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EDITORIAL

Are T cell repertoires useful as diagnostics for SARS- CoV- 2 infection?

Tim Harris and Karsten Sauer

Repertoire Immune Medicines, Cambridge, MA, USA

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Conventional diagnostic tests for SARS-CoV-2 infection include PCR-based tests to measure viral RNA and various serologic tests to measure antibodies arising from the infection [1]. Much of the testing that has been put in place as part of measures to design isolation and social distancing strategies during the COVID-19 pandemic is based on PCR tests.

Among them, the CDC's 2019 Novel Coronavirus (2019-nCoV) Real-Time Reverse Transcriptase (RT)–PCR Diagnostic Panel detects the SARS-CoV-2 virus in both nasal and bronchial lavage specimens. The test is designed to be used with existing RT-PCR instruments used to test for other RNA virus infections. Point of care (POC) PCR tests have been and are being developed for rapid testing at airports and other locations. Commercially available PCR tests detect at least two virus targets by using three primer–probe mixes including probes for the virus nucleocapsid gene and include a control for RNA integrity. These PCR tests are highly reliable and sensitive but, owing to their high sensitivity, can suffer from issues including persistently low-level-positive results with no clear correlation with the clinical picture and most importantly, with the real infectiousness of the patients. False negative results also occur owing to poor sampling and/or very low virus titers in some infected individuals.

Serological tests measuring anti-virus antibodies are generally a good marker of virus infection and typically arise 1 to 3 weeks after infection . SARS-CoV-2 first induces IgM responses which are then rapidly replaced by IgG and subsequently IgA. The two major virus proteins to which antibodies are generally raised and measured are the spike (S) protein and the immunodominant nucleocapsid (N) protein. The tests are designed to test either binding or neutralizing antibodies. Binding assays are usually in the ELISA format, although POC lateral flow assays are available to measure both IgM and IgG. Neutralizing antibody tests can either measure inhibition of the binding of the RBD domain of the S protein to the ACE-2 receptor or can measure inhibition of viral replication *in vitro*. Antibody tests are useful for measuring levels of immunity in the population (herd immunity) but are particularly important for assessing vaccine efficacy. Neutralization assays using pseudotyped virus (usually based on VSV and MLV) are also used to determine neutralizing antibody levels but are not performed routinely. The efficacy of the different vaccines being developed against SARS-CoV-2 was initially assessed by measuring their ability to raise antibodies in animals before any human vaccine trial could take place [2]. Only once this has been demonstrated in humans in Phase I and II trials will event-based Phase III trials take place: for example those that were completed with the mRNA-based vaccines and now with the DNA based spike encoding vaccines. There are currently no *routine* assays used to unequivocally determine the level of protection conferred by any of the vaccines in use or in development. This is determined in Phase 3 trials or by the collection of real world data.

Most infected individuals will raise antibodies to virus proteins but, owing to highly variable pathology, going from asymptomatic disease in children and younger people to very serious disease and death in older patients, the actual antibody levels vary considerably. It is also presently unclear how long antibodies and protective immunity last in infected or vaccinated individuals, with some reports of short-lasting antibody responses after mild disease [3,4].

The role of T cells in SARS-CoV-2 infections and their importance for vaccines is now much more appreciated than at the beginning of the pandemic. In many virus infections, T cells potentially target all virus antigens and contribute significantly and, in some cases, exclusively to virus clearance and protection even in the absence of antibody responses. T cells are also likely major mediators of long-term protective memory and persist much longer than antibodies [5]. Many groups worldwide have analyzed T cells from PBMCs and BAL from both convalescent and acutely infected SARS-CoV-2 patients [6–8]. Both CD4 and CD 8 T cells are found in patients and recognize epitopes from most viral proteins. This was predicated to some extent by what was seen when examining T cell responses to infection with the original SARS-CoV causing the 2002/2003 SARS epidemic. Here, specific T cells were recognized for their ability to respond to peptide pools derived from viral proteins [9]. T cells recognized mostly N, M, and S proteins. CD8 T cell responses were more common than CD4 T cell responses. There was also profound lymphodepletion in patients with severe disease. This is almost exactly what is now seen in SARS-CoV-2 infections. Additional data also suggest that aberrant features of T cell responses correlate with disease severity. The reasons for these aberrations are presently unclear [5].

