

Powerful Populations Respond to Viruses and Vaccines

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<http://dx.doi.org/10.1016/j.immuni.2015.11.018>

In this issue of *Immunity*, [Andres-Terre et al. \(2015\)](#) and [Nakaya et al. \(2015\)](#) perform multi-cohort meta-analyses of immune responses to viruses and vaccines. With increased statistical power and more diverse sampling populations, their findings promise to be more generally applicable and suggestive of novel mechanisms for regulating immunity.

A central problem in human immunology is to understand why some individuals mount productive immune responses to vaccines and pathogens and others do not. The move toward systems immunology stems from the idea that emergent immune system behaviors, such as coordinated immune responses, can best be understood by discerning the interactions between cells, proteins, and genes that collectively give rise to these responses. Systems analyses aim to quantify as many components of the system as possible in the same sample and apply high-dimensional data analysis techniques to infer probable relationships between the components. By doing this in the context of specific immune responses, the interactions of cells, proteins, and genes underlying the response of interest can be understood. Systems approaches are particularly well suited for studies in human subjects because of the significant inter-individual variation in immune cell frequencies, protein concentrations, and transcript abundances. By correlating measurements across individuals, their probable relationships can be inferred and the regulation of the system as a whole investigated ([Brodin et al., 2015](#)). One implication thereof is that these systems immunology analyses can prove more informative when performed in heterogeneous populations than in more homogenous populations.

In these early days of systems immunology, whole blood or blood mononuclear cell gene expression analysis has been the most widely used technology. It provides a powerful yet convenient way to globally profile blood immune systems in humans. The generated blood transcriptome data represent a composite measure of genes expressed within cells and relative frequencies of different

cell populations in the blood ([Whitney et al., 2003](#)). By analyzing modules of genes rather than individual genes and by relating the genes within modules to known cellular pathways and biological functions, the gene signatures identified can become more interpretable ([Subramanian et al., 2005](#)).

In systems immunology as in systems biology in general, the ability to perturb the system is key for inferring functional relationships between system components. Vaccination represents the most widely used immune perturbation in humans and several recent studies have utilized systems immunology analyses before and after vaccination to understand immune responses to vaccines. Gene expression signatures predictive of either cellular or humoral immune responses to influenza, meningococci, and yellow-fever vaccines have all been reported and reviewed elsewhere ([Hagan et al., 2015](#)). Such predictive signatures have also provided novel insights into the regulation of immunity, and some have been pursued mechanistically in follow-up studies ([Furman et al., 2014](#); [Ravindran et al., 2014](#)). This illustrates the potential of using gene expression analyses during immune system perturbation as a means for generating new knowledge about the regulation of the immune system.

Meta-analyses are analytical techniques used to summarize the results of multiple studies. As such, they hold the potential for allowing conclusions to be drawn from more diverse sampling populations that better represent the population as a whole. The increased total number of individuals analyzed can also increase the statistical power of the conclusions. Finally, meta-analyses can be particularly useful in systems immunology and the inference of functional relationships be-

tween immune system components by allowing analyses of data from diverse populations. Meta-analyses are not trivial or without complications and major obstacles have to be overcome to realize the potential of meta-analyses across public datasets. Many of the obstacles are technology specific and dependent on the experimental method used for data collection. Efforts to standardize immunological assays are ongoing ([Janetzki et al., 2009](#)), and a public repository for immunological data—the ImmPort database—has been established and is being populated with cytometry data and more ([Bhattacharya et al., 2014](#)). Gene expression databases such as the NCBI Gene Expression Omnibus, GEO, and the ArrayExpress are more mature and well-established repositories with impressive amounts of data available to query. For example, the ArrayExpress currently contains more than 1.8 million assays from more than 60,000 experiments, an incredible resource for those with the right know-how to harvest its full potential through meta-analyses ([Figure 1](#)).

