Mining the Antibody Repertoire for Solutions to SARS-CoV-2

Wenzhao Meng,1 Aaron M. Rosenfeld,1 and Eline T. Luning Prak1,2,*
1Perelman School of Medicine, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
2Institute for Immunology University of Pennsylvania, Philadelphia, PA 19104, USA
*Correspondence: luning@pennmedicine.upenn.edu
https://doi.org/10.1016/j.chom.2020.09.010

In this issue of Cell Host & Microbe, Nielsen and colleagues sequence antibody repertoires of patients with severe COVID-19 to reveal potentially convergent features on the background of a larger, polyclonal response. Their findings suggest that, as databases improve, it may be possible to monitor virus-specific B cells after infection or vaccination using antibody sequencing.

The antibody repertoire, or collection of different B-cell clones that produce distinct antibodies, is large, diverse, and complex. Antibody repertoire diversity is achieved through the recombination of Variable, Diversity, and Joining gene segments during primary B-cell maturation as well as by DNA point hypermutation (somatic hypermutation [SHM]) during an immune response. The unit of B-cell selection is the clone, or a collection of B cells that produce highly related antibodies and derive from the same precursor cell. During the immune response to a pathogen such as a virus, B cells that recognize the virus typically undergo clonal expansion and SHM. However, in the case of patients with severe SARS-CoV-2 infection, the dynamics of the B-cell immune response are incompletely understood. Several early repertoire studies on COVID-19 homed in on virus-specific antibody responses (Ju et al., 2020) (Cao et al., 2020) (Robbiani et al., 2020) with the goal of generating candidate antibodies for passive immunotherapy. The important contribution of the paper by Nielsen et al. (2020) is that the investigators profile the overall immune repertoire but also attempt to link specific clones within this larger repertoire to virus-binding antibodies. The long-term aim of antibody repertoire studies, coupled with antigen specificity data, is to arrive at immune profiles that could reveal who is immune and who is at risk of developing immunopathology.

Nielsen and colleagues profiled the antibody repertoires of 13 hospitalized patients with PCR-confirmed COVID-19 infection. A significant strength of their study is that their patients were surveyed at multiple time points, providing an in-depth view of how the antibody repertoire changes during early infection. All of their patients had detectable antibodies to the receptor binding domain (RBD) portion of the SARS-CoV-2 spike protein at one or more time points. Peripheral blood mononuclear cells were analyzed by sequencing from both genomic DNA, providing general insights into the clonal landscape (diversity, clone burst size), and from RNA, providing antibody isotyping (e.g., IgM, IgG, IgA, etc.) and high-fidelity sequences for SHM analysis. Their dataset comprised over 1.2 million clones in a total of 38 samples. For comparison, they used a control dataset derived from 114 healthy adults from one of their previous studies. In addition, selected samples were subjected to single-cell sequencing to obtain paired antibody heavy-chain and light-chain reads for antibody cloning and specificity analysis.

When Nielsen and colleagues surveyed the repertoire as a whole in COVID-19 patients, they noted a diverse collection of expanded B-cell clones. Their findings are consistent with other reports, which also document a diverse polyclonal response accompanied by large clonal expansions that are associated with high levels of circulating plasmablasts (Kuri-Cervantes et al., 2020) (Kaneko et al., 2020). Furthermore, Nielsen and colleagues noted that several of the expanded clones harbored no or low levels of SHM. Even many of the class-switched clones had low SHM, and the authors commented that they have observed similar low-SHM class-switched clones in immune responses to other severe infections, such as Ebola. The plasmablast expansion and low level of SHM could be due to an extrafollicular immune response in the context of severe or early disease. Indeed, Woodruff and colleagues report an increased frequency of double-negative (CD27–IgD–) B cells and plasmablasts in severe COVID-19 infection, commenting that similar abnormalities occur in the patients with systemic lupus erythematosus who are experiencing disease flares (Woodruff et al., 2020). Also consistent with an extrafollicular immune response, Kaneko and colleagues recently reported the loss of T follicular helper cells and germinal centers in the tissues of COVID-19 autopsy cases (Kaneko et al., 2020). An extrafollicular immune response could represent an “all hands on deck” response that is characterized by bystander activation of whatever is available, with viral injury and inflammation short-circuiting the conventional T-cell-dependent immune response. It is not yet known if this response eventually becomes T-cell and germinal-center dependent in individuals who recover from the virus.

