From Finger Pricks to Point-and-Click

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In this issue of *Immunity*, Obermoser et al. (2013) systematically analyze and compare the blood-transcriptomic response of the pneumococcal and influenza vaccines in humans over multiple time points spanning hours to tens of days. They then present web-based interactive figures that facilitate exploration of this large, complex data set.

Advances in high-throughput multiplexed technologies for measuring states of biological systems from molecules, to cells, to whole organisms are rapidly turning biology into a "big data" science. Immunology is no exception. Although the immune system and its response to perturbations such as infection and cancer are invariably complex, involving intricate molecular and cellular orchestrations over space and time, the increasingly comprehensive view offered by global studies of immunity complements more traditional approaches to chip away at this complexity. Microarray profiling of peripheral blood before and after yellow fever and influenza vaccination, for instance, has revealed both expected (e.g., plasmablast) and new response signatures and has led to novel hypotheses on the genetic regulation of antibody and $CD8^+$ T cell responses, which in turn could help facilitate rational design of new vaccines (Pulendran et al., 2010). The extent to which immune responses vary among different vaccines, however, remains largely unexplored at the genome-wide scale. In this issue of *Immunity*, Obermoser et al. (2013) systematically and comprehensively compare the blood-transcriptomic responses of two widely used vaccines: the pneumococcal vaccine, composed of polysaccharide extracts, and the influenza split vaccine, which comprises inactivated viral components.

In addition to providing a comprehensive analysis of the shared and distinct transcriptome signatures induced by two important vaccines, this work contributes a rich data set for the investigation of vaccine responses in humans. However, exploration of such large data sets requires custom bioinformatics analysis, which is typically accessible only to individuals with computational and statistical expertise. Here, the authors provide an innovative solution: for the first time in *Immunity* (and to the best of our knowledge, in any scientific journal), readers can further explore the data (not just the images) presented in all of the figures through an aesthetically appealing and functionally rich set of web tools called interactive figures (iFigures). These interactive data-browsing and analysis tools render such complex data sets immediately accessible to a diverse biological audience.

The design of this study is noteworthy (Figure 1). In addition to the typical time points (days 0, 1, 3, and 7) profiled in previous studies of yellow fever and influenza vaccination, this work investigates longer-term responses by sampling from days 10, 14, 21, and 28. To examine innate responses over a dense set of early time points in the scale of hours after vaccination, the authors profile minute amounts of blood from finger-prick samples in a separate cohort of individuals. Also included is a parallel placebo arm with the same set of time points wherein saline was administered in lieu of the vaccine. The lack of substantial response activity following saline injection provides reassurance that the data-generation platforms are robust; it also indicates that intramuscular injection elicits minimal transcriptomic activities in the blood; thus, those observed in the main arms are vaccine specific. Another nice feature is the inclusion of an extra prevaccination time point (day −7) for obtaining more robust baseline estimates. The cohort sizes are relatively small, making robust inference of correlates of immunogenicity and control for confounding by outlier individuals difficult. However, the sample size is sufficient for deriving robust vaccine signatures, and the careful design and methodology of the study will serve as an invaluable reference for future blood-transcriptomic studies in humans, such as ones involving pediatric subjects, dense time points, and minute blood volumes.

A major challenge in any study involving large-scale data is the transformation of such data sets into biological insights, and one of the main obstacles is the curse of the high dimensionality of such data sets. The authors address this in a couple of ways. First, they reduce the dimensionality of transcriptomic responses from tens of thousands of genes to 62 functionally annotated gene modules, which are sets of coexpressed genes derived from multiple independent blood-transcriptomic data sets (Chaussabel et al., 2008). Similarly, they draw insights from a host of early responding genes by comparing them to a manageable set of innate transcriptomic signatures, which are obtained from the stimulation of in vitro blood cell culture with diverse stimuli, including cytokines, Toll-like receptor (TLR) ligands, live viruses, and killed bacteria. Second, iFigures provide a point-and-click web interface to expand the static views of traditional figures and allow the reader to explore the data from different perspectives, thus offering a great level of flexibility and transparency. The reader can now test the robustness of the authors' conclusions to different p value or fold-change cutoffs, inspect the flow-cytometry gating details when examining figures

