Rapid flow cytometric whole blood assay for assessment of SARS-CoV-2-reactive T cell immunity

Poster ID: #192

Philipp David Gert, Verena Traska, Sofia Chatzopoulou, Christian Dose, Anne Richter, Marc Schuster
R&D Chemical Biology, Miltenyi Biotec, Bergisch Gladbach, Germany

Scan the QR code to reach our website:

Introduction

As of May 2021, the viral pathogen severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing the coronavirus disease 2019 (COVID-19), has infected more than 150 million individuals worldwide and killed over 3.2 million people. Therefore, understanding the nature of the accompanying immune response is essential, especially the role of T cells for successful virus defense but also during immunopathology leading to severe disease outcomes. Furthermore, as neutralizing antibodies are reported to decay within months post infection or vaccination, SARS-CoV-2-specific T cell persistence is discussed as indicator of long-term protective immunity. Therefore, we developed a rapid and easy flow cytometric assay for the analysis of SARS-CoV-2-reactive CD4$^+$ and CD8$^+$ T cells directly from whole blood.

Methods

Figure 1

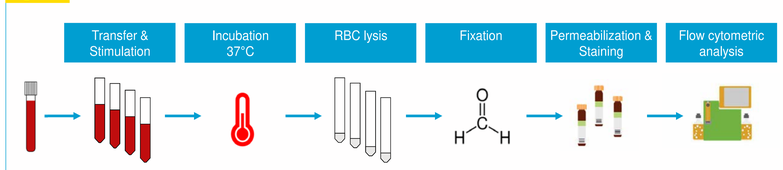


Table 1

Specificity	Clone	Fluorochrome
CD3	REA613	APC
CD4	REA623	Vio® Bright B515
CD8	REA734	VioGreen™
CD14	REA599	VioBlue®
CD20	REA780	VioBlue
CD154	REA238	APC-Vio 770
IFN-γ	REA600	PE
IL-2	REA689	PE-Vio 615
TNF-α	REA656	PE-Vio 770

As shown in figure 1, heparinized whole blood of e.g. healthy, COVID-19 convalescent or SARS-CoV-2 vaccinated donors is transferred to 5ml FACS™ tubes and stimulated with different SARS-CoV-2 PepTivator® Peptide Pools for 8h at 37°C and 5% CO_2 together with Brefeldin A. Afterwards, a red blood cell lysis is performed and the cells are fixed with Inside Fix solution. Subsequently, cells are permeabilized, stained with the REAfinity™ antibodies shown in table 1, and SARS-CoV-2 reactive T cells are detected using the MACSQuant® 16.

A variety of SARS-CoV-2 PepTivators peptide pools were used. PepTivators for the nucleoprotein (N), the membrane glycoprotein (M), and the spike glycoprotein (S, S1, complete S) consists of 15mer peptide having an 11 amino acid overlap, which cover the complete protein sequence (see figure 2). The PepTivator SARS-CoV-2 Select encompasses MHC-I and MHC-II restricted immunodominant epitopes of the whole viral proteome, i.e. peptides from structural as well as non-structural proteins and the other open reading frames (see figure 3).

Figure 2

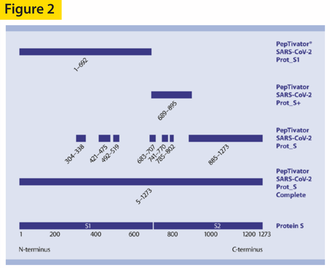
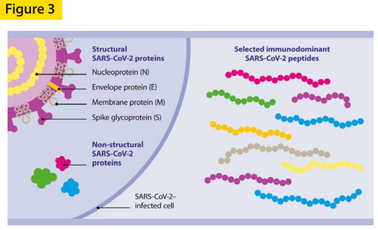


