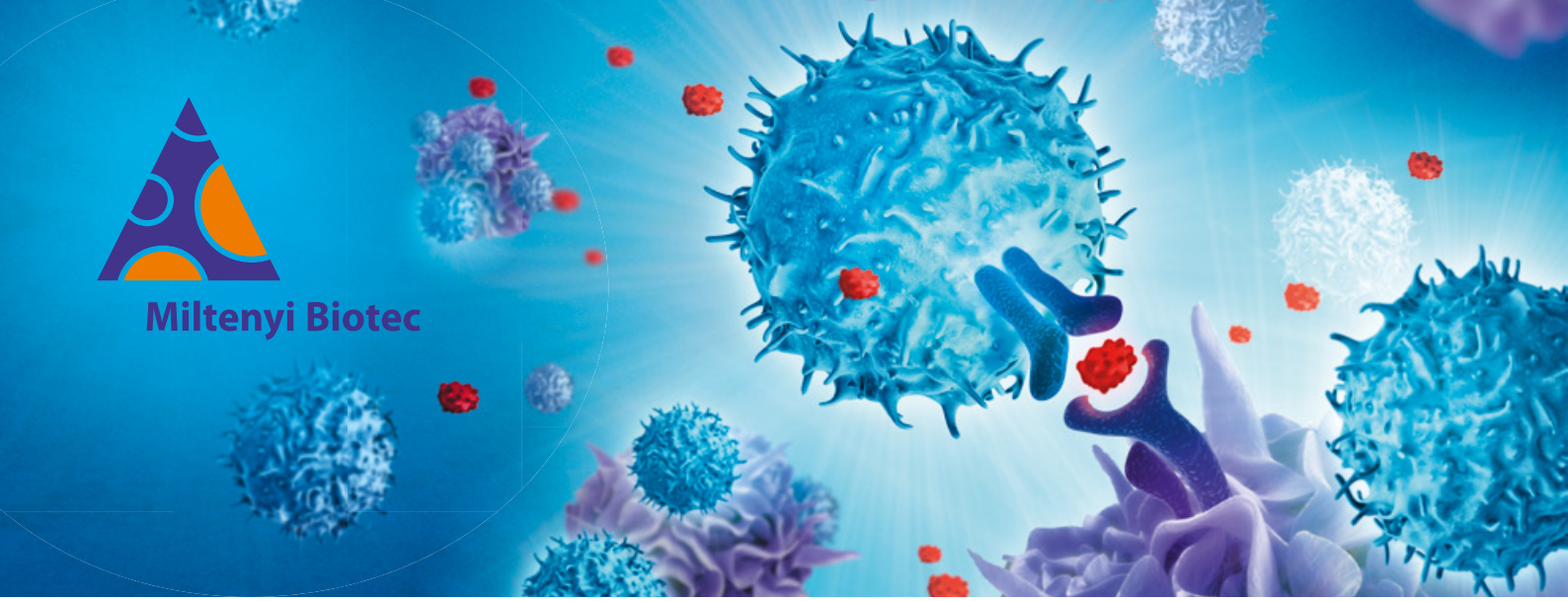

Fast and efficient detection of SARS-CoV-2–reactive T cells via antigen-specific stimulation

Background

T lymphocytes execute and control immunological reactions with a repertoire of cytokines, cytotoxic substances, and other mediators. The quantitative and qualitative analysis of $CD4^+$ and $CD8^+$ T cells that recognize and react towards a defined antigen provide important information to understand their immunological function. Furthermore, the presence of these cells indicates an infected or convalescent donor and may also allow conclusions on disease progress, specific immune reaction, and immune status[1].

Antigen-reactive T cells can be identified and characterized by analyzing their effector function, such as upregulation of activation markers and production of cytokines. Here, the SARS-CoV-2 T Cell Analysis Kit (PBMC), human has been used to efficiently asses the SARS-CoV-2–reactive T cell response in the context of COVID-19 based on a sensitive and precise multiparameter flow cytometry assay.

