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Humoral fingerprinting of immune responses: “super-resolution”, high-dimensional serology

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Abstract

In a recent study, Chung *et al.* report the development of a high-dimensional approach to assess humoral responses to immune perturbation that goes beyond antibody neutralization and titers. This approach enables the identification of potentially novel correlates and mechanisms of protective immunity to HIV vaccination, thus offering a glimpse of how dense phenotyping of serological responses coupled with bioinformatics analysis could lead to much-sought-after markers of protective vaccination responses.

Recent advances in high-throughput, multiplexed technologies and computational approaches for analyzing the resulting large-scale datasets are beginning to provide an increasingly comprehensive view of human immune “states” in health and disease, as well as before and after perturbations [1]. In a recent paper by Chung *et al.* published in *Cell* [2], serological responses to HIV vaccination were put under a similar “systems immunology” lens, where 64 biophysical and functional properties (or features) of antibodies (Ab) from recipients of HIV vaccines from multiple trials were measured and analyzed. Antigen-specific Ab levels or their neutralization capabilities have long been used as a correlate of protection. However, the absence of neutralization is not equivalent to the lack of protection [3], because Abs can confer protective benefits via interaction with other immune-system components, including binding to Fc receptors on the surface of immune cells to mediate targeted killing of infected cells [4]. While many of the Ab features measured by Chung *et al.*, including Ab subclasses, antigen specificity, and cell-mediated functions, have been examined previously to assess vaccination outcomes [3], they have not been examined simultaneously in this manner. Obtaining such a high-dimensional view of humoral responses offers new insights and data analysis possibilities, for example, by enabling statistical modeling to assess the correlative relationship among different Ab features and to more comprehensively screen for robust correlates of protection.

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Chung *et al.* applied their approach to four HIV vaccine trials by assessing plasma samples taken two weeks after the final dose of vaccination in each trial. The trials had distinct but also shared features: for example, subjects in the RV144 trials were administered a viral-vector prime at weeks 0 and 4, followed by a prime/protein-boost combination at weeks 12 and 24, while the same protein immunogen (gp120) was administered in the VAX003 trial between 0 and 30 months; in the IPCAVD001 trial—a phase I safety, first-in-human study—a single dose of adenovirus serotype-26 (Ad26) vector (containing a modified gp140 protein) was administered, while the HVTN204 trial tested another adenovirus boost (rAd5) administered at month 6 after a HIV-1 DNA prime at months 0 to 2.

To analyze this complex dataset, the authors employed several machine-learning techniques, which can uncover associations between multiple variables (in this case, Ab features) to outcomes. Here, the main goal of the authors was to extract correlates of Ab-mediated protection, for example, by asking what distinguishes RV144 from VAX003 given that the former had an estimated efficacy of more than 31.2% while the latter had none. As expected, substantial inter-trial and-subject heterogeneities were observed, where hierarchical clustering largely grouped the samples based on the trial/immunogen type. The authors then turned to dimensionality reduction and classification techniques to identify Ab features that discriminate between sample classes. Interestingly, only seven features were needed to nicely differentiate between samples from RV144 and VAX003 regimens. Analysis with all four regimens revealed similarities between RV144 and Ad26, due in part to their shared IgG3 responses and Abs targeting the V1V2 domain of the HIV-1 envelope. Together with existing evidence that links these two features with reduced risk of infection and data from a regimen similar to the Ad26 trial showing protective effects in animals [2], these results offer hope that Ad26 may elicit similar or better protection in humans. Questions remain on whether the observed grouping of samples was primarily driven by the vaccine or by some latent variable(s) associated with individual trials/cohorts. For example, samples from the four trials were taken at different time points with respect to the initial vaccination, subject to varying degrees of additional exposure during the vaccination period. Ultimately, hypotheses generated from these analyses should be tested using additional, independent cohorts.

The authors next focused on the RV144 trial and applied machine-learning approaches to ask whether additional Ab features correlate with IgG V1V2 and IgG3 V1V2 responses, which are known to be positively associated with protection. Strong IgG V1V2 and IgG3 V1V2 responses (i.e., in the top 33% of each respective variable) correlated with higher Ab-dependent cellular activities. Specifically, IgG3 V1V2 responders tend to carry Abs that bound more effectively to the activating Fc receptors FCGR2A and FCGR3A, which are involved in Ab dependent cellular phagocytosis, NK cell degranulation and chemokine secretion [2]. Interestingly, poor responders as determined by amounts of IgG V1V2 had increased levels of gp120-specific IgA, which is associated with elevated risk of infection following vaccination. Furthermore, antibodies from the plasma of these patients bound strongly to the only known inhibitory Fc γ receptor (FCGR2B), which can attenuate B-cell receptor signaling upon binding to immune complexes [4].

