Original Article

T-Cell Activation: Post-Infection Diagnostic Tool for COVID-19

(COVID-19 / SARS-CoV-2 / T-cell activation)

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Abstract. COVID-19 is caused by the SARS-CoV-2 virus and has spread globally in 2020. Cellular immunity may serve as an important functional marker of the disease, especially in the asymptomatic cases. Blood samples were collected from 46 convalescent donors with a history of COVID-19 and 38 control donors. Quantification of the T-cell response upon contact with SARS-CoV-2 proteins *in vitro* was based on IFN-γ. Significantly higher numbers of activated cells were measured in patients who underwent COVID-19. Anti-SARS-CoV-2 T cells were detected weeks after the active virus disappeared from the organism. Repeated sample collection after five months proved that the T-cell activation was weaker in time in 79 % of the patients. In the majority of cases, the $CD4^+$ helper T-cell subpopulation was responsible for the immune reaction. Moreover, different viral proteins triggered activation in $CD4^+$ helper and in $CD8^+$ cytotoxic T cells. Together, these findings suggest that the T-cell activation level identifies the individuals who underwent COVID-19 and may become a diagnostic tool for the disease.

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Abbreviations: COVID-19 – coronavirus disease 2019, ELISA – enzyme-linked immunosorbent assay, GVHD – graft-versus-host disease, IFN-γ – interferon γ, ORF – open reading frame, PBMCs – peripheral blood mononuclear cells, RT-PCR – real-time polymerase chain reaction, SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2, T_H – helper T cells, T_C – cytotoxic T cells, WHO – World Health Organization.

Introduction

In the end of the year 2019, a new type of human virus from the coronavirus family emerged. The virus is called Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and the disease was named Coronavirus Disease 2019 (COVID-19). The infection quickly spread around the globe and caused unparalleled public health and economic upheaval. According to the World Health Organization (WHO), the current number of confirmed cases is around 70 million with over 1.5 million deaths (in December 2020).

SARS-CoV-2 is a positive-sense single-stranded RNA virus with a single linear RNA segment. It consists of structural proteins, including (i) the membrane glycoprotein (M) that shapes the viral envelope, (ii) the nucleocapsid phosphoprotein (N) that is responsible for the packaging of its genome, (iii) the surface glycoprotein or the "spike protein" (S) that the virus uses to gain entry into host cells, and (iv) the envelope protein that is embedded in the lipid envelope (E). Coronaviruses have their replicase-transcriptase proteins, including their RNA-dependent RNA polymerase (RdRp), encoded in open reading frame 1a (ORF1a) and ORF1b (Ahmed et al., 2020). The above-mentioned coronaviral genes and proteins serve as important targets for the research of vaccines, therapeutic antibodies, and diagnostics.

Three methods are being routinely used to diagnose COVID-19. Real-time polymerase chain reaction (RT-PCR) detects the viral RNA, antigen tests detect viral proteins, and enzyme-linked immunosorbent assay (ELISA) detects virus-specific serum antibodies. RT-PCR is performed with nasopharyngeal samples and identifies currently infected patients. Several companies manufacture and distribute RT-PCR kits for routine diagnostics of symptomatic COVID-19 patients. The most targeted genes are ORF1ab/RdRp, E, N, and S (Callow et al., 1990). Antigen tests are faster and less expensive than RT-PCR, but may be less reliable. ELISA testing is performed with serum specimens. Protein S has been shown to be an ideal target for virus-specific IgG and IgM antibody detection (Cohen and Kessel, 2020; Freeman et al., 2020). Seroconversion may occur as late as

1–3 weeks after infection, and therefore the ELISA method is not suitable for patients in the acute phase of the disease. However, antibody detection is highly appreciated as a tool for population surveillance. The question mark remains on how long after infection the SARS-CoV-2 antibodies remain in the serum to be detected.

