Measuring SARS-CoV-2 T cell immunity with a scalable qPCR-based assay

We developed two quantitative PCR-based assays to detect SARS-CoV-2-specific T cell immunity: qTACT and dqTACT. The assays quantify *CXCL10* mRNA, after incubation of whole blood with viral peptides, as a proxy of an antigen-specific T cell response, and will allow population-level monitoring of cellular immunity to SARS-CoV-2.

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The problem

As the deployment of vaccines attenuates the healthcare burden of the pandemic caused by SARS-CoV-2, the duration of protective immunity needs to be reliably assessed and monitored at a global level. Long-term protection from viral infection is mediated by both humoral (antibody) and cellular (T cell) immunity. While antibodies can protect against infection, recent evidence points to the importance of T cell function in preventing severe health consequences in patients with COVID-19. Because of the heterogeneity of individual immune responses, humoral and cellular immune measurements do not always correlate, as seen in patients who do not become seropositive, those with low neutralizing antibody titers, or those with cancer, who can mount a SARS-CoV-2-specific T cell response in the absence of antibodies[1,2]. Importantly, several groups have demonstrated that, unlike antibodies, cross-reactive T cells recognizing shared epitopes between betacoronaviruses and SARS-CoV-2 can be detected in a large proportion of the healthy population[2,3].

Although quantification of SARS-CoV-2-specific antibodies is often used as a marker of immune protection[4], the measurement of T cell responses is rarely performed at scale, owing to technical challenges and lack of appropriate diagnostic tests. Therefore, it is paramount to deploy cheap, standardized and scalable assays to measure T cell functionality to fill this critical diagnostic gap[5].

The solution

As we have shown previously, SARS-CoV-2-specific T cells can be activated in vitro following overnight incubation of whole blood with synthetic viral peptides. Leveraging this finding, we developed a probe-based quantitative PCR (qPCR) rapid T cell activation (qTACT) assay to measure *CXCL10* mRNA, a transcript expressed by monocytes in response to T cell activation, as a proxy of functional virus-specific T cells (Fig. 1a). A further technical implementation of the assay, dqTACT, allows quantification of cellular immunity directly from blood, bypassing the need for RNA extraction (Fig. 1a). Results obtained with both qTACT and dqTACT are concordant and comparable in terms of accuracy and sensitivity when compared against gold standard ELLA (enzyme-linked lectin assay) and ELISpot (enzyme-linked immune absorbent spot) assays[4].

Using multiple large cohorts, we demonstrated that levels of induced *CXCL10* transcripts correlate robustly with interferon (IFN)-γ molecules produced by activated antigen-specific T cells, serving as a proxy to detect a helper T cell response in people who have recovered from COVID-19 or have been vaccinated against SARS-CoV-2 (Fig. 1b–d). We have used the qTACT and dqTACT assays to quantify the induction and persistence of SARS-CoV-2-specific T cells in both convalescent and vaccinated individuals, including older adults. In both cohorts, T cell immunity can be robustly detected for over 8 months (Fig. 1b–d). We also demonstrated the utility of the dqTACT assay in measuring the presence of T cells specific for peptides mutated in variants of concern.

The implications

A lack of affordable, accessible, scalable and accurate diagnostic methods to quantify SARS-CoV-2 cellular immunity has prevented large population studies, affecting long-term vaccination strategies and public health responses to the current pandemic[5]. Because diagnostic centers around the world have ramped up the setup of reverse transcription (RT)-qPCR-based facilities, we believe that the qTACT and dqTACT assays, which require only 1 ml of blood and a 24-hour turnaround time, could help to close this knowledge gap.

A caveat to using *CXCL10* as a proxy to measure T cell activity is that we cannot phenotype the functional T cells, but rather we assess their ability to produce IFN-γ in response to MHC-presented viral peptides.

In the near future we hope to extend our findings to quantify the T cell response to other viruses by similarly implemented qPCR-based assays. Future work for the SARS-CoV-2 qTACT and dqTACT tests will include the use of peptide pools directed towards multiple viral proteins[2,3], including those mutated in variants of concern, and the implementation of qPCR-based detection of T cells present in the mucosa, as opposed to whole blood.