The second area of focus of the Nielsen paper was a description of potentially convergent features of the immune repertoire in SARS-CoV-2, using three different approaches. Their first approach was to look for IGHV (human immunoglobulin heavy chain variable) genes that were enriched in patients than in controls. They observed increased usage of certain IGHV genes among IgG clones in comparison with healthy controls, including
IGHV1-24, IGHV3-9, IGHV3-13, and IGHV3-20. However, the same IGHV genes were decreased in expanded versus non-expanded B-cell populations in their COVID-19 patients. Furthermore, several other IGHV genes that have been documented in other studies of SARS-CoV-2 RBD-binding antibodies, including IGHV1-2, IGHV1-69, IGHV3-30-3, IGHV3-23, IGHV3-48, IGHV3-53, and IGHV3-66, were not enriched in expanded versus non-expanded clones. Their second approach was to scrutinize the amino acid sequence of the third complementarity-determining region (CDR3) for evidence of similarities among different individuals. This analysis revealed that many clones from patients harbored elongated CDR3 sequences, some with hydrophobic amino acids, consistent with other published findings (Cao et al., 2020; Robbiani et al., 2020). However, very few antibodies with specific long CDR3 sequences are shared between different individuals with severe COVID-19 (Kuri-Cervantes et al., 2020). Nevertheless, the frequent finding of longer CDR3-containing antibodies in severe COVID-19 infection could point to a shared mechanism for their genesis. One possibility is that B cells with long CDR3 antibodies are multi-reactive (Wardemann et al., 2003) and thereby are preferentially recruited into the response. Nielsen’s third approach was to use a combination of IGHV, IGHJ, and CDR3 sequence features to map the repertoire into “clusters” that were shared by SARS-CoV-2 patients but not by controls. Working from two sets of IGHV-IGHJ-CDR3 clusters, the authors generated monoclonal antibodies that were found to bind to the SARS-CoV-2 spike protein and S1 portion of the spike protein, but not to the RBD. Although this result suggests that these clusters are related to the immune response to SARS-CoV-2, how useful these clusters are as a general signature of SARS-CoV-2 infection is unclear, because even the most shared clusters were only found in five out of the 13 patients. Finally, the authors were able to recover antibodies that were similar to RBD-binding clones found in the literature in most but not all of their COVID-19 individuals.

Defining an immune signature for SARS-CoV-2 is challenging. There is considerable disease heterogeneity in COVID-19, and antibody responses vary. Global repertoire studies have focused mostly on early disease, but anti-viral antibodies could be more distinctive if they are acquired later in the immune response, after rounds of selection and affinity maturation. Most studies have sampled the blood, which is filled with numerous B- and T-cell clones, most of which are unrelated to SARS-CoV-2. Tissue-based B-cell clones differ from those in the circulation (Meng et al., 2017) and could be more relevant for disease. Compounding all of these difficulties, the most relevant antibodies for SARS-CoV-2 neutralization (e.g., those that bind to the RBD), as Nielsen and colleagues note, are very infrequent in the blood, despite readily detectable serum antibodies. Finally, antibodies that are produced in immune responses can often be private, e.g., individuals generate their own immune “solutions” to the virus rather than necessarily producing an antibody that “read the textbook.” But inherent in this final limitation is the alternative possibility that the textbook isn’t detailed enough. Public databases of antigen-specific SARS-CoV-2 antibody sequences were still quite limited when these studies were initiated. As the authors point out, their study represents a starting point and a useful reference library of sequences that could be related to the virus. How exactly they are related awaits further studies of the immune response to the virus—not just to the spike protein, but to other viral antigens as well.

To accelerate progress toward defining the most relevant immune repertoire features of SARS-CoV-2, data need to be shared, as Nielsen and colleagues have done in exemplary fashion. They shared their data before publication using Adaptive Immune Receptor Repertoire (AIRR) Community metadata information standards, so that their data could be compared to data from other studies (Rubelt et al., 2017). Their data are available on the NCBI Sequence Read Archive as well as on the AIRR Data Communications via the iReceptor portal. COVID-19 immune repertoire data and publications are being compiled at the B–T.CR website and in partnerships such as “ImmuneCODE” between Adaptive Biotechnologies and Microsoft for T–cell receptor rearrangements. Parallel efforts are underway to generate datasets on antigen-specific B-cell and T–cell motifs through the Immune Epitope Database (IEDB), VDJdb, and CoV-AbDab, to name a few. As more data are made available via public repositories, immune repertoires from different studies can be merged to provide a better reference library of antigen-specific and disease-associated clonotypes. This shared undertaking will result in a more comprehensive understanding of the immune response in SARS-CoV-2 for all.

ACKNOWLEDGMENTS
E.T.L.P. is funded by NIH grants P01 AI106697, UM1 AI44288, P30 AI045080, P30 CA016520, and UC4 DK112217.

DECLARATION OF INTERESTS
E.T.L.P. is a paid consultant for Enpicom, B.V., a software company that develops tools for data analysis and visualization of immune repertoires.