Monitoring of the cellular immunity response to SARS-CoV-2 infection represents an alternative approach to serum antibody detection. Virus-specific effector/memory T cells from peripheral blood can be *in vitro* activated to secrete interferon γ (IFN-γ) upon contact with short sequences of viral proteins M, N, and S (Kaeuferle et al., 2019; Grifoni et al., 2020). The pool of peptides of 15 amino acid length with 11 amino acid overlap enables efficient *in vitro* activation of antigen-specific T cells. The percentage of activated IFN-γ-secreting T cells can be visualized and quantified using flow cytometry with superior sensitivity. This method reveals whether the tested individual underwent SARS-CoV-2 infection in the past, similarly as antibody detection. However, memory T cells probably remain in the human body long after plasma cells ceased to release antibodies.

In this study, we aimed to measure the response of T cells to the activation with SARS-CoV-2 proteins *in vitro*. We hypothesized that T cells from patients who underwent COVID-19 would be activated to secrete IFN-γ upon contact with viral proteins. This would allow us to reliably identify the disease based on the functional response of immune cells. Hence, T-cell activation may become an important diagnostic tool for COVID-19.

Material and Methods

Donors

Forty-six convalescent donors with a history of COVID-19 and 38 control donors were recruited for this study. The samples were collected between 6th of May and 23rd of June 2020. Paired samples were analysed in 29 individuals. Donors were anonymized under the ID code "Cov-" followed by a letter or a number. The eligibility criteria for COVID-19 included positive RT-PCR test for SARS-CoV-2, but negative two last tests. The controls proved no positive RT-PCR test for SARS-CoV-2 and declared no symptoms of COVID-19. All donors met the standard criteria for blood donation and signed an informed consent approved by the St. Anne's Hospital Ethic Committee Board (2020/05/02). Ten ml of peripheral blood was collected from each donor. The details about donors are given in Supplement 1 and summarized in Table 1.

Cell isolation

The blood was processed immediately after collection. Peripheral blood mononuclear cells (PBMCs) were isolated from the blood samples using Ficoll®-Paque (Merck, Darmstadt, Germany) density gradient centrifugation in SepMate™ tubes (Stemcell Technologies, Vancouver, Canada). Before activation, PBMCs were cultured overnight in 5 ml of Roswell Park Memorial Institute medium supplemented with 10% foetal bovine serum (Thermo Fisher Scientific, Waltham, MA), 2 mM L-glutamine (Thermo Fisher Scientific), and ZellShield® anti-

Table 1. Details about donors. Data were disclosed by donors in a personal survey

		Ctrl	COVID
	Number	38	46
	Gender; M:F	8:30	22:24
	Age; mean ± SD	38.89 ± 12.63	41.36 ± 13.11
	Days of symptoms; mean (range)	–	16.23 (0–94)
	Days between tests*; mean (range)	–	27.22 (6–79)
	Days between test and sampling**; mean (range)	–	62.24 (19–117)
Severity of the symptoms (%)	None	–	4 %
	Mild	–	54 %
	Medium	–	22 %
	Severe	–	13 %
	Hospitalization	–	7 %
Symptoms (%)	Temperature > 37 °C	–	74 %
	Cough	–	37 %
	Headache	–	43 %
	Aqeusia	–	41 %
	Anosmia	–	54 %
Suspected source of infection (%)	Unknown	–	35 %
	At work	–	22 %
	Family centre	–	20 %
	Room-mate	–	13 %
	Friend	–	7 %
	Other	–	4 %

*Days between the first positive and the last negative RT-PCR test; **Days between the first positive RT-PCR test and the sampling

biotics (Minerva Biolabs, Berlin, Germany) in a humidified 37 °C incubator with an atmosphere of 5% (v/v) CO_2.

