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Systems immunology

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Systems biological approaches to immunology have grown exponentially in the past decade, especially as broad approaches to data collection have become more accessible. It is still in its infancy; however, largely descriptive, and looking for the main drivers of particular phenomena, such as vaccination effects or pregnancy. But this lays the ground work for an increasingly sophisticated appreciation of subsystems and interactions and will lead to predictive modeling and a deeper understanding of human diseases and interactions with pathogens.

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Edited by **Petter Brodin** and **Lluís Quintana-Murci**

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“Imagine that the gods are playing some great game like chess . . . you don’t know the rules of the game, but you’re allowed to look at the board at least from time to time and . . . from these observations, you try to figure out what the rules are . . .” Richard Feynman

While this quote is meant to apply to science generally, I find it especially relevant to where we are now in applying Systems approaches to immunology. It also is an important reminder of how all science begins with observation. This contradicts the conventional wisdom that one must start with a hypothesis and quickly get to ‘mechanistic’ data by the end of the paper. Hypotheses and mechanisms are surely where we want to go, but careful observation and analysis must come first. Only as more data accumulates are hypotheses worth having. I am biased, but I believe the modern form of Systems Immunology began in 2008–2009, with a relatively concurrent

publication that I wrote entitled ‘A Prescription for Human Immunology’ [1] together with the first data papers by Sekaly [2] and Pulendran [3], where both groups analyzed Yellow Fever Vaccine responses using gene array data and other data. The theme of all of these was that we needed new approaches to human immunology, since so much of what we do in mouse work cannot be done in humans. Furthermore we needed data that was not dependent on what we know about the mouse immunology, because many failed translational efforts suggest that the mouse is not a reliable predictor of human results (and not just in immunology). And also that at least part of the way forward was to look closely at vaccine responses, since these stimulate the immune system broadly and are relatively easy to get underway.

But first some definitions and the overarching logic. I think that the success of a systems approach to an area depends on how well the data to be obtained captures the most important aspects of system. Early systems work focused on signaling pathways in yeast for example [4] which makes sense for a single cell organism, where the cells can be thought of as uniform entities (although they were not!). But the immune system is much more complex, with many very different cell types and subsets and activation state differences. Furthermore these cells ‘talk’ to each other with many different cytokines. And develop different antigen receptor repertoires that are shaped by experience. Luckily, we now have very sophisticated methods to phenotype the different cell types and quantify most of the different cytokines. And new methods to characterize the TCR and BCR repertoires. Importantly, this can be done at the single cell level, which is important in distinguishing what different subpopulations may be doing versus the aggregate. Much less accessible in humans are the different tissues and organs where immune cells mature and mount responses in, but there has been considerable progress recently here as well [5*]. But why study the immune system as a system? Because that’s what it is—the different cell types and molecules work together to mount and modulate responses. While it has been necessary to take a piecemeal approach to something as complicated as the immune system for most of the history of our field, it shouldn’t stop there, and the next order of business (and much ongoing work) is to understand the interplay of its various components such that we can actually predict the contribution of each part to a given response. Here blood is the most valuable resource in that it is one of the mostly easily obtainable clinical material and contains both most of the key cell types and many of the cytokines. And these cells are circulating, especially after any immunological

stimulations, such as a vaccination. Another reason why this approach is particularly valuable for human work is that the key interactivities of a system are revealed by perturbations—thus the more and the more varied perturbations employed, the more can be learned. Here humans have an embarrassment of riches, with thousands of drug treatments, dozens of vaccines, thousands of infectious diseases and so on. And there is broad genetic variation and environmental exposures in different parts of the world. Plus humans typically live a long time and so longitudinal studies can be undertaken to see how the immune system varies with age.

But inbred mice and monkeys can be very useful in a systems approach as well, there are many manipulations, both immunological, infectious and genetic that are well known in mice and would reveal immune circuitry quickly and efficiently. And it would be much easier to go into mechanistic depth than with humans, of course. But another reason for parallel systems studies in mice is to discover differences in mouse versus human responses that might explain why mouse models of disease fail to predict human responses or their heterogeneity so often. Monkeys are attractive because they are much more likely to produce similar responses to humans (although this is not a given) but they are a more limited option because of their expense.

I think of systems immunology as divisible into four phases, as depicted in Table 1. The first being a ‘discovery’ phase where you use the many available technology platforms to ‘cast a big net’ over an area or phenomenon of interest and see what you catch. This is mostly where we are now, finding expected or unexpected connections between parts of the immune system and a disease for example. A second phase would be where attention is paid to the interactions of different parts of the system in order to discern which parts correlate or anti-correlate. The third and fourth phases are where we have enough understanding of the major drivers of activity to predict what will happen, first in a subsystem and ultimately in the whole thing. A spoiler alert at this point would be that three and four are still quite distant! An excellent early example of phase 1 was the observation of Pulendran *et al.* that the stimulation of TLR5 on murine B cells was critical for them to progress to

antibody secretion and the plasma cell stage. And that this stimulation was dependent on flagellin produced by the microbiome [6]. Interestingly, while the mouse experiment was suggested by gene expression data from human vaccination, thus far this same relationship does not seem to be operative in human vaccination, although the microbiome does have interesting effects in human vaccines [7*].

