T cell testing in SARS-CoV-2

Accurate identification of SARS-CoV-2 infection is crucial to track viral spread throughout populations and to formulate successful strategies for large scale containment of, treatment for and potential vaccination against this virus. While both PCR and antibody tests can detect current and post-infection respectively, these technologies also have specific challenges. There is strong evidence to suggest that SARS-CoV-2 triggers robust T cell responses. Measuring T cell responses to SARS-CoV-2 may be reliable at detecting infection and demonstrating immunity, and may thus be an important adjunct to current diagnostic methods. This white paper will examine the specific challenges surrounding the current diagnostic landscape and present emerging evidence for the importance of T cell testing along the continuum of COVID-19 pathology.

Introduction

Coronavirus disease 2019 (COVID-19) was first identified in late 2019 in the Wuhan province of China, although its exact origin remains uncertain. As the virus that causes COVID-19, SARS-CoV-2, spread rapidly around the world it gave rise to the most severe pandemic in recent history. It poses a major threat to public health and the global economy, as a result of ever-growing cases and COVID-19-related deaths.

A major challenge for governments is gaining the ability to accurately measure and detect how widespread the disease is among the population. Routine diagnostic tests that are currently available include molecular tests (PCR antigen tests), which indicate current SARS-CoV-2 infection, and serology tests (ELISA antibody tests), which can indicate current and previous SARS-CoV-2 infection (as antibodies become detectable during infection, and remain detectable for a period of time after the infection has been eradicated). The ability to perform accurate, large-scale testing of individuals is vital to allow a precise understanding of individual risk. Such testing could lead to a reduction in the restrictive interventions in society, have a positive economic impact and ultimately, permit normal life to resume. However, there is growing evidence that PCR and antibody results are dependent on the timepoint post infection, and questions remain about the validity of these tests.

Although our understanding of the virus is evolving rapidly, there is much that remains unknown about the way individual immune systems respond to the virus—which can vary substantially from asymptomatic infection to respiratory failure. Recent studies have investigated T cell responses in patients with COVID-19 and the accumulating evidence clearly supports a role for T cells in the immune response that forms during, and following recovery from SARS-CoV-2 infection. Identifying the T cell response in patients could be an important part of SARS-CoV-2 testing, with potential for use in vaccine development, therapeutic monitoring and possibly even the identification of those with immunity.

The current testing landscape

Diagnostic tests for SARS-CoV-2 infection fall into two main categories: molecular tests used to confirm whether an individual is infected with SARS-CoV-2, and antibody tests that tell whether an individual has been infected previously by detecting anti-SARS-CoV-2 antibodies. Molecular tests (PCR) are highly sensitive, specific and efficient at detecting viral RNA present in nasal or salivary swabs early on. They are also relatively fast to process, giving them a distinct advantage in controlling rapid outbreaks. However, a major limitation of PCR tests is that they are not able to detect whether individuals have been infected in the past or, more importantly, whether the virus is still active. Also, they can sometimes give false negative results in symptomatic patients when the viremia has dropped.

The key advantages of antibody tests are that they are relatively cheap and quick to process. However, a fundamental drawback is the fact that false negative results may occur if a patient has a delayed immune response or is able to eliminate SARS-CoV-2 infection without an antibody-mediated response. As a consequence, SARS-CoV-2 antibody tests may significantly underestimate the number of people previously infected.

Furthermore, recent findings suggest that SARS-CoV-2 antibodies decline significantly post infection, and at an even faster pace than what was found for MERS and SARS-CoV-1 infections. This means that the protective
role of antibodies against SARS-CoV-2 may not be as strong as expected, particularly in individuals suffering with mild illness, who make up the majority of people with COVID-19.

This transient antibody response could be a feature that low-severity COVID-19 shares with seasonal coronaviruses that cause common colds. People with mild disease might be producing fewer and shorter-lasting antibodies compared to those suffering from more severe disease. All of these findings have important implications when considering widespread serological testing, antibody protection against re-infection with SARS-CoV-2, and the durability of vaccine protection.

Taken together, these results contribute to the growing evidence base that shows that the reliability of PCR and antibody tests depends on when after infection the sample is taken. This factor should be taken into consideration in routine clinical practice, and in research that focuses on cell-mediated immune responses alongside antibody-mediated responses to SARS-CoV-2.

The need for T cell testing in SARS-CoV-2

It is too early to know whether antibody responses give a reliable representation of a previous SARS-CoV-2 infection. However, studies investigating the importance of T cell responses in patients with COVID-19 have confirmed that T cells do play an important role in the immunological memory that forms following recovery from SARS-CoV-2 infection. For example, most patients who are hospitalised with COVID-19 displayed both CD8 and CD4 T cell responses.

Another interesting aspect to consider in cell-mediated immunity to SARS-CoV-2 is the T cell response observed in seronegative (antibody negative) individuals. Almost twice as many exposed people and healthy individuals were found to generate memory T cells compared to antibody responses. This implies that antibody testing could seriously underestimate the extent of population-level immunity to SARS-CoV-2.

There are also findings that suggest SARS-CoV-2 elicits robust memory T cell responses akin to those observed in the context of successful vaccines, suggesting that natural exposure or infection may prevent recurrent episodes of severe COVID-19, even in seronegative individuals.

Applications of T cell testing

Potential applications for T cell testing include use in vaccine development and therapeutic monitoring. Potentially, it can also help identify those with immunity to SARS-CoV-2 or at lower risk of severe COVID-19.

Vaccine development

T cell testing may play a crucial role in determining people’s immune status before enrolment in vaccine trials, since T cell responses provide baseline information on any preexisting immunity, or exposure to SARS-CoV-2. T cell testing could help identify both uninfected individuals with no natural immunity for participation in vaccine trials, and those with prior natural immunity that may be at risk of T cell dependent hypersensitive immune reactions when exposed to specific antigens in the vaccines. It can help in making decisions on whether or not to enrol specific individuals in a trial.