T-cell activation and labelling

T cells were activated to secrete IFN-γ by adding peptide sequences from SARS-CoV-2 proteins M, N, and S (PepTivator® SARS-CoV-2, product numbers 130-126-702, 130-126-698, and 130-126-700 for proteins M, N, and S respectively, Miltenyi Biotec, Bergisch Gladbach, Germany). The mix of the three proteins was added to each sample. In six samples, each protein was also tested separately. The level of activation was expressed as the number of T cells that secreted IFN-γ. The Rapid Cytokine Inspector (CD4/CD8 T Cell) Kit (Miltenyi Biotec) was used according to manufacturer's instructions to analyse the samples. Briefly, 1,000,000 cells per well were seeded in a V-bottom 96-well plate and PepTivator® was added. As a positive control, CytoStim™ (Miltenyi Biotec) was used instead. After two hours, 0.2 µg of brefeldin A was added to each well to trap IFN-γ inside the cells. After four more hours, the cells were fixed and permeabilized. A cocktail of fluorochrome-conjugated monoclonal anti-human antibodies consisting of CD3-VioBlue® (clone: BW264-56, isotype: mouse IgG2a), CD4-APC (clone: VIT4, isotype: mouse IgG2a), CD8-FITC (clone: BW135/80, isotype: mouse IgG2a), CD14-PerCP (clone: TÜK4, isotype: mouse IgG2a), CD20-PerCP (clone: LT20, isotype: mouse IgG1), and IFN-γ (clone: 45-15, isotype: mouse IgG1) was added. Samples were analysed in a CytoFLEX Flow Cytometer (Beckman Coulter, Brea, CA).

Data analysis

Using CD14 and CD20 markers, we gated-out monocytes and B cells. IFN-γ secretion was measured in the T-cell population ($CD3^+$), helper T cells (T_H, $CD4^+$), and cytotoxic T cells (T_C, $CD8^+$). For each donor, the percentage of IFN-γ^+ T cells was assessed in a non-activated sample (negative control), CytoStim™-activated sample (positive control), and PepTivator®-activated sample. To exclude possible IFN-γ background caused by non-SARS-CoV-2 viral infection, the percentage of IFN-γ^+ T cells in the negative control was subtracted from the percentage of IFN-γ^+ T cells in PepTivator®-activated sample. The data sets were statistically analysed by D'Agostino & Pearson normality test, Mann–Whitney test, Wilcoxon test, and Spearman correlation test. $P < 0.05$ was considered statistically significant (*) and $P < 0.005$ highly statistically significant (***). The data were processed using GraphPad Prism and Excel software.

Results

COVID-19 patients developed cellular immunity against SARS-CoV-2

PBMCs were isolated from 84 samples of peripheral blood with an average yield of 0.79×10^6 PBMCs per 1 ml of blood (range between 0.14 and 2×10^6). The samples were subsequently analysed by flow cytometry (Fig. 1). Activation of the T-cell population ($CD3^+$) and