Methods

PBMCs were freshly prepared from a SARS-CoV-2–reactive donor's blood sample. Subsequently, 10^6 PBMCs per well were seeded in a 96-well plate in a total volume of 100 µL cell culture medium. Next, cells were stimulated for 2 hours. SARS-CoV-2 PepTivator® Peptide Pools were used to stimulate SARS-CoV-2–reactive T cells, and polyclonal anti-CD3/CD28 stimulation using T Cell TransAct™, human was used as a positive control. After the addition of Brefeldin A, cells were incubated for an additional 4 hours. Subsequently, the cells were stained with the live/dead marker Viobility™ 405/452 Fixable Dye, fixed, and permeabilized for intracellular staining according to the optimized intracellular staining protocol provided in the SARS-CoV-2 T Cell Analysis Kit (PBMC), human. Cells were stained with the flow cytometry antibody panel provided in the kit, consisting of fluorophore-conjugated antibodies directed against CD3, CD4, CD8, IFN-γ, TNF-α, CD14, CD20, and CD154 (see table 1).

The free PE-Vio® 615 channel, which is not occupied by a fluorophore-conjugated antibody in the kit, allows the customer to add a further antibody of choice. In this example, the IL-2 Antibody, anti-human, PE-Vio 615, REAfinity™ was included in the staining panel.

The kit contains a SARS-CoV-2 PepTivator Peptide Pool of choice, the Viobility 405/452 Fixable Dye, the 8-color flow cytometry antibody panel (see table 1), buffers for fixation and permeabilization, and a positive control (CytoStim™ Reagent). Additional PepTivator Peptide Pools as well as further fluorophore-conjugated antibodies to stain for supplementary parameters in the free PE-Vio 615 channel may be purchased separately (see table 2). Additional specificities may include IL-2, IL-4, IL-17, IL-21, CD69, CD134 (OX-40), CD137 (4-1BB), Granzyme-B, HLA-DR, Perforin, and TIGIT.

Finally, cells were analyzed using the MACSQuant® Analyzer 16. First, doublets, debris, and dead cells were excluded, and after pre-gating on CD3 as well as CD4 or CD8, activation marker and cytokine expression was assessed.

Marker	Fluorophore
CD3	APC
CD4	Vio Bright B515
CD8	Vio Green
IFN-γ	PE
TNF-α	PE-Vio 770
CD14	VioBlue®
CD20	VioBlue
CD154 (CD40L)	APC-Vio 770
Live/dead	Viobility 405/452 Fixable Dye
Free channel*	PE-Vio 615

Table 1: Flow panel for the analysis of SARS-CoV-2–reactive T cells (included in kit).

* Can be used for additional specificities of choice.

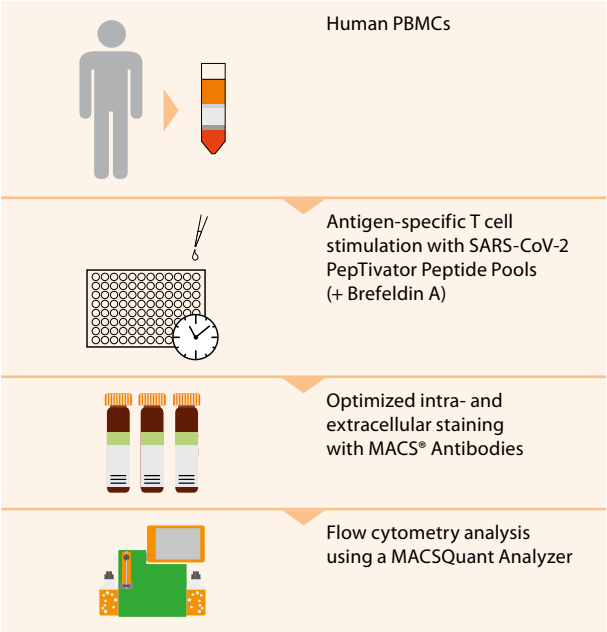

Figure 1: Schematic overview of the workflow for stimulation and analysis of SARS-CoV-2–reactive T cells using the SARS-CoV-2 T Cell Analysis Kit (PBMC), human.