T cell testing could also be crucial in vaccine development during phase 1-3 studies by confirming that T cells specific to SARS-CoV-2 are present after vaccination, and not before. Considering the evidence indicating that SARS-CoV-1 T cells remain present in some individuals up to 17 years after initial exposure, it is clear that the inherently robust longevity of SARS-CoV-2 T cells should be considered in the global COVID-19 public health response. Understanding the duration of specific T cell responses after vaccination is of equal importance in understanding and predicting how much immunity is provided by vaccines.

Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) helps determine the optimal therapeutic dose of a drug to achieve the best clinical outcomes in patients. This is particularly relevant to COVID-19 where patient responses are highly variable and drugs are either new or are being applied in a new context. T cell responses to SARS-CoV-2 can also vary substantially between patients depending on the viral proteins they respond to and evaluating the strength of this response is a key element of TDM in vitro.

Population immunity

T cell testing could complement antibody testing to better understand how many people within the population have been infected, including asymptomatic infections where fewer antibodies are produced. This would help build a more reliable picture of population level immunity.

T cell testing could also identify cohorts of people who have some degree of natural protection from developing severe types of COVID-19, and conversely those who are more likely to develop a severe form of the disease.
In turn, this may support decisions on best policies to mitigate the impacts on the most vulnerable and help clinicians make informed decisions about the best course of treatment for COVID-19 patients, based on their risk profile.

**About T-SPOT® Discovery SARS-CoV-2**

The T-SPOT Discovery SARS-CoV-2 research use only (RUO) kit has been developed to help detect and measure the strength of the T cell response to SARS-CoV-2 infection. The kit is a modified ELISPOT assay where samples prepared from peripheral blood are stimulated in vitro by peptide pools from SARS-CoV-2. This re-stimulation allows the number SARS-CoV-2 specific T cells to be counted to assess the T cell response (Figure 1). The standardisation brought by washing the cells and using a specific number of cells in each assay and the high analytical sensitivity of ELISPOT means that the technology is able to maintain its performance even in samples from immunosuppressed individuals.

The T-SPOT Discovery SARS-CoV-2 is based on the proven T-SPOT technology platform from Oxford Immunotec, the only globally regulated ELISPOT platform. It is currently used clinically for identifying T cells made in response to another respiratory pathogen (the pathogen responsible for tuberculosis). It has been approved for clinical use to detect TB infection in over 50 countries, including the US, EU, China and Japan. T-SPOT technology is manufactured under a quality management system, and product is transported worldwide. Over 20 million T-SPOT tests have been manufactured, and this experience translates to high-quality, reliable and reproducible results.

A key advantage of the RUO T-SPOT Discovery SARS-CoV-2 kit is its ability to count the number of SARS-CoV-2 specific T cells responding to the various SARS-CoV-2 antigens in the test. The optimised antigen mix enables investigation of the maximum breadth of the immune response to SARS-CoV-2, since all antigens are based on structural proteins of the SARS-CoV-2 virus and span the full length of those proteins. Regions of high homology to other endemic coronaviruses have been removed. Each antigen mix is plated in separate wells, allowing scientists to determine responses to individual SARS-CoV-2 proteins, which builds a reliable picture of the diversity of immune responses and potential T cell immunity at both the individual and population level (Figure 2). The kit can also easily be automated, making it ideal for use in labs processing a high volume of samples. The automation platform also allows for an extended sample stability which enables the easy centralisation of fresh blood samples from different study locations. These attributes are essential in large-scale studies, or for more widespread testing.

While serology is able to detect antibodies to SARS-CoV-2 in the blood of some individuals after infection, little is currently known about how this confers immunity.

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**Figure 1:** T-SPOT Discovery SARS-CoV-2 utilises proven T.SPOT technology based on a modified ELISPOT assay. The test employs a standardised number of washed cells to circumvent sample variation and maintain performance.

**Figure 2:** T-SPOT Discovery SARS-CoV-2 result. The number of SARS-CoV-2 specific T cells responding to the various antigens in the test can be easily visualised and counted.
T-SPOT technology goes further than serology by interrogating the immune system’s T cell response and enabling measurement of the strength of that response. As such, T cell testing could provide an important adjunctive test to antibody testing when investigating immunity to SARS-CoV-2. This may be particularly important for studies on population immunity thresholds and projections for the COVID-19 pandemic where it is critical to understand the full extent of immunity, including in seronegative individuals.

**Summary**

PCR and antibody tests are seen by many as the standard tests for detecting SARS-CoV-2 infection. PCR tests can determine whether a person is currently infected, and while antibody tests can determine whether someone was infected in the past, they can present some challenges. Using only these tests may not give a complete overview of the immune status of an individual or a population. T cell testing can be considered a reliable adjunctive test to antibody testing in SARS-CoV-2 infection, and may have particular importance in individuals who do not develop detectable antibody responses. It can have important implications in vaccine trials, where scientists need the most comprehensive data about whether subjects may have any preexisting immunity, and have been exposed to SARS-CoV-2 before enrolment, and about how individuals respond to a vaccine. Studying T cell responses may also play a role in SARS-CoV-2 therapeutic monitoring. Whether T cell testing could act as a reliable predictor of long-term immunity, is yet to be determined, but if it can, it may allow more precise modelling of the pandemic and potentially pave the way for ‘SARS-CoV-2 immunity passports’ permitting protected individuals to resume a normal life.

**References**

10. Doshi, P. Covid-19: Do many people have pre-existing immunity? BMJ. 2020; 370:m3563