Published in final edited form as:

Trends Microbiol. 2014 September ; 22(9): 488–489. doi:10.1016/j.tim.2014.07.005.

Random yet deterministic: convergent immunoglobulin responses to influenza

Andrew Martins¹ and John S. Tsang^{1,2}

¹Systems Genomics and Bioinformatics Unit, Laboratory of Systems Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health

²Trans-NIH Center for Human Immunology, National Institutes of Health

Abstract

B-cell clonal expansion is a hallmark of host-defense and vaccination responses. Given the vast immunoglobulin repertoire, individuals may expand B cells carrying largely distinct immunoglobulin genes following antigenic challenge. Using immunoglobulin-repertoire sequencing to dynamically track responses to influenza vaccination, Jackson *et al.* find evidence of convergent immunoglobulin responses across individuals.

Immunologists have long been fascinated by the immune system's ability to generate antibodies specific to diverse arrays of unforeseen antigens. B cells are at the center of this 'magic': individual B cells express rearranged, mutated versions of the germline-encoded immunoglobulin (IG) genes via V-(D)-J recombination and somatic hypermutation (SHM) to produce antibodies that recognize distinct antigens (Figure 1A). Upon pathogen or vaccine exposure, for example, B cells capable of binding antigens undergo clonal expansion, further maturation of their antigen-recognition abilities via SHM, and differentiation into (and further expansion as) plasmablasts (i.e., antibody-producing factories) or long-lived plasma/memory cells [1]. As a key mechanism underlying host-defense and vaccination responses, a detailed understanding of IG responses to immune challenges in humans is of upmost medical importance. However, despite decades of advances, open questions remain. For instance, how do the quantity and diversity of B-cell clones evolve upon challenge? To what extent are clonal responses shared across individuals for a given antigen? How do inter-subject variations correlate with differences in outcome, e.g., level of protection or disease severity? Using high-throughput sequencing of the IG variable regions (Figure 1B), these issues can now be investigated in great depth by large-scale assessment of the IG repertoire and its clonal structure [2]. This powerful approach was recently utilized by Jackson *et al.* to examine the B-cell response to influenza vaccination in humans [3].

Corresponding author: Tsang, J.S. (john.tsang@nih.gov).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

A hallmark of vaccination responses is the generation of short-lived plasma cells and formation of germinal centers, from which high affinity long-lived antibody-producing plasma and memory B cells derive. By one week after influenza immunization, a strong but transient plasmablast response can be detected in the blood [4]. Accordingly, Jackson *et al.* observed a substantial increase in the number and size of B cell clones [inferred by sequence clustering (Figure 1B)] seven days post vaccination, particularly in high serological responders. Similar to the level of plasmablast expansion, increases in the number of clones correlate with the magnitude of the vaccine-specific antibody response. In addition, poor responders show lower levels of SHM. By comparing the sequences to those obtained from single plasmablasts isolated from the same subjects with previously determined antigen specificity [5], the authors estimated that a sizable fraction of the expanded clonal populations are influenza specific. However, many sequences also do not overlap with those from plasmablasts. Characterizing additional single plasmablasts could lead to more overlaps, but this result also suggests that some clones could have expanded prior to plasma cell differentiation or in other B cell subsets (e.g., memory cells). IG repertoire sequencing of sorted subsets or single B cells could shed further light on this issue.

The authors next obtained a high-resolution view of clonal expansion by following the response of one individual daily over a period of 14 days after immunization with the single-agent 2009 pandemic H1N1 (pH1N1) vaccine. Mirroring the dynamics of blood plasmablast frequencies following vaccination [6], the number of expanded clones peaks around day 7, rapidly subsides towards day 10, and falls out of detection by day 14. Of the 256 clonal lineages detected, 11 were strongly induced by the vaccine. Over half of these shared a stereotypic immunoglobulin heavy-chain (IGH) gene usage pattern, suggesting that this rearrangement may be particularly effective for recognizing pH1N1 antigens. Remarkably, this usage pattern and much of the mutated positions are shared by pH1N1-reactive clones obtained from independent, pH1N1-infected donors in two previous studies [7,8]. Otherwise, they have rarely been seen in this or other studied subjects prior to pH1N1 emergence. Of the over 500 000 IGH sequences obtained from different individuals before 2009, only one appeared similar to the stereotypic clones, further highlighting the rarity of circulating B cells carrying these particular IGH genes before pH1N1 exposure. Intriguingly, these stereotypic clones were not induced following 2010 seasonal influenza vaccination (which includes the pH1N1 strain) in the same subject. Instead, clones with the same gene usage but distinct somatic mutations were detected, which could reflect, for example, expansion of new clones or further maturation of clones induced by the 2009 vaccine.