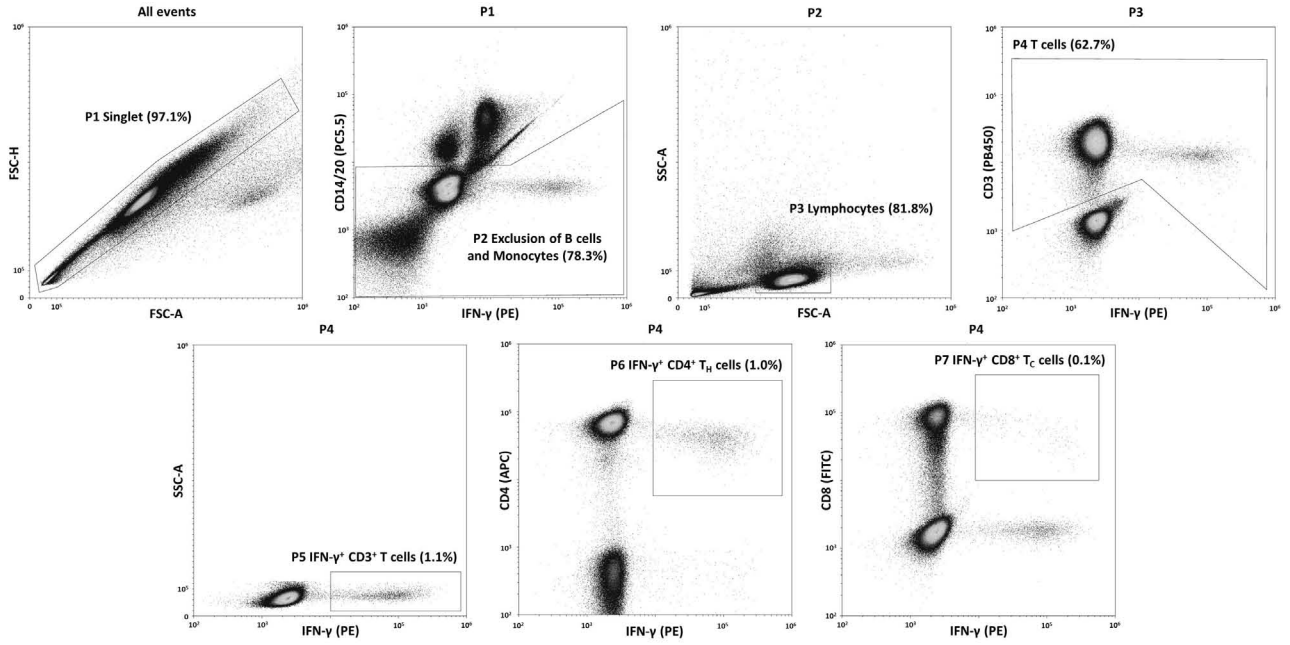

Fig. 1. Gating strategy. Upper dot plots from left to right: Gate P1: singlets were selected within a FSC-A/FSC-H dot plot. Gate P2: monocytes and B cells were excluded using CD14 and CD20 antibodies. Gate P3: lymphocytes were selected with a FSC-A/SSC-A dot plot. Gate P4: T cells were gated within the $CD3^+$ population. Lower dot plots from left to right: cells secreting IFN-γ were gated in P4 in T cells ($CD3^+$), T_H cells ($CD4^+$), and T_C cells ($CD8^+$). Data are shown for representative sample Cov-33.

its subpopulations, helper T cells ($CD4^+$; T_H) and cytotoxic T cells ($CD8^+$; T_C), upon contact with viral peptides was studied. The percentage of cells secreting IFN-γ was plotted for each cell type separately for control (38) and COVID-19 (46) donors (Fig. 2). Higher activation was observed for COVID-19 cell samples than for control samples.

In the $CD3^+$ T-cell population, the median value of cells secreting IFN-γ upon SARS-CoV-2 encounter was 0.01 % for the control samples and 0.15 % for the COVID-19 samples. The majority of the control sample T cells ranged between −0.04 % and 0.06 % except for three samples (Cov-1: 0.12 %; Cov-44: 0.25 %; Cov-64: 2.64 %). These three samples above the threshold constitute 8 % of our tested control samples (the threshold for COVID-19-positive samples was set ≥ 0.08% of IFN-γ^+ $CD3^+$ T cells). None of these donors declared any symptoms of COVID-19, and it is therefore possible that these donors underwent asymptomatic and undetected COVID-19 disease. The majority of the COVID-19 sample $CD3^+$ T cells ranged between 0.08 % and 1.46 % except for seven samples (Cov-29: −0.02 %; Cov-30: 0.04 %; Cov-61: 0.06 %; Cov-65: 0.04 %; Cov-67: 0.02 %; Cov-70: 0.04 %; Cov-71: 0.01 %). These seven samples below the threshold constitute 15 % of our tested COVID-19 samples. It is possible that the result of RT-PCR might be false-positive for the minority of the tested samples. According to the survey, all seven "false-positive" COVID-19 donors had none or minor symptoms.