An example of the second phase has been in the immunology of pregnancy, where Brodin *et al.* on the differences from birth to early life of children born naturally versus caesarian births is very interesting, in that they start out having very distinct but reproducible phenotypes but then converge soon afterwards [8**]. Other groups have also developed very deep ‘omics approaches to pregnancy, identifying the major immunological and other shifts that occur and relating the immunological data with microbiome and metabolomic data [9*,10*] reviewed in Brodin [11]. These analyses clearly set the stage for further work on what can go wrong in pregnancy, which if one could know in advance would be important clinically. Another area of longstanding interest centers around vaccination and infectious diseases. The recent SARS-CoV-2 pandemic where different individuals have very different outcomes is a dramatic example of individual immune variation, but not enough data is available at this point to say much, or to cite papers. But we do have very interesting systems studies of other diseases to mention, particular those of Chien and Khatri [12**] on latent infection with *Mycobacterium tuberculosis*. Here an extensive mass cytometry panel analysis comparing latently infected with uninfected individuals to show distinct differences in peripheral B cells and T cell types. Most importantly they used a program to convert gene expression data in other studies to cell subsets [13*] to find (and confirm) that NK cell levels drop dramatically in subjects that develop active TB disease, and that they return to baseline after antibiotic treatment. In the context of vaccination, there are many reports, but perhaps the most important of the most recent crop is that of Tsang and co-workers who followed up their earlier correlation of successful influenza vaccine responses with higher baseline levels of memory B cells in the periphery [14] with a very recent study expanding on that theme and extending it to an autoimmune disease [15**]. That higher levels of the raw material of an antibody response at the outset would be this predictive seems simplistic, but there is a definite link here and this may reflect an underlying dynamic within the system. Whether it applies to other parts of an immune response is less clear. Another important area to be tackled is the general influence of the immune system on aging and health problems in general. The absence of almost any medically validated biomarkers has fostered a whole industry of ‘Immune Boosters’ that while popular with the public, have no basis in fact. Here the earlier work of Furman and colleagues [16]

Table 1

Systems immunology stages

1. Broad survey to look for most prominent components in a phenomenon.
2. Looking for interacting components within a phenomenon (e.g. Pregnancy, a particular infection etc.).
3. Predictive modeling within a phenomenon.
4. System-wide predictive modeling.

established an important link between the inflammasome pathway and cardiovascular disease. Also noteworthy is a time course of stroke patients that uses machine learning of large immunological datasets (as do many of those already cited) to show a very dynamic of immune activity. These early studies lay the groundwork for potential interventions, as well as identifying useful biomarkers that have predictive potential.

Here it is worth noting how valuable, but rare, longitudinal studies are. There is no better control for an individual than themselves, and following them over an extended time can be very revealing. The Framingham study is one of the longest running and has been very valuable for general medicine over time. But there are few studies that use modern immunological assays over time. Here the Stanford-Ellison study is small (~135 individuals) but has been going since 2007 and has been a rich source of insight into how peoples immune systems age and the relationships to common diseases. A number of papers have emerged from this cohort, including the Furman paper cited above, most recently Alpert *et al.* [17**] have seen that older individuals have a specific ‘trajectory’ in their cellular phenotype where different individuals have different immune starting points, but generally change with the same slope. They also derived an inflammatory cell type score that was more predictive of cardiovascular disease than one derived from methylation patterns (considered the best indicator prior). Combining both the S-E longitudinal data and much larger cross sectional cohorts to cover over one thousand individuals, Sayed *et al.* [18**], used machine learning and other computational methods to subset individuals and linked these subsets to specific disease risks and inflammation associated with aging.

Also critically important in the development of systems immunology is the development of specialized computational and statistical methods to extract meaning from the large amounts of data that are generated and solve some of the many problems that come up. Here the widespread use of gene arrays (and now single cell RNAseq) expression data has resulted in masses of data. Being able to simultaneously assay all the genes in the genome was a revolutionary advance, and it is still the richest data source obtainable from blood or tissues, but gene expression data is inherently noisy and there is also no guarantee that a given transcript is translated. But here Khatri and colleagues have developed innovative ways to combine study results such that common themes and gene signatures emerge clearly. This they have done in multiple studies, most importantly in TB, where they have been able to re-analyze multiple conflicting studies to derive a three-gene signature for predicting who will develop TB disease versus the vast majority of people who will not [19**,20].

Other important tools for systems analysis are programs that combine different data sets to identify important relationships between the immune system and metabolism for example, or the microbiome [9*,10*]. Another area of immunology where computational tools have been sorely needed has been in the area of TCR and BCR repertoire analysis. While it is easy now to generate massive data sets of millions of these sequences, parsing them into useful insights has been a struggle. Particularly in human T cell responses there can be hundreds or thousands of TCR sequences for each ab TCR specificity peptide-MHC. Here several new programs have come to the fore [21,22,23**] that allow one to reduce even very large TCR sequence sets into a much smaller set of shared specificities.

In closing I see a bright future for systems immunology, which will only become more useful with time. I am reminded of the famous words of Theodosius Dobzhansky, who said that “Nothing in biology makes sense except in the light of evolution.” To which I would say that the equivalent here would be that “Nothing in immunology makes sense except in the context of infectious diseases and self-nonself discrimination.” In the case of the former influence, the review by Prof. Quintana-Merci is very timely [24**].

Conflict of interest statement

Nothing declared.

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