High-resolution snapshots of antibody repertoires as potential correlates of protection

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Klaus Eyer from ETH Zurich describes high-resolution snapshots of antibody repertoires as potential correlates of protection

The induction and long-term presence of key adaptive immune cells are essential for vaccine-mediated protection. As a direct, causal measure of protection is often challenging; finding and defining correlates of protection are critical for vaccine development, approval, and efficacy testing. Correlates of protection are quantifiable signs of being protected from becoming infected and/or developing disease that may be causal to protection itself.

Antibodies in serum are often assessed as potential correlates as immunisations often lead to generating a vaccine-specific, functionally active and diverse antibody repertoire. In its simplest form, a potential correlate could be their presence if binding by the antibody is sufficient. This is assessed in the so-called titer measurements. Here, a serum sample is sequentially diluted, and the binding of the antibodies towards the vaccine is evaluated and compared to an often empirically derived threshold. This gold-standard measure has many advantages: it is fast, reliable and easily compared.

However, binding itself is often insufficient, and titer measures are often not a good correlate for antibody-mediated protection. Indeed, binding often initiates a complicated cascade of secondary antibody functions, such as neutralisation, antibody-dependent cytotoxicity, complement activation or phagocytosis. These are potent secondary effects potentially induced by certain binding antibodies.

Therefore, specific functionalities are often assayed, such as a virus neutralisation titer or complement activation. This is important as not every specific antibody can induce all functionalities, and certain functionalities can also be beneficial for particular pathogens, leading to antibody-dependent enhancement. Therefore, the induced functionalities must be carefully balanced, and specific functionalities must be often measured.

Antibody repertoires: Why do serum measures not always provide the necessary protection?

Serum measurements do not always provide good correlates of protection, even for vaccines where protection is supposed to be, at least partly, antibody-mediated. This could have several reasons, and only a selection is named here.

Firstly, the assays might not test the responsible immune functionality, or protection could be mediated by a mix of functionalities.

Secondly, these assays do not decipher the extensive antibody heterogeneity. Essentially, thousands of different antibodies are present in blood at a given time, and only a few tens to hundreds will bind to the antigens present in the vaccine. Even within this group, only a small fraction will be functionally active.

Thirdly, rapid changes and dynamics of an active immune response, when the antibody-secreting cells are generated, selected, matured, and differentiated, are lost in large in vivo distribution volumes and half-lives, leading to the masking of critical intermediary stages and immune decision points. Therefore, rapid processes and changes are masked, and the titer represents an integrated measurement of the recent and, sometimes, distant history of experienced infections and immunisations.

However, there are alternative approaches to this problem. Each antibody present in serum is produced by a specialised cell, and interestingly, each cell will only secrete an individual antibody at a given time. Therefore, instead of measuring the functional average in serum, a single-cell-based analysis will provide a functional overview of all antibodies individually, dissecting the complex response into its components. This analysis, however, is not a standard off-the-shelf analysis.

Measuring the quality and functionality of antibodies

Therefore, researchers in the FuncMab project started in 2019 to develop microfluidic technologies and specific assays that allow for the functional characterisation of each secreted antibody individually. The developed systems measure the presence and functionality of antibodies on the cellular level and employs a microfluidic technique to create small containers, each containing one cell and a surrogate assay for the functionality of interest.

The secreted antibody accumulates within the droplet, and the surrogate assay determines the functionality of the secreted antibody, ranging from simple binding to complement activation to neutralisation. At its core, this approach generates a high- resolution snapshot of the induced antibody repertoire and deciphers for each antibody quantity, quality, and functionality.

Over the last few years, the members of the FuncMAb team have developed and validated various assays to measure and determine antibody functions after immunisation, intending to provide additional insights into these highly complex responses, firstly in murine immunisation studies. Various vaccination components are systematically altered in these studies, and their influence on the repertoire's quantity, quality, and functionality are assessed.

Here, the analysis of protein secretion with single-cell resolution can provide additional analytical and kinetic insights into the highly dynamic and complex immune response. Exemplarily, antibodies that activate the complement system are critical in the immune response against pathogenic bacteria and could be used as a potential correlate of protection. Dr Nathan Aymerich, an involved researcher in the FuncMAb project, developed a bioassay to quantify complement activation at the single-antibody level and applied this assay to characterise antibacterial repertoires focusing on the pathogenic bacteria P. aeruginosa, B. pertussis, and N. meningitidis.

This allowed us to gain novel insights into the capability of monoclonal antibodies to induce complement activation, and the variation of immunisation allowed us to study the influence of bacterial vaccine compositions on the frequency and efficiency of this process. These findings are currently under review and represent an illustrative example of the insights generated by this project.

Early stages of Experimentation

However, the FuncMAb findings are unlikely to be immediately translated into clinical applications, as this research is still at an early stage and the experiments performed in murine models. The developed assays might be interesting additions, not a replacement, to serum measurements, as several critical components are absent in the single-cell approach, such as collaborative binding or competition.

Interestingly, we regularly observe an absence of correlation between our immunological snapshots and the conducted titer measurement. This is somewhat expected and illustrates the potential to look for correlates of protection in the highly-resolved antibody repertoires even if the titer does not show any.

However, these assays are broadly applicable, and the team has started to apply them in various proof-of-concept studies beyond the initial scope, studying antibody-repertoires in auto-immune patients, clinical studies for anti-allergic treatments, and fundamental studies of rare genetic immune disorders. The developed assays are of additional interest for functional antibody screenings, potentially leading to novel therapeutic candidates to overcome antibacterial-resistant infections in the example mentioned above.

Furthermore, the high-resolution images of the antibody repertoire allowed us to generate models for in silico modeling. Also, they resulted in a significant reduction of needed animals in the project. The concepts have also led to the creation of a start-up, Saber Bio, with the goal of developing instruments enabling other researchers, clinicians and companies to perform these measures.

Understanding post-vaccination protection and lack thereof

Lastly, the enhanced precision in assessing individual antibody repertoires could also potentially lead to new insights into the reasons behind the lack of post-vaccination protection and vaccine safety, especially when combined with the analysis of other critical immune functions.

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