CONTACT Tim Harris tharris@repertoire.com Repertoire Immune Medicines, Cambridge, MA 02139, USA

The peptide pool technology, where T cell stimulation is measured by interferon-γ release via ELISPOT assay, is still used today but has been largely superseded by single-cell methods based on DNA bar-coded tags and nucleic acid sequencing. This allows for the derivation of information not only about T cell immuno-phenotypes (including CD4, CD8, helper, effector, cytotoxic, regulatory, naive, activated, memory, or exhausted T cells) but also about the antigen epitopes that are bound by specific human leukocyte antigen (HLA) complex class I or II molecules and the sequence of the T cell receptors (TCRs) binding them. Key technologies for doing this at large scale include for HLA Class I analysis, the binding of libraries of DNA bar-coded HLA-peptide complex tetramers (or larger multimers) to T cells. Sequencing of bar-coded, TCR genes and transcriptome of the T cell bound can then associate HLA-haplotype, peptide sequence, TCR clonotype, and T cell phenotype. Analyses of HLA Class II peptide–TCR interactions employ peptide-based methods and newer technologies where T cell clones are co-cultured with reporter cells expressing libraries of HLA Class II–peptide fusion proteins attached to signaling domains that trigger expression of fluorescent reporter proteins when the HLA–peptide fusion is engaged by the TCR of the co-cultured T cell clone. Repeated enrichment cycles of co-culture and sorting of fluorescent reporter cells followed by sequencing of the encoded peptide allow association of the specific TCR clonotype, peptide, and HLA allele used for screening [10].

All the data that have been obtained by these and other methods show that T cell responses, including memory T cell induction, are an important feature of the immune response to SARS-CoV-2 and occur in almost all infected patients, regardless of whether antibodies are detected. Furthermore, contrasting with the relatively restricted specificity of antibodies primarily for S and N proteins, most virus proteins do elicit responsive T cell populations [7]. Thus, the T cell response to SARS-CoV-2 is broader than the antibody response. These data have led to the proposal that the analysis of T cell 'repertoires' could be useful in the detection and diagnosis of SARS-CoV-2 and provide answers to some important questions regarding infection that will not be answered solely by the analysis of antibody responses.

One approach to diagnostic profiling is based on understanding the TCR clonotype usage in T cells from infected and convalescent COVID-19 patients by TCRβ chain sequencing [11]. When the sequence data sets are deep enough to capture both HLA Class I and Class II restricted TCRs, diagnostic profiles can be developed based on the frequencies of particular β-chain sequences seen in the infected population compared to a control population. From these, an 'infected person profile' can be derived. Some level of epitope definition can be obtained by analyzing what the TCRs are binding to, but answering the more important questions about the virus infection will not be done by TCRβ chain sequencing alone.

Some of these questions include identifying the immunodominant T cell epitopes of all virus antigens in the context of all common Class I and Class II HLA alleles that are recognized in infected patients with different disease courses by both BAL T cells and peripheral blood-derived T cells. It has also been suggested that infection with the related human common-cold-coronaviruses may affect the susceptibility or clinical outcome of subsequently SARS-CoV-2-infected patients, and it has been shown that there are T cells in uninfected people that cross-react with SARS-CoV-2 peptide epitopes [12,13]. Whether or not these clones are responsible for any measure of protection from severe COVID-19 remains to be seen. It will also be important to determine which HLA haplotypes (if any) correlate with disease severity or protection, what the immunodominant epitopes are, and whether there are epitopes that are not seen in T cells in patients with particular outcomes – implying that there might be 'repertoire holes' that have pathological consequences. It should be possible with rich enough data sets to define the disease in different people by their T cell responses in a 'constellation of epitope reactivities' format. This value would come from knowing what T cell reactivity or clonotype profile correlates with disease outcome.

From a vaccine perspective, it will be important to know what the overlap is between T cells, HLA-restricted epitopes, and levels of protection. Most current, S-protein-targeted vaccines by design will only invoke T cells against S [14]. A vaccine that invokes a T cell epitope profile that is similar to the protective T cell epitope profile in patients who recovered sequelae free, including recovered patients where no antibodies were detected, would be expected intuitively to give the best protection. This may be best achieved by a killed virus vaccine although fortunately, the S-protein-targeted mRNA and protein vaccines seem to induce high levels of protective immunity in animals, and antibody responses lasting at least 4 months in people [15]. Conversely, there is the need to understand whether certain T cell reactivities or epitopes might cause hyperactivity-related side effects or worsen disease.

In conclusion, measuring the T cell responses in SARS-CoV-2-infected individuals will help us understand the pathology of the disease better than looking only at antibody and B cell responses, and T cell repertoires will also be useful diagnostic tools for identifying infected individuals and classifying their disease and its prognosis [16]

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Declaration of Interest

T Harris and K Sauer are both employees of Repertoire Immune Medicines. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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