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FROM THE EDITOR

"Assessing cellular immunity resulting from prior infection or vaccination requires analysis of the specificity of immune cells — a more challenging undertaking than quantifying antibody-based immunity, which can be measured via protein-based assays. The methodology described in this paper, based on whole blood cells incubated with a relevant peptide and standard PCR-based amplification of selected immune cell markers, provides a strategy for assessing cellular immunity that is both fast and straightforward."
Joao Duarte, Senior Editor, *Nature Biotechnology*

FIGURE

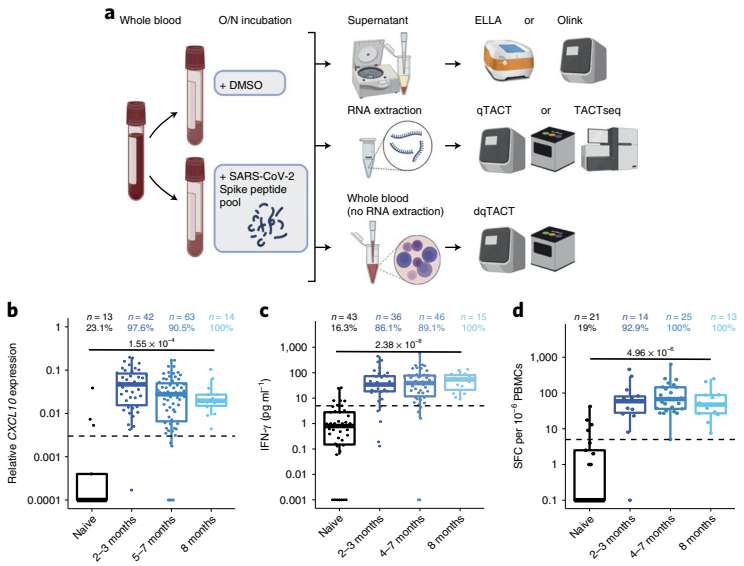

Fig. 1 | qPCR-based T cell activation assays to detect SARS-CoV-2 cellular immunity. a, Schematic showing workflow for the T cell activation assays. All assays begin with whole blood collection followed by overnight (O/N) stimulation with DMSO (negative control) or SARS-CoV-2 spike peptide pools. Next, supernatants are collected for ELLA or the Olink multiplex assay; RNA is extracted and used for probe-based qPCR (qTACT) or next -generation sequencing (TACTseq); or whole blood is diluted and used directly for qPCR (dqTACT). Schematic created with Biorender.com. **b**–**d**, Detection of *CXCL10* mRNA by dqTACT (**b**), IFN-γ protein secretion by ELLA (**c**), and IFN-γ producing cells by ELISpot (**d**) in vaccinated individuals over time. The *x* axis shows time after vaccination. Dashed lines represent the threshold for each method. The number of participants is shown above each box plot, along with the percentage who fall above the threshold. PBMCs, peripheral blood mononuclear cells; SFC, spot-forming cell. *P*-values represent the result of Wilcoxon rank-sum test, corrected using the Benjamini–Hochberg method, comparing naive versus 8 months.

BEHIND THE PAPER

At the start of the pandemic, cancer researchers like myself were in self-quarantine. During that time, I had discussions with a close collaborator, A. Bertoletti, who described his attempts to develop rapid screening methods to detect SARS-CoV-2-specific T cells by ELISA. I then asked: "Why don't you use sequencing or PCR-based methods?" He replied: "Because immunologists don't normally do that, but it looks like a good idea; let's try it!" From there, M. Schwarz spearheaded the effort to develop the TACT assay. By adapting the ELISA-based method developed in the Bertoletti lab, we identified *CXCL10* mRNA as a proxy marker quantifiable by qPCR. It then took months of validation efforts, thanks to collaborations with J. Ochando, who coordinated testing across multiple hospitals, Synlab and, finally, Hyris, a global biotech company, who helped us commercialize and bring to market our initial idea that had started over a phone call on a sunny morning in April 2020. **E.G.**

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