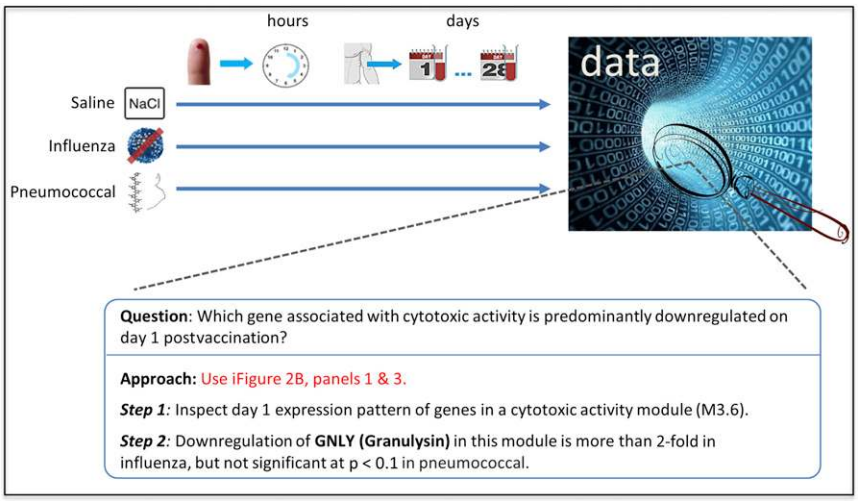

Figure 1. Study Design and an Example of Using the Interactive Figures to Explore the Data
The top section depicts the study design: three parallel arms for saline, influenza, or pneumococcal vaccine injections. These arms were performed twice in different cohorts: once for profiling samples from early time points in the scale of hours and the other for time points in the scale of days following injection. This study generates large-scale data that can be explored further with interactive figures (Obermoser et al., 2013) that accelerate finding answers to specific questions. Here, we illustrate the use of the interactive figures by inspecting the specificity of expression patterns of a gene in a cytotoxic activity module that was mentioned, but not elaborated on, in the paper. Source of the "data" image: luckey_sun at Flickr.com.

depicting cell-population frequencies, and select and further investigate the genes inside modules of interest (Figure 1)—these are but a few of the myriad exploratory possibilities. Although releasing the raw data, as the authors are also doing with this work, is crucial for enabling unrestricted exploratory analysis, model building, integration with data from other studies, and further hypothesis generation, iFigures extend the reach of many of these exercises from computational experts to biologists with limited bioinformatics expertise.

The comparative exercise undertaken in the paper revealed that within hours both vaccines can induce meaningful transcriptomic responses in the blood, with interferon and inflammatory/TLR-inducible genes predominant for the influenza and pneumococcal vaccines, respectively. For both vaccines plasmablast responses were apparent by day 7, but the pneumococcal vaccine had a more sustained response that lasted until day 10, as seen in both microarray and flow-cytometry data. These interesting observations raise further questions: e.g., how do differences in early responses affect the duration and level of the plasmablast response? Answers to this question can point to strategies for designing more potent vaccines. The current data also leave open the question of the mechanisms that drive the marked differences between the vaccines. Expression profiling of sorted cell subsets from the influenza vaccinees indicated that the interferon signature originated from neutrophils and monocytes, but which cell subset produced the interferon is less clear. A related question is where neutrophil stimulation took place—at the site of injection or further downstream? To begin to answer these questions, future studies could consider simultaneous profiling of blood and tissue biopsies from the injection site; utilizing mouse models from which tissue samples can be obtained more readily could also prove valuable. In general, correlating responses in tissues to those in blood would be highly informative and could start to reveal molecular and cellular mechanisms that give rise to blood response signatures.