Correlation among Ab features can be depicted as a functional interaction network wherein nodes are Ab features and a connection (or an edge) between two nodes denotes statistically significant correlation. This analysis can be performed within distinct groups of subjects and the resulting networks (one per subject group) can be comparatively assessed to reveal functional interactions that are potentially specific to a group of subjects [5,6] (Figure 1). This type of differential network analyses have, for example, provided insight into the mechanisms driving distinct cancer subtypes [5]. Chung *et al.* applied this approach to compare the correlation network across trials, and to assess network-level differences between good vs. poor responders in the RV144 trial, where the quality of the response was defined based on the levels of IgG V1V2 and gp120-specific IgA. Interestingly, this analysis revealed that in the good-responder group (i.e., carrying lower risk for infections, defined as IgG V1V2^{high}/IgA gp120^{low}), IgA features were isolated, forming an “island”, and were not linked to the more connected IgG and IgG3 features. The poor-responder group (IgG V1V2^{low}/IgA^{high}) had a more fragmented network, wherein some of the Ab-dependent functional properties were disconnected from the IgG and IgG3 features. By similarly comparing networks constructed from each of the four vaccine regimens, the authors also observed regimen specific patterns of functional connections among Ab features. While it remains to be evaluated, e.g., by using quantitative network comparison methods [5,6], the extent to which such network signatures of good vs. poor vaccination responses can be attributed to random effects (e.g., measurement and subject sampling noise), this study points the way to the use of network-based features for assessing vaccination outcomes.

Past approaches for evaluating vaccine efficacy have largely focused on probing a few aspects of neutralization and Ab functions. Chung *et al.* have nicely illustrated the potential of a high-dimensional approach in deciphering robust correlates of protection following HIV vaccination. Further integration of these Ab features with other data types (e.g., blood transcriptomes) will offer opportunities for uncovering molecular and cellular predictors of protection from early time-points following vaccination, or even at baseline before vaccination [7,8]. This approach can also be used to assess humoral responses in a longitudinal manner, particularly given that the timing of peak Ab responses can vary across subjects and vaccines [1]. Integration with Ab repertoire data provides further intriguing possibilities, such as linking specific Ab clones and past exposure of an individual to Ab-dependent cellular function [9]. Continued development and application of such systems immunology approaches will help us achieve an increasingly detailed view of the immune system that may ultimately lead to better vaccine designs and a more quantitative understanding of how immune responses are orchestrated.

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References

1. Tsang JS. Utilizing population variation, vaccination, and systems biology to study human immunology. *Trends Immunol.* 2015; 36:479–493. [PubMed: 26187853]

2. Chung AW, et al. Dissecting Polyclonal Vaccine-Induced Humoral Immunity against HIV Using Systems Serology. *Cell*. 2015; 163:988–998. [PubMed: 26544943]
3. Plotkin SA, Plotkin SA. Correlates of Vaccine-Induced Immunity. *Clin Infect Dis*. 2008; 47:401–409. [PubMed: 18558875]
4. Takai T. Roles of Fc receptors in autoimmunity. *Nat Rev Immunol*. 2002; 2:580–592. [PubMed: 12154377]
5. Ideker T, Krogan NJ. Differential network biology. *Mol Syst Biol*. 2012; 8
6. de la Fuente A. From “differential expression” to “differential networking” identification of dysfunctional regulatory networks in diseases. *Trends Genet*. 2010; 26:326–333. [PubMed: 20570387]
7. Tsang JS, et al. Global Analyses of Human Immune Variation Reveal Baseline Predictors of Postvaccination Responses. *Cell*. 2014; 157:499–513. [PubMed: 24725414]
8. Furman D, et al. Apoptosis and other immune biomarkers predict influenza vaccine responsiveness. *Mol Syst Biol*. 2013; 9:659. [PubMed: 23591775]
9. Robinson WH. Sequencing the functional antibody repertoire—diagnostic and therapeutic discovery. *Nat Rev Rheumatol*. 2015; 11:171–182. [PubMed: 25536486]

Figure 1. Constructing and comparing correlation networks across groups of human subjects Here nodes denote antibody features (e.g., binding affinity to certain antigens) and an edge between a pair of nodes denotes statistically significant correlation between the two features among individuals in a particular group (depicted here are good vs. poor responders to vaccination.) The two correlation networks depicted here have both shared and distinct edges, such as the edge between features A and B where a significant correlation can be detected in the good responder group (blue subjects), but not in the poor responder group (red subjects). The presence of significant correlation (or connection) between features A and B as well as between C and D in the good-responder network bridges the green and orange subnetworks, which are disconnected in the poor-responder network. Since different sources of noise can contribute to correlation strength among variables, quantitative analyses are needed to formally assess whether an observed difference in correlation between two

nodes is statistically significant (see refs. [5,6] for further details and references to analysis approaches.)