Results

Elevated levels of CD4⁺ and CD8⁺ T cells expressing activation markers and cytokines, such as CD154 and IL-2 as well as TNF-α and IFN-γ, respectively, can be assessed upon stimulation with the SARS-CoV-2 PepTivator Prot_N, Prot_M, Prot_S, and Prot_S1 when compared to the unstimulated control. As expected, polyclonal anti-CD3/CD28 T cell stimulation as positive control (T Cell TransAct, human) resulted in a strong CD4⁺ and CD8⁺ T cell activation.

These results confirm the presence of SARS-CoV-2–reactive T cells in the donor's sample.

T cell reactivities vary between donors and individual PepTivator Peptide Pool specificities depending on disease severity and individual MHC background. In the presented case, stimulation with SARS-CoV-2 PepTivator Peptide Pools resulted in a stronger specific response in CD4⁺ T cells when compared to CD8⁺ T cells. CD8⁺ T cells reacted predominantly to stimulation with Protein N and exhibited low reactivity towards the other tested SARS-CoV-2 PepTivator Peptide Pools.

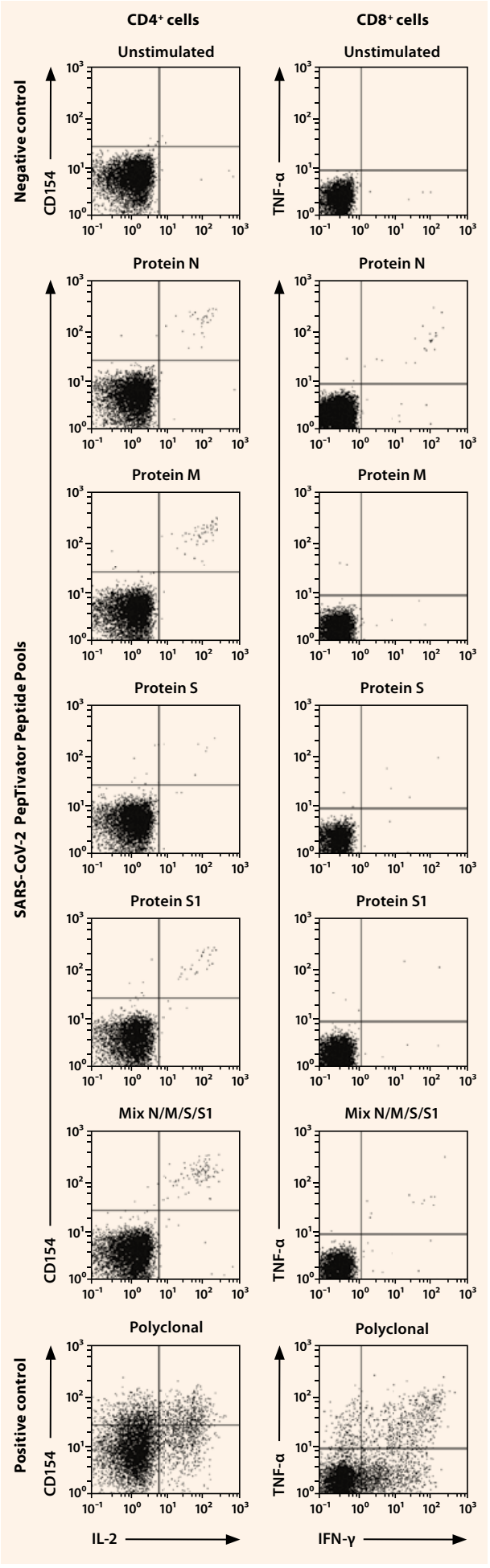

Figure 2 (right): Detection of SARS-CoV-2–reactive T cells with the SARS-CoV-2 T Cell Analysis Kit (PBMC), human. PBMCs from a SARS-CoV-2–reactive donor were stimulated for 6 hours with the respective PepTivator Peptide Pools or left unstimulated (negative control). Polyclonal anti-CD3/CD28 T cell stimulation (T Cell TransAct, human) was used as positive control. Subsequently, cells were stained with the flow cytometry antibody panel included in the SARS-CoV-2 T Cell Analysis Kit (PBMC), human, with the addition of the IL-2 Antibody, anti-human, PE-Vio 615, REAfinity. After pre-gating on live CD4⁺ or CD8⁺ cells, expression of CD154 and IL-2 (for CD4⁺ cells) and TNF-α and IFN-γ (for CD8⁺ cells) amongst other markers was assessed on the MACSQuant Analyzer 16. The plots clearly show that both CD4⁺ and CD8⁺ SARS-CoV-2–reactive T cells express activation markers and cytokines in dependence of stimulation with the respective PepTivator Peptide Pools.

Conclusions

Upon stimulation with SARS-CoV-2 PepTivator Peptide Pools, $CD4^+$ and $CD8^+$ T cells expressing specific activation markers were detected. The results indicate the presence of SARS-CoV-2–reactive T cells in the donor's sample. Interestingly, the overall $CD4^+$ T cell response upon stimulation with the SARS-CoV-2 PepTivator Peptide Pools was stronger when compared to $CD8^+$ T cells. This is in line with literature indicating a more severe course of disease[2,3,4]. However, stimulation results vary from donor to donor and between individual antigen specificities.