Data from this and other large-scale studies of human immunity together with ongoing efforts (e.g., those from the Human Immunology Project Consortium and the National Institutes of Health's Center for Human Immunology, Autoimmunity, and Inflammation [Leslie, 2010]) will begin to provide increasingly comprehensive characterizations of the human immune system, e.g., before and after vaccine and drug intervention in healthy and disease-affected individuals. Proper integration of these dense data sets will start to provide a human "immune state" atlas—akin to the Connectivity Map for functional genomics and drug discovery (Lamb et al., 2006)—useful for probing and comparison of immune statuses under diverse settings and for reverse engineering functional interactions among immune-system components based on correlations among the observed phenotypes across individuals (Marbach et al., 2012). In addition to proper design (e.g., sufficiently large cohort sizes) and execution at the individual study level, achieving this goal requires overcoming several obstacles, such as the standardization of experimental practices and technology platforms, the identification of confounding covariates, and the development of approaches for normalization across studies, as well as agreeing to common data-dissemination and replication standards. Some of these, such as data-processing standards and sharing policies, can be partially borrowed from similar efforts in related fields (e.g., The Cancer Genome Atlas [Chin et al., 2011]); others need further research innovation as well as collaboration and dialog across the research community. The biggest challenge and opportunity, however, lies in the development of novel concepts and approaches to turn such data compendia into immunological insights and predictive constructs for unseen perturbations. For example, predictive models have been built for disease prognosis (van 't Veer et al., 2002) and are being explored at levels ranging from molecular interactions to whole cells (Bonneau et al., 2007; Karr et al., 2012). Extending these by incorporating key elements of immunity (e.g., cell-cell interactions; spatial and temporal considerations) and proper abstraction and modeling across different biological scales will help usher in an era of immunology in which the systematic collection of large-scale data sets such as the one presented in this study is combined with computational and mathematical modeling to systematically chip away at the complexity of the human immune system in health and disease.

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Roquin Paralogs Add a New Dimension to ICOS Regulation

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Two studies in the current issue of *Immunity* (Pratama et al., 2013; Vogel et al., 2013) demonstrate that the RNA-binding proteins Roquin-1 and Roquin-2 have redundant function in the posttranscriptional repression of *Icos* messenger RNA by CD4$^+$ T cells.

CD4$^+$ follicular helper T (Tfh) cells promote maturation of B cells into memory or antibody-secreting plasma cells in germinal centers (GCs) of secondary lymphoid organs (SLOs) (Craft, 2012). Similarly, autoantibodies in diseases such as systemic lupus erythematosus (SLE, or lupus) are produced via GC B cells, with dysfunctional Tfh cells necessary for their development (Vinuesa et al., 2009). Two papers in this issue of *Immunity* provide new insights into the regulation of Tfh cell help for GC B cells and how this regulatory pathway might be subverted in autoimmune conditions such as lupus (Pratama et al., 2013; Vogel et al., 2013).

For naive CD4$^+$ T cells fated to the Tfh developmental pathway, antigen priming by dendritic cells (DCs) in T cell zones of SLOs results in upregulation of the inducible costimulator (ICOS). ICOS signaling, delivered by ICOS ligand (ICOS-L) expressed on T-zone DCs, is required for development and expansion of Tfh cells and expression of their canonical transcription factor Bcl6 (Choi et al., 2011). ICOS, a member of the CD28-superfamily of costimulatory molecules, is upregulated by T cells upon activation, unlike its constitutively expressed sister molecule CD28 (Linterman et al., 2009). The *Icos* gene originated through duplication of the *Cd28* locus, making the differential expression of their protein products a curiosity. CD28 provides the crucial second step in T cell activation upon binding its cognate ligands CD80 and CD86, upregulated on DCs following recognition of microbial products. Unlike CD28 ligands, however, ICOS-L is constitutively present on DCs; consequentially, inducible ICOS expression imparts regulation of T cell activation and subsequent Tfh cell induction. The RNA-binding protein Roquin-1, a RING (really interesting new gene)-domain containing product of the *Rc3h1* gene, posttranscriptionally regulates *Icos* messenger RNA (mRNA), maintaining activation-induced expression of ICOS in CD4$^+$ T cells (Vinuesa et al., 2005) (Figure 1). Recognition of the 3′ untranslated region of this mRNA by the RNA-binding domain of Roquin-1 facilitates degradation of the transcript through interactions with the decapping enzyme Edc4 and helicase Rck (Glasmacher et al., 2010). Roquin expression is repressed upon activation of CD4$^+$ T cells via degradation of the *Rc3h1* transcript by the miR-223 microRNA (miRNA), allowing for the regulated expression of target transcripts.

Previous studies by Vinuesa and colleagues identified a role for the Roquin protein in mRNA regulation by using mice with a single-base substitution in the *Rc3h1* locus, leading to alterations in Roquin structure and activity (Vinuesa et al., 2005). Animals with homozygosity of the mutant allele, *Rc3h1*$^{san/san}$ or *sanroque*, had ICOS overexpression on CD4$^+$ T cells, a consequence of the mutant Roquin protein, with Bcl6 upregulation and Tfh cell expansion upon immunization, leading to robust GCs and plasma cells producing isotype-switched antibodies and a lupus-like phenotype (Figure 1). Roquin-1 localized to cytosolic