Neutralizing Antibodies to SARS-CoV-2: An Important Mechanism of Immunity

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The global COVID-19 pandemic has put the entire scientific and medical community to test with the urgent need to understand this novel virus (SARS-CoV-2) and the new disease (COVID-19).[1,2] While the diagnostic industry has responded to the call in a record speed and gained substantial information, an emerging focus is to understand whether patients recovered from COVID-19 will develop protection or immunity.[3,4] Knowledge about acquired immunity to COVID-19 not only provides valuable insights for the development of vaccines and therapeutics but also enable the implementation of suitable policies and strategies for effective pandemic control.[5,6]

WHAT DO WE MEAN BY IMMUNITY? AND WHAT ARE NEUTRALIZING ANTIBODIES?

Immunity is the ability to resist a disease or an infection. Immunity to viral infection is a combined outcome of both *cellular* and *humoral* (antibody) immune responses. While cellular immune response is often difficult to measure, antibody production serves as a hallmark of humoral immune response.[6]

Neutralizing antibody is a subset of antibody that can inhibit viral replication and represents a major mechanism of humoral immunity against viral infection.[7] The protective function of neutralizing antibodies is mainly mediated by the blocking of the interaction between the virus and its host cells, resulting in the inhibition of viral entry to cells, thus, prevents viral infection.[7] Because of the mechanism of action, it is not surprising that most neutralizing antibodies are against *viral surface proteins.*[7]

WHAT DO WE KNOW ABOUT NEUTRALIZING ANTIBODIES TO SARS-CoV-2?

The virus causing COVID-19, SARS-CoV-2, uses the Spike protein (S) to bind to the receptor on host cells to trigger cell entry and infection. S protein consists of S1 and S2 subunits, and S1 interacts with the host cells via the Receptor Binding Domain (RBD).[8] Monoclonal antibodies to S1 protein that exhibit neutralizing activities are being developed as potential therapeutics for COVID-19.[9,10] Neutralizing antibodies to the S protein are the key active ingredients of convalescent plasma used to treat severe COVID-19 patients.[11] In addition, almost all the COVID-19 vaccines currently under development target the S protein with the goal as to induce neutralizing anti-S antibodies.[12] Preliminary vaccine studies showed a correlation between neutralizing antibody titers with protective efficacy in animal models.[13]

All these observations suggest that S protein is a primary target for neutralizing antibodies against SARS-CoV-2, and anti-S neutralizing antibodies play a key role in COVID-19 immunity.

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Humoral immune response and antibody production after SARS-CoV-2 infection have not been fully understood in various COVID-19 patient populations. Existing studies clearly showed that not all COVID-19 patients produced detectable antibodies, and certainly not all patients developed neutralizing antibodies.[14,15] Even among convalescent plasma donors, around 10% samples did not contain detectable neutralizing activities, putting the therapeutic efficacy of these convalescent plasma in question.[16] Therefore, measuring neutralizing antibody activities is important to ensure qualified convalescent plasma are used to achieve desirable therapeutic outcomes as well as to support the development of COVID-19 vaccines.[16] In addition, given the heterogeneity of the immune responses among COVID-19 patients, it is important to assess neutralizing antibody activities for individual patient as well as in populations to help to address the key questions about COVID-19 immunity:

1. **What is the neutralizing antibody level sufficient to provide a complete protection?**
2. **How long does the protection last?**

HOW ARE NEUTRALIZING ANTIBODIES TYPICALLY MEASURED? WHAT ARE THE CHALLENGES?

Neutralizing antibody activity is typically measured by biological assays mimicking viral infection in cultured cells. The tests are time-consuming, labor-intensive, and are low throughput. The operational complexities of the tests make them unfeasible for scaled up routine testing in large populations.[16,17] A recent publication reported a modified neutralization assay with increased throughput.

There is a clear need to develop automated, high throughput, and easy to operate serological tests as potential surrogate tests to evaluate neutralizing antibody activities in a large patient population.

However, the test system still requires viral particles and live cells, which can be difficult to implement in a typical clinical laboratory.[18] Therefore, there is a clear need to develop automated, high throughput, and easy to operate serological tests as potential surrogate tests to evaluate neutralizing antibody activities in a large patient population.

HOW CAN NEUTRALIZING ANTIBODIES BE MORE EASILY ASSESSED? CAN SOME SEROLOGICAL TESTS SERVE AS A SURROGATE FOR NEUTRALIZATION ASSAYS?

In responding to this need, a group of scientists and physicians at New York Blood Center evaluated six different serological tests for their correlation with neutralization assays.[16] The tests included in this study represent a variety of technology platforms (lateral flow assay, ELISA, and automated chemiluminescent tests) with different viral targets (S1, nucleocapsid (N), and RBD). VITROS® Anti-SARS-CoV-2 Total assay targeting the S1 protein of SARS-CoV-2 was among the 6 tests evaluated. The study tested 370 convalescent plasma on the serological tests and the results were correlated with neutralizing titers generated from two different neutralization assays.[16] In general, ELISA and chemiluminescent assays showed better correlation with neutralizing titers than the lateral flow assay, and assays targeting the S1 protein exhibited better correlation than assays targeting the N protein. Among all 6 tests evaluated, the VITROS Anti-SARS-CoV-2 Total test demonstrated the strongest correlation with neutralizing titers and produced the highest predictive value for neutralization assays. The authors concluded that tests such as the VITROS Anti-SARS-CoV-2 Total test may thus serve to predict antiviral activity against SARS-CoV-2.[16]

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It is expected that the VITROS Anti-SARS-CoV-2 Total test that targets the S1 protein demonstrates the strongest correlation with neutralization assays, as most, if not all, neutralizing antibodies bind to S1 protein and should be detected by the VITROS assay. Assays targeting the N protein, on the other hand, rely mainly on indirect correlations. In the New York Blood Center study,[16] 14% of the convalescent plasma samples contained only anti-N but no detectable anti-S antibodies, which would make it hard to use results from an anti-N assay to predict neutralizing titers in these samples.

In addition, the high sensitivity, semi-quantitative capability, and the wide dynamic measuring range of the VITROS Anti-SARS-CoV-2 Total test are all crucial design features enabling more accurate prediction of a wide range of neutralizing titers observed among convalescent plasma donors in this study.[16]

The validation of suitable serological tests as surrogates for SARS-CoV-2 neutralization assays will be a substantial advancement in antibody testing. It will make the assessment of neutralizing activities in a large patient population possible to gain better understandings on community-based immunity or herd immunity. Six months into the COVID-19 pandemic, understandably there are still major knowledge gaps of immunity to SARS-CoV-2.

The possibility of predicting neutralizing activities at both individual and population level using an automated high-throughput high performance serological test will bring us one step closer to the understanding of COVID-19 immunity.

We do not yet know the minimum neutralizing activity required for protection from re-infection or the sustainability of the protection. However, the possibility of predicting neutralizing activities at both individual and population level using an automated high-throughput high performance serological test will bring us one step closer to the understanding of COVID-19 immunity.

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