In this issue of *Immunity*, two such groups present their recent findings using data from the NCBI GEO. In the first study, [Khatri and colleagues](#) make use of an elegant and recently described meta-analysis approach ([Khatri et al., 2013](#); [Andres-Terre et al., 2015](#)). By combining two different methods for finding differentially regulated genes across multiple studies, more robust signatures can be identified. By performing such meta-analyses in an iterative leave-one-study-out cycle, the authors prevent individual studies from inadvertently dominating the resulting signatures. Using this analytical pipeline, the group studies immune responses to respiratory viral infections and is able to identify a signature specific to such responses

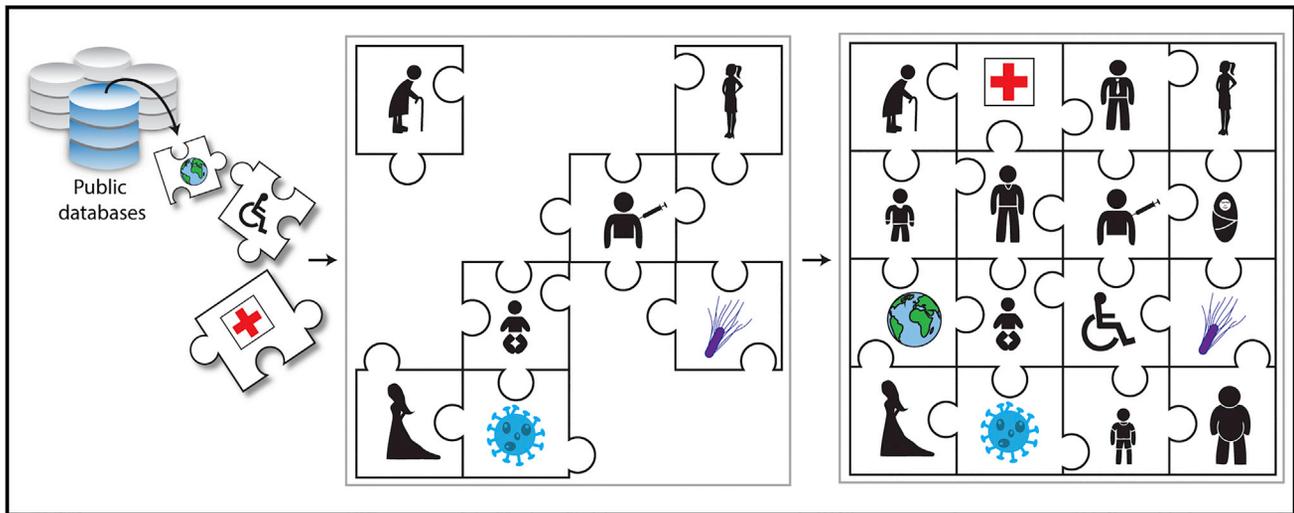


Figure 1. Understanding Immune Responses to Viruses and Vaccines through Analyses of Diverse Populations

With the use of clever strategies for meta-analysis of public gene expression data, a puzzle can be put together to identify shared or contrasting gene expression signatures among diverse populations, represented by the characters in each puzzle piece. These signatures can predict important clinical outcomes and suggest novel mechanisms of immune system regulation.

with important diagnostic potential in patients. Using this signature, patients with respiratory viral infection can be distinguished from healthy controls and, importantly, also from patients with bacterial infections. This important finding made possible through public data meta-analysis should inspire immunologists to formulate novel, testable hypotheses about the role of these identified genes in regulating antiviral immune responses. Moreover, using the same strategy, the group also presents a smaller, 11-gene signature specific to influenza virus infection that is predictive of clinical outcome as well as flu-vaccine responses. Although these 11 genes in the flu-specific signature were differentially expressed across age groups, gender, and disease severity, none of the individual studies included in the meta-analysis had been able to identify all 11 genes, suggesting that the meta-analysis approach provides enhanced precision over previous published reports. Also, the genes in this signature probably contain important clues to the mechanisms of flu-specific immune responses in the context of both infection and vaccination.