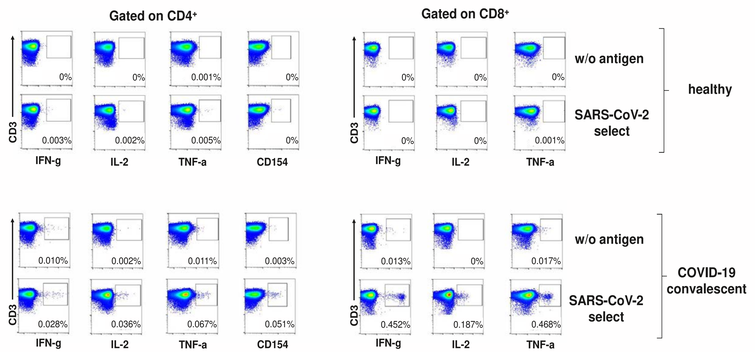
Figure 3



Results

1 Flow cytometric identification of SARS-CoV-2-reactive T cells

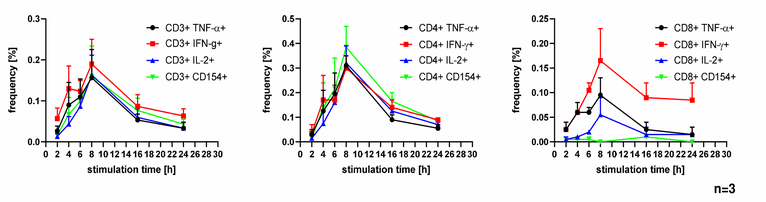
Figure 4



Whole blood samples of a healthy - presumably unexposed - and a COVID-19 convalescent donor were incubated for 8 hours with SARS-CoV-2 PepTivator Select or without antigen as negative control in the presence of Brefeldin A. After fixation, permeabilization and staining (see table 1), the samples were analyzed and expression of the activation markers IFN-γ, IL-2, TNF-α, and CD154 determined in both the CD4$^+$ and CD8$^+$ T cell compartment. Figure 4 shows exemplary pseudocolor dotplots for analyzing the frequencies of activated T cells according to each of the activation markers for a healthy and a convalescent donor. A specific T cell response can be identified for the COVID-19 convalescent donor in both T cell compartments, with frequencies of e.g. 0.051% CD4$^+$ CD154$^+$ or 0.452% CD8$^+$ IFN-γ$^+$ T cells.

2 Kinetics of SARS-CoV-2 PepTivator stimulation

Figure 5

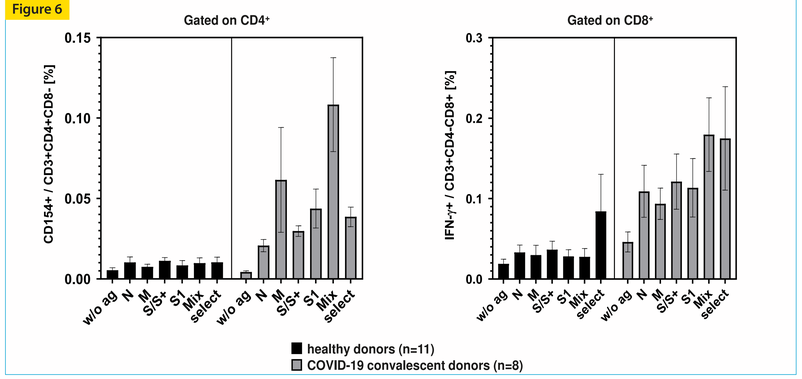


To investigate the kinetics of T cell activation, whole blood samples of COVID-19 convalescent donors were stimulated with the mix of PepTivators N, M, S1, S, and S+ (see figure 2) for several incubation times ranging from 2h to 24h. As illustrated in figure 5, the kinetics vary slightly between the different activation markers and T cell subsets. Nevertheless, an incubation time of approximately 8h was identified to be optimal to analyze T cell activation in all the indicated subsets as the highest frequency of reacting T cells was detectable. For each time point the background of the unstimulated control was subtracted. Error bars shown indicate standard deviation of the mean.

3 Analysis of SARS-CoV-2-reactive T cell response in convalescent donors

Blood of healthy and COVID-19 convalescent donors was stimulated with SARS-CoV-2 PepTivator Peptide Pools or without antigen. Blood was drawn between three and six month post infection. Figure 6 shows the frequencies of CD4$^+$ CD154$^+$ and CD8$^+$ IFN-γ$^+$ T cells after stimulation with the indicated peptide pools, including a mix of the PepTivators N, M, and S1, S+ and S, as a bar diagram. This mix results in the highest reactivity for both CD4$^+$ and CD8$^+$ T cells. The quantity of the response and the patterns regarding the different peptide pools are highly donor dependent, possibly due to differences in severity and timepoint of analysis post infection.

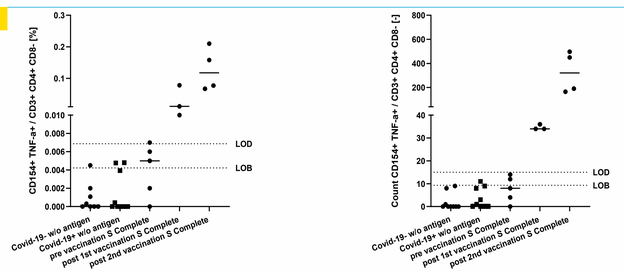
Figure 6



4 Analysis of SARS-CoV-2-reactive T cell response post-vaccination

Whole blood samples from donors, either pre-vaccination, after the first or second vaccination with Vaxzevria or Comirnaty, were stimulated with the PepTivator SARS-CoV-2 Prot_S Complete. This PepTivator covers the entire sequence of the spike protein and resembles the amino acid sequence which is used for vaccination. As figure 7 shows, there is an increase in reactive CD4$^+$ T cells (CD154$^+$ TNF-α$^+$) after the initial as well as after the booster vaccination when compared to donors pre-vaccination. Unstimulated whole blood samples from healthy and convalescent donors were used to determine limit of detection and limit of blank.

Figure 7



Summary

- Assay enables quantification of SARS-CoV-2-reactive CD4$^+$ and CD8$^+$ T cells responses directly from whole blood
- Discrimination of convalescent and unexposed donors based on activation marker expression
- Monitoring of SARS-CoV-2-reactive T cells during and post vaccination
- Tool to evaluate the impact of T cell immunity for protection after natural infection and vaccination

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. For further regulatory notes, refer to miltenyibiotec.com/regulatory-notes.
CytoStim, MACSQuant, MACSQuantify, the Miltenyi Biotec logo, PepTivator, REAfinity, Vio, Viobility, VioBlue, VioBright, and VioGreen are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide.
All other trademarks mentioned in this document are the property of their respective owners and are used for identification purposes only.
Copyright © 2021 Miltenyi Biotec and/or its affiliates. All rights reserved.