REFERENCES
Cao, Y., Su, B., Guo, X., Sun, W., Deng, Y., Bao, L., Zhu, Q., Zhang, X., Zheng, Y., Geng, C., et al. (2020). Potent Neutralizing Antibodies against SARS-CoV-2 Identified by High-Throughput Single-Cell Sequencing of Convalescent Patients’ B Cells. Cell 182, 73–84.e16.
Ju, B., Zhang, Q., Ge, J., Wang, R., Sun, J., Ge, X., Yu, J., Shan, S., Zhou, B., Song, S., et al. (2020). Human neutralizing antibodies elicited by SARS-CoV-2 infection. Nature 584, 115–119.
Kaneko, N., Kuo, H.H., Boucau, J., Farmer, J.R., Allard-Chamard, H., Mahajan, V.S., Piechocka-Trocha, A., Lettler, K., Osborn, M., Bals, J., et al.; Massachusetts Consortium on Pathogen Readiness Specimen Working Group (2020). Loss of BCL6-Expressing T Follicular Helper Cells and Germinal Centers in COVID-19. Cell 182, S0092-8674(20)31067-9.
Kuri-Cervantes, L., Pampena, M.B., Meng, W., Rosenfeld, A.M., Ittner, C.A.G., Weisman, A.R., Agyeukum, R.S., Mathew, D., Baxter, A.E., Vella, L.A., et al. (2020). Comprehensive mapping of immune perturbations associated with severe COVID-19. Sci. Immunol. 5, 5.
Meng, W., Zhang, B., Schwartz, G.W., Rosenfeld, A.M., Ren, D., Thome, J.J.C., Carpenter, D.J., Matsuoka, N., Lerner, H., Friedman, A.L., et al. (2017). An atlas of B-cell clonal distribution in the human body. Nat. Biotechnol. 35, 879–884.
Nielsen, S.C.A., Yang, F., Jackson, K.J.L., Hoh, R.A., Rödtgen, K., Stevens, B., Lee, J.Y., Rustagi, A., Rogers, A.J., Powell, A.E., et al. (2020). Human B cell clonal expansion and convergent antibody responses to SARS-CoV-2. Cell Host Microbe 28, this issue, 516–525.
The intestinal microbiota promote myriad functions that affect the host. In this issue of Cell Host & Microbe, Caballero-Flores et al. (2020) demonstrate that resident microbes can thwart gut colonization by the enteric bacterial pathogen Citrobacter rodentium by consuming amino acids, thus starving the invading organism of essential nutrients.

Microbes of the gut serve their animal hosts well: present in vast numbers, they provide essential nutrients, foster the development of intestinal tissues, and modulate immune responses (Pickard et al., 2017; Sekirov et al., 2010). Among their most important benefits, however, is their ability to ward off attacks by enteric pathogenic bacteria, preventing the proliferation of these disease-causing invaders of the intestine. This function, termed colonization resistance, is itself multi-faceted, as resident microbes both bolster the immune responses of the host as well as create a chemical and physical environment conducive to pathogen proliferation (Bäumler and Sperandio, 2016). In this issue, Caballero-Flores et al. propose an additional mechanism by which gut microbes resist such attacks; the microbiota compete with bacterial pathogens for amino acids essential for survival, and thus starve them into submission.

The authors employed as their model Citrobacter rodentium, a pathogen of mice long used to study human infections with enteropathogenic and enterohemorrhagic E. coli, with which it shares virulence mechanisms (Collins et al., 2014; Kaper et al., 2004; Mundy et al., 2005). They sought to discover metabolic functions required for the survival of this enteric pathogen within the gut in the presence of the microbiota, but not in its absence, thus identifying means of colonization resistance that require the resident microbes. To accomplish this, they used the powerful tool of transposon sequencing (van Opijnen et al., 2009), randomly generating an exhaustive library of C. rodentium mutants and using this pool to infect either germ-free mice or conventional mice harboring an intact microbiota. Upon sequencing the feces of both groups, they discovered in the conventional mice a lack of C. rodentium mutants defective in a wide range of amino-acid synthesis pathways, including those for arginine, threonine, histidine, and tryptophan, as well as the branched-chain amino acids. This paucity of mutants, not observed in the germ-free mice, suggested that the synthesis of amino acids was required for C. rodentium to effectively compete with the normal microbial inhabitants of the gut. To confirm these findings, the authors next tested specific mutants deficient in the production of arginine, threonine, histidine, tryptophan, or isoleucine for their ability to survive in mice. Although these mutants demonstrated no defect when grown in a rich laboratory medium, they competed poorly for growth in conventional mice when compared to wild-type C. rodentium. No growth difference, however, existed between the wild-type and mutant strains in germ-free mice, again implicating the microbiota as essential to colonization resistance. If the synthesis of amino acids were required for this pathogen to proliferate in competition with the microbiota, one might reasonably expect these same metabolic functions to be induced in C. rodentium while residing within a mouse. This was indeed the case, as expression of genes from these same pathways was found to be higher in conventional mice than in germ-free mice. Finally, the authors posed a pointed and important question: Can colonization resistance be overcome by diet? To answer this, they compared mice fed a typical laboratory chow with those fed a diet containing on average 3.5-fold more protein. When administered a high inoculum of C. rodentium (~10^7 cf.u), mice fed the protein-rich diet were colonized more heavily during the first week after infection, an effect that was lost in mice treated with antibiotics to reduce the density of the microbiota. Remarkably, feeding this same diet for a single day,