In the second study, Nakaya et al. (2015) address the important issue of whether immune responses to flu vaccines are similar across multiple flu seasons and across diverse populations of young and old, healthy or diseased. Flu

viruses change from one year to the next through antigenic drift and antigenic shift. Whether previously reported gene signatures predictive of early humoral vaccine responses would also be robust enough to predict responses across different years despite antigenic changes was unknown. The authors perform meta-analyses of blood gene expression data collected across multiple flu seasons and in different studies and reveal a signature that was indeed able to predict the humoral response at day 28 after vaccination across these multiple seasons. The same signature was not predictive of the later day 180 humoral response, suggesting that different mechanisms and pathways are involved in regulating the longevity of vaccine responses. These important findings suggest that core mechanisms determine the immune system's early response to flu vaccines—mechanisms that could potentially be the target of a universal influenza vaccine in the future. Another important issue addressed by Nakaya et al. (2015) relates to the known reduction in flu-vaccine responsiveness among many elderly, a key target population for this vaccine. It is important to understand whether signatures predictive of responses are equally predictive in the young and the elderly, and if not, whether other signatures could better predict the responses in the elderly and provide an explanation to the observations that

some elderly individuals respond while many others do not. The authors present a predictive gene signature that is equally predictive in the young, the elderly, and also in a smaller group of patients with type II diabetes, suggesting that vaccine responders across these different groups respond by similar mechanisms. This also suggests that by exploring the genes in this signature, one could learn a lot more about these shared vaccine-response mechanisms.

Together, the studies of Andres-Terre et al. (2015) and Nakaya et al. (2015) have used innovative ways to generate new insights from public datasets that will now have immunologists sifting through supplementary gene lists in search of novel hypotheses regarding the regulation of antiviral and flu-vaccine immune responses for future follow-up experiments.

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Small but Mighty: Selected Commensal Bacterial Species Determine the Effectiveness of Anti-cancer Immunotherapies

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<http://dx.doi.org/10.1016/j.immuni.2015.11.014>

Commensal microorganisms influence malignant progression by altering systemic inflammation. New data from two groups (Vétizou et al., 2015; Sivan et al., 2015) indicate that the abundance of specific commensal bacterial species enhances the anti-cancer activity of immune checkpoint inhibitors.

Humans co-exist in a symbiotic relationship with trillions of bacteria, viruses, and fungi that populate the intestine, skin, and upper respiratory and genitourinary tracts. Technological advances have unveiled that the normal flora of humans is remarkably complex in that its components outnumber human cells at a ratio of 10:1. The intestine alone harbors up to 1,000 commensal bacterial species (Tud-denham and Sears, 2015), mostly from four major lineages (termed phyla): Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes. Variations in the composition of the bacterial flora have been associated with metabolic changes and obesity, as well as inflammation, autoimmunity, and infectious diseases. Studies with germ-free mice have shown that forging a robust immune system and a broad repertoire of T cell receptors requires colonization by commensal microorganisms. The role of the microbiota in the progression of extra-intestinal tu-

mors, however, has only emerged in the last 2 years.

Commensal bacteria have been found to influence distal malignant evolution by modulating systemic tumor-promoting inflammation and, subsequently, the magnitude of T-cell-dependent anti-tumor immunity (Rutkowski et al., 2015). In this study, tumor growth depended upon host commensal microbes and was independent of measurable bacterial translocation. Intrinsic differences in some bacterial genera, such as *Bacteroides*, were identified in co-housed mice that developed tumors at different growth rates in a toll-like receptor 5 (TLR5)-dependent manner (Rutkowski et al., 2015). However, the abundance of segmented filamentous bacteria (SFBs), an obvious target identified to drive interleukin-17 (IL-17) production and autoimmunity in tumor-free mice (Ivanov et al., 2009; Sano et al., 2015), was unchanged in mice showing TLR5-dependent acceler-

ated malignant progression. Therefore, changes in the equilibrium between commensal microbial communities (or dysbiosis) influence the tumor growth distally from places of bacterial colonization. Elegant independent studies have also demonstrated that the effectiveness of immunotherapy against different tumors also requires the presence of commensal bacteria (Iida et al., 2013; Vaud et al., 2013). However, understanding how the repertoire of commensal microbes can be specifically manipulated to synergize with available anti-cancer interventions has remained elusive.

In a recent issue of *Science*, data from two groups demonstrate how individual bacterial species can be used to enhance the effectiveness of immune checkpoint inhibitors. Vétizou et al. (2015) and Sivan et al. (2015) independently demonstrate that the abundance of distinct species from the phylum Bacteroidetes and distinct genera from the phylum Actinobacteria is