Resource

Cell

Global Analyses of Human Immune Variation Reveal Baseline Predictors of Postvaccination Responses

John S. Tsang,[1,2,*] Pamela L. Schwartzberg,[1,3,*] Yuri Kotliarov,[1] Angelique Biancotto,[1] Zhi Xie,[1,9] Ronald N. Germain,[1,4] Ena Wang,[1,5,10] Matthew J. Olnes,[1,6] Manikandan Narayanan,[2] Hana Golding,[7] Susan Moir,[8] Howard B. Dickler,[1] Shira Perl,[1] Foo Cheung,[1] The Baylor HIPC Center, and The CHI Consortium

[1]Trans-NIH Center for Human Immunology, Autoimmunity and Inflammation, National Institutes of Health, Bethesda, MD 20892, USA
[2]Systems Genomics and Bioinformatics Unit, Laboratory of Systems Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA
[3]Genetic Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892, USA
[4]Lymphocyte Biology Section, Laboratory of Systems Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA
[5]Department of Transfusion Medicine, Clinical Center, National Institutes of Health, Bethesda, MD 20892, USA
[6]Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA
[7]Laboratory of Retrovirus Research, US Food and Drug Administration, Bethesda, MD 20892, USA
[8]Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA
[9]Present address: Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou 510275, People's Republic of China
[10]Present address: Research Branch, Sidra Medical and Research Centre, Qatar
*Correspondence: john.tsang@nih.gov (J.S.T.), pams@nhgri.nih.gov (P.L.S.)
http://dx.doi.org/10.1016/j.cell.2014.03.031

SUMMARY

A major goal of systems biology is the development of models that accurately predict responses to perturbation. Constructing such models requires the collection of dense measurements of system states, yet transformation of data into predictive constructs remains a challenge. To begin to model human immunity, we analyzed immune parameters in depth both at baseline and in response to influenza vaccination. Peripheral blood mononuclear cell transcriptomes, serum titers, cell subpopulation frequencies, and B cell responses were assessed in 63 individuals before and after vaccination and were used to develop a systematic framework to dissect inter- and intra-individual variation and build predictive models of postvaccination antibody responses. Strikingly, independent of age and pre-existing antibody titers, accurate models could be constructed using pre-perturbation cell populations alone, which were validated using independent baseline time points. Most of the parameters contributing to prediction delineated temporally stable baseline differences across individuals, raising the prospect of immune monitoring before intervention.

INTRODUCTION

The development of accurate models that predict biological responses is one of the major goals of systems biology. Such models have the potential to increase our understanding of pathophysiology and contribute to the development of improved therapeutics (Kitano, 2002; Schadt, 2009). The human immune system provides an excellent context for developing such approaches: many immune cells and molecular components are readily accessible from blood, permitting collection of samples from individuals across multiple time points, followed by in-depth data generation and analyses (Davis, 2008; Pulendran et al., 2010). Furthermore, it is increasingly clear that the immune system and inflammation contribute not only to the pathogenesis of autoimmune and infectious disease, but also to cancer, cardiac disease, diabetes, obesity, neurodegeneration, and other chronic illnesses (Germain and Schwartzberg, 2011). Thus, a more comprehensive and quantitative understanding of how immune responses are orchestrated, together with identification of predictive parameters of effective versus damaging responses, could have implications for the prevention and treatment of diverse diseases.

Building quantitative models often involves the application of perturbations to the system and comprehensive measurements of the initial and resulting states (Chuang et al., 2010). Although advances in high-throughput technologies have made such measurements more routine, utilization of appropriate and ethical perturbations in humans is often a challenge. Here, the

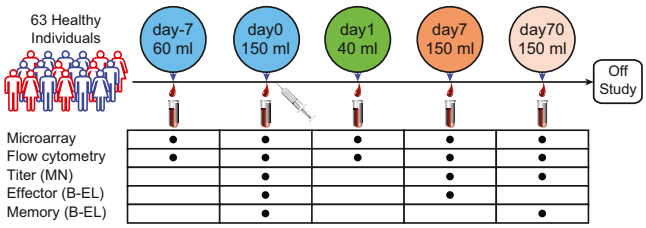

Figure 1. Study Design

Study design indicating blood collections and assays performed. Each subject was vaccinated with the seasonal and pandemic H1N1 influenza vaccines right after the day 0 blood draw.

