

## Difference in sensitivity between SARS-CoV-2–specific T cell assays in patients with underlying conditions. Reply.

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*J Clin Invest.* 2021;131(24):e155701. <https://doi.org/10.1172/JCI155701>.

Letter to the Editor

COVID-19

The authors reply: We thank Rory D. de Vries and colleagues for bringing attention to results showing that measurement of SARS-CoV-2–specific T cell responses in whole blood can be influenced by variables like the presence of immunosuppressive drugs (1). This is an important point. Nevertheless, whether the influence of “environmental factors” makes the rapid cytokine release assay (CRA) performed by measuring cytokines in the plasma of blood stimulated with peptides (2) less “accurate” and less able to measure the overall SARS-CoV-2–specific T cell response is a matter of debate. Overall, any assay designed to measure the frequency and function of T cells in vitro is performed to gauge the level of T cell response in vivo. As such, we could argue that testing SARS-CoV-2–specific T cell function directly in the whole blood of patients treated with immunosuppressive drugs (and thus in the presence of the drug) is a closer mimic of the in vivo conditions than the testing of T cells purified from whole blood with an ELISpot assay. In the latter case, the drug has been removed and as such, the assay can overestimate the real in vivo functionality of such T cells. Van Baarle and colleagues, however, correctly pointed out that in individuals under treatment with immunosuppressive drugs, T cell assays performed in whole blood (CRA) and in [...]

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**The authors reply:** We thank Rory D. de Vries and colleagues for bringing attention to results showing that measurement of SARS-CoV-2-specific T cell responses in whole blood can be influenced by variables like the presence of immunosuppressive drugs (1). This is an important point. Nevertheless, whether the influence of “environmental factors” makes the rapid cytokine release assay (CRA) performed by measuring cytokines in the plasma of blood stimulated with peptides (2) less “accurate” and less able to measure the overall SARS-CoV-2-specific T cell response is a matter of debate.

Overall, any assay designed to measure the frequency and function of T cells *in vitro* is performed to gauge the level of T cell response *in vivo*. As such, we could argue that testing SARS-CoV-2-specific T cell function directly in the whole blood of patients treated with immunosuppressive drugs (and thus in the presence of the drug) is a closer mimic of the *in vivo* conditions than the testing of T cells purified from whole blood with an ELISpot assay. In the latter case, the drug has been removed and as such, the assay can overestimate the real *in vivo* functionality of such T cells.

Van Baarle and colleagues, however, correctly pointed out that in individuals under treatment with immunosuppressive drugs, T cell assays performed in whole blood (CRA) and in isolated PBMCs (ELISpot) give different results, and thus these differences need to be correctly interpreted. The CRA using whole blood might more accurately measure the overall *in vivo* potency of SARS-CoV-2-specific T cell responses; ELISpot or other assays performed using PBMCs (like quantification of activation-induced markers) might better quantify the numbers and the intrinsic function of SARS-CoV-2-specific T cells present in such individuals.

Having said that, we should not forget that SARS-CoV-2 primarily infects cells present in the upper and lower respiratory tract and not in the blood. As such, as we argued before (3), any measurement of circulating SARS-CoV-2-specific T cells performed using either whole blood or purified circulating PBMCs has limitations and is in any case likely to represent only a dis-

tant proxy of the function and quantity of the T cells that are targeting SARS-CoV-2-infected cells *in vivo* and that are known to reside in tissues and in associated lymph nodes (4, 5).

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Conflict of interest: ATT, NLB, and AB report a pending patent for a method to monitor SARS-CoV-2-specific T cells in biological samples. AB reports personal fees from Oxford Immunotech and Qiagen.

Reference information: *J Clin Invest.* 2021;131(24):e155701. <https://doi.org/10.1172/JCI155701>.

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