In conclusion, the SARS-CoV-2 T Cell Analysis Kit (PBMCs), human is a promising approach for the effective stimulation and sensitive detection of SARS-CoV-2–reactive T cells in human PBMC samples.

Product	Order no.
SARS-CoV-2 Prot_S T Cell Analysis Kit (PBMC), human	130-127-586
SARS-CoV-2 Prot_S1 T Cell Analysis Kit (PBMC), human	130-127-585
SARS-CoV-2 Prot_S+ T Cell Analysis Kit (PBMC), human	130-127-584
SARS-CoV-2 Prot_N T Cell Analysis Kit (PBMC), human	130-127-583
PepTivator SARS-CoV-2 Prot_S, 6 nmol, research grade	130-126-700
PepTivator SARS-CoV-2 Prot_S1, 6 nmol, research grade	130-127-041
PepTivator SARS-CoV-2 Prot_S+, 6 nmol, research grade	130-127-311
PepTivator SARS-CoV-2 Prot_N, 6 nmol, research grade	130-126-698
PepTivator SARS-CoV-2 Prot_M, 6 nmol, research grade	130-126-702
IL-2 Antibody, anti-human, PE-Vio 615, REAfinity	130-111-307
IL-4 Antibody, anti-human, PE-Vio 615	130-107-144
IL-17A Antibody, anti-human, PE-Vio 615, REAfinity	130-118-247
HLA-DR Antibody, anti-human, PE-Vio 615, REAfinity	130-111-797
CD69 Antibody, anti-human, PE-Vio 615, REAfinity	130-112-617
CD137 Antibody, anti-human, PE-Vio 615, REAfinity	130-110-766
TIGIT Antibody, anti-human, PE-Vio 615, REAfinity	130-116-816
REAfinity Recombinant Antibodies	*
Antigens and peptide pools	*

Table 2: Related products.

* For more information on REAfinity Recombinant Antibodies and on antigens and peptide pools visit **miltenyibiotec.com/antibodies** and **miltenyibiotec.com/peptivators**

References

1. Thieme, C.J. *et al.* (2020) The SARS-CoV-2 T cell immunity is directed against the spike, membrane, and nucleocapsid protein and associated with COVID-19 severity. medRxiv: Epub, May 16.
2. Swadling, L. and Maini, M.K. (2020) T cells in COVID-19 – united in diversity. Nat. Immunol.: Epub, Sep. 7.
3. Kusnadi, A. *et al.* (2020) Severely ill COVID-19 patients display augmented functional properties in SARS-CoV-2–reactive $CD8^+$ T cells. bioRxiv: Epub, Jul. 10.
4. Meckiff, B. J. *et al.* (2020) Imbalance of regulatory and cytotoxic SARS-CoV-2–reactive $CD4^+$ T cells in COVID-19. Cell: Epub, Sep. 30.

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