This duality of convergent (across subjects) but yet divergent (even within the same subject following second immunization) IGH responses to pH1N1 highlights the complexity of immune responses to influenza in humans. It is interesting to see convergent responses at the sequence level given the potentially large number of IGH gene-rearrangement and somatic-mutation combinations capable of robust antigen recognition. On the other hand, annual boosting by seasonal vaccination and occasional emergence of antigenic shifts (e.g., 2009 pH1N1) can give rise to complex memory responses upon antigen encounter [9], including the convergent (across subjects), broadly reactive (across influenza strains), hemagglutinin stem-targeted responses following pH1N1 infection reported previously [8]. It will be interesting to assess, for example, whether or not the convergent responses seen by Jackson

and colleagues originated from memory or naïve cells. Applying the authors' approach to antigens not previously seen by the host (e.g., the yellow fever vaccine) could reveal the extent and prevalence of convergent responses originating from cells naïve to the antigen. In general, a more comprehensive understanding of the mapping between antigens and their stereotypic responses could allow the reconstruction of an individual's exposure history via the sequencing of his/her IG repertoire, which could have important epidemiologic and diagnostic value.

Building upon Jackson *et al.*'s findings, additional larger-scale studies will help shed light on the type of stereotypic responses most beneficial to target for optimal vaccine effectiveness. With continuing advances in sequencing technology, we will soon be able to obtain deep IG repertoires before and after diverse immune perturbations across a large number of subjects with high temporal resolution. It will be of great interest to use such data to assess the intra- and inter-subject variations as well as the extent of convergent IG responses at the sequence level. Together with simultaneous multi-modal monitoring of immune statuses (e.g., cell subset frequencies, blood transcriptomes and serum cytokine levels) [10], we will be poised to uncover biomarkers and build quantitative, predictive models of IG responses in health and disease.

Acknowledgments

This work was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases at the National Institutes of Health.

References

1. LeBien TW, Tedder TF. B lymphocytes: how they develop and function. *Blood*. 2008; 112:1570–1580. [PubMed: 18725575]
2. Georgiou G, et al. The promise and challenge of high-throughput sequencing of the antibody repertoire. *Nat Biotechnol*. 2014; 32:158–168. [PubMed: 24441474]
3. Jackson KJL, et al. Human responses to influenza vaccination show seroconversion signatures and convergent antibody rearrangements. *Cell Host Microbe*. 2014; 16:105–114. [PubMed: 24981332]
4. Wrammert J, et al. Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. *Nature*. 2008; 453:667–671. [PubMed: 18449194]
5. Moody MA, et al. H3N2 influenza infection elicits more cross-reactive and less clonally expanded anti-hemagglutinin antibodies than influenza vaccination. *PLoS ONE*. 2011; 6:e25797. [PubMed: 22039424]
6. Obermoser G, et al. Systems scale interactive exploration reveals quantitative and qualitative differences in response to influenza and pneumococcal vaccines. *Immunity*. 2013; 38:831–844. [PubMed: 23601689]
7. Krause JC, et al. Epitope-specific human influenza antibody repertoires diversify by B cell intraclonal sequence divergence and interclonal convergence. *J Immunol*. 2011; 187:3704–3711. [PubMed: 21880983]
8. Wrammert J, et al. Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 influenza virus infection. *J Exp Med*. 2011; 208:181–193. [PubMed: 21220454]
9. Palese P, Wang TT. Why do influenza virus subtypes die out? A hypothesis. *mBio*. 2011; 2:e00150–11. [PubMed: 21878571]
10. Tsang JS, et al. Global analyses of human immune variation reveal baseline predictors of postvaccination responses. *Cell*. 2014; 157:499–513. [PubMed: 24725414]

Figure 1.

Probing immunoglobulin diversity and clonal structure using high-throughput sequencing. **(A)** Combinatorial joining of different V, D, and J gene segments, the removal of and/or addition of non-templated nucleotides at junctions, together with somatic hypermutation at the immunoglobulin heavy-chain (IGH) locus generate tremendous sequence diversity (and therefore antigen recognition diversity) among different B cells. **(B)** To analyze the B-cell repertoire by sequencing, the IGH locus is PCR amplified from genomic DNA isolated from pools of B cells to generate sequencing libraries. These libraries are subjected to 454 pyrosequencing, where a random subset of the amplified IGH fragments is sequenced. As B-cell clones have largely unique IGH sequences, clustering analysis of the sequences can reveal the clonal structure of the B cells in the sample.