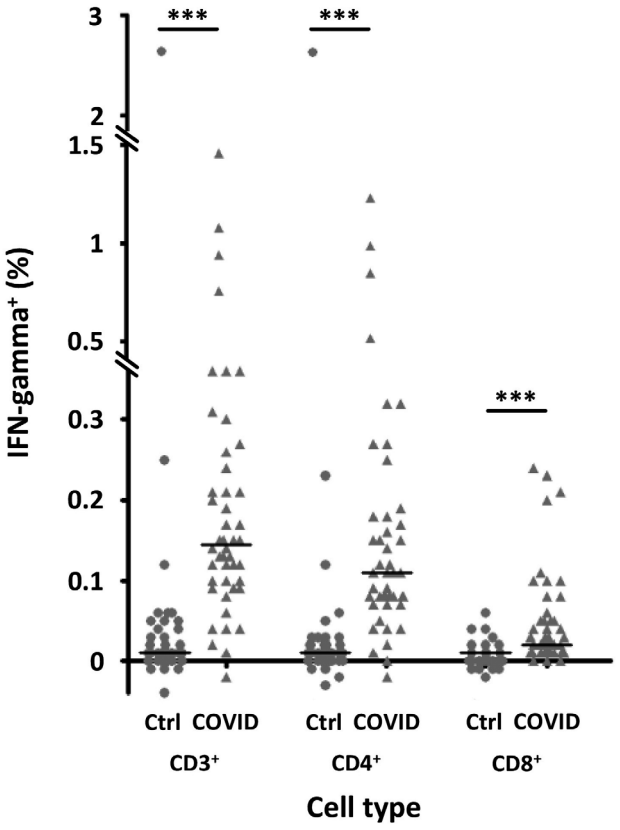

Fig. 2. IFN-γ secretion. Visualization of the percentage of cells secreting IFN-γ in T cells ($CD3^+$), T_H cells ($CD4^+$), and T_C cells ($CD8^+$). Data are shown for the control group (Ctrl) and the COVID-19 group of donors. Bars represent the median. Asterisks indicate statistical significance according to the Mann-Whitney test.

Our data revealed that of the two subpopulations of T cells, the major contributors to cellular immunity reaction against SARS-CoV-2, were $CD4^+$ T_H cells in the majority of patients. As shown in Fig. 2, the IFN-γ pattern for $CD4^+$ T_H cells resembled that of $CD3^+$ T cells with a median of 0.01 % for controls and 0.11 % for COVID-19. In the case of $CD8^+$ T_C cells, the difference between COVID-19 and control samples was subtle, although statistically significant (0.01 % for control and 0.02 for COVID-19).

These data suggest that COVID-19 patients develop virus-specific cellular immunity, which can be detected by the flow cytometry technique after acute infection. Importantly, this approach may serve as a diagnostic tool complementing RT-PCR and antibody testing.

The strength of the anti-SARS-CoV-2 cellular immunity response decreases in time

Twenty-nine COVID-19 donors were invited after five months for repeated test to find out how the cellular immunity response develops in time. Our results show that the specific anti-SARS-CoV-2 immune reaction of T cells decreases in time. After five months since the first testing, the decrease in the cells secreting IFN-γ upon SARS-CoV-2 encounter was statistically significant in the $CD3^+$ and $CD4^+$ populations (Fig. 3). No decrease was observed in the $CD8^+$ subset. Seventy-nine percent of COVID-19 donors showed a decrease in anti-SARS-CoV-2 $CD3^+$ T cells, 3 % showed no change, and in 17 % cases, the number of virus-specific active $CD3^+$ T cells increased. The median value of the decrease in IFN-γ-secreting $CD3^+$ T cells was 40 %, and in the rare cases of increase there was 58 % more $CD3^+$ T cells secreting IFN-γ.

Our data indicate that anti-SARS-CoV-2 cellular immunity becomes weaker in time in the majority of COVID-19 patients and may result in a risk of second infection after a certain time span since the first infection.

T_H and T_C subsets respond to different coronaviral proteins

Our main experiment was designed to observe T-cell activation upon encounter with the mixture of SARS-CoV-2 proteins M, N, and S. This setting ensures detection of the cellular response with maximal sensitivity. To further investigate which of the three coronaviral proteins activates T cells the most, we incubated six samples (Cov-1, Cov-18, Cov-19, Cov-15, Cov-25, and Cov-33) individually with each of the proteins.

