which can be linked to two scales of selection. This raises additional questions on the nature of selection, both somatically in the body, and through the evolution of diseases and adaptive immune systems, which are beyond the scope of this forum piece (Figure 1).

Immune cells have a wide array of potential dynamics and interactions. While this has long been suggested, the wave of novel high-throughput experimental and computational methods, for the first time, enable us to fully characterize the breadth and nature of stochasticity of cellular behaviors. They also show us that the diversity of cell dynamics is not a hindrance to immune function, acting as 'noise' in the system, but rather, that it is part of what makes it robust [15]. To fully understand the 'how' requires us to ask questions about the immune system across multiple scales of biological interactions and selection, and connect them all. Only then will we be able to truly advance our ability to understand immune function and create successful and nuanced methods for the modulation of immune responses (Figure 1).

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