immune system again offers an advantage, as it is amenable to experimental manipulation. The inactivated influenza vaccine, in particular, is used routinely in healthy and ill populations (Fiore et al., 2009) and provides an attractive perturbation for global data collection and systematic modeling. Upon vaccination, the immune system responds with coordinated changes that reflect the activation and interaction of distinct cell populations and pathways, culminating in the generation of short-lived plasma cells and the formation of germinal centers, from which high-affinity long-lived antibody-producing plasma and memory B cells derive (Pulendran and Ahmed, 2011). By one week post-immunization, a strong but transient plasmablast response can be detected in the blood (Cox et al., 1994; Pulendran et al., 2010), accompanied by increased antibodies in the serum (de Jong et al., 2003). Accordingly, transcriptional profiling of peripheral blood mononuclear cells (PBMCs) revealed substantial changes on days 1, 3, and 7 postvaccination, reflecting both early innate immune activation and day 7 plasmablast responses (Bucasas et al., 2011; Nakaya et al., 2011; Obermoser et al., 2013). Thus, influenza vaccination provides an excellent model of coordinated immune activity involving innate and adaptive responses.

While perturbation analysis is a cornerstone of systems biology, another critical factor for building models in humans is natural population variation. Differences in genetics and environment result in substantial diversity in molecular and cellular states among individuals before and after perturbation. Through correlation analysis, heterogeneity among individuals provides raw ingredients to infer functional relationships among system components—links that cannot be drawn if the parameters analyzed have insufficient diversity in a population. For example, intersubject variation in PBMC gene expression after vaccination has helped to identify postvaccination transcript correlates for antibody responses to yellow fever or influenza vaccination (Gaucher et al., 2008; Nakaya et al., 2011; Querec et al., 2009). However, with the exception of age, how intersubject differences at baseline contribute to outcome has not been well examined. A better characterization of immune variation in healthy individuals is critical not only for the identification of correlates and model building, but also for biomarker development, the definition and characterization of pathological states, and eventually, personalized medicine.

Here, we present a computational framework that utilizes vaccination and multiplexed measurements (gene expression, high density analyses of cell populations, and cellular and serological responses) to quantify baseline and response heterogeneity in a cohort of individuals and systematically identify correlates, build predictive models of vaccination response quality, and infer functional connectivities in the immune system. Using antibody responses as an exemplar endpoint, our analyses confirmed previously reported post-vaccination transcriptome correlates (Gaucher et al., 2008; Nakaya et al., 2011; Querec et al., 2009). Importantly, after accounting for the influence of pre-existing serology, age, ancestry, and gender, we have successfully constructed predictive models and have identified correlates of antibody responses based on prevaccination parameters alone. The robustness and translational potential of these findings is emphasized by our demonstration that the parameters playing essential roles in accurate prediction were cell subsets with temporally stable baseline values within individuals, raising the prospect of predicting the quality of immune responses in the clinic. The data and analytic framework presented provide a potential resource for studying human immunity in health and disease.

RESULTS

In-Depth Analysis of Human Immune Status before and after Vaccination

As a first step toward developing a systems-level understanding of human immunity, we generated a database of immunological measurements using samples drawn from healthy volunteers before and after administration of the 2009 seasonal and pandemic H1N1 (pH1N1) vaccines (Fiore et al., 2009; Table S1 available online and Figure 1). To facilitate the evaluation of intra-individual variation, two baseline blood samples were obtained: one a week before and the other immediately prior to vaccination. Responses were evaluated on days 1, 7, and 70 postvaccination to examine innate, adaptive, and long-term responses, respectively (Figure 1). Purified PBMCs and sera were frozen to allow subsequent assessments to be performed at the same time for a given individual, thereby minimizing batch effects. For the present study, assays included antibody-forming cell responses, influenza-specific serum neutralization titers, multiple 15 color flow cytometric analyses (examining 126 cell subpopulations), and transcriptome analyses using microarrays (Figures 1 and S1A and S1B). Analyses were performed on the 63 subjects from whom we were able to obtain samples at all scheduled time points.

Substantial Immune Baseline Variations in Healthy Subjects

We first examined baseline variation in immune parameters. As expected for subjects from the general population, we found a wide range of baseline titers to the seasonal vaccine, with corresponding large variations in vaccine-specific memory B cell numbers (Figure S2A) (Sasaki et al., 2008). In contrast, most of the cohort was naive for the pH1N1 virus based on titers, despite some potential cross-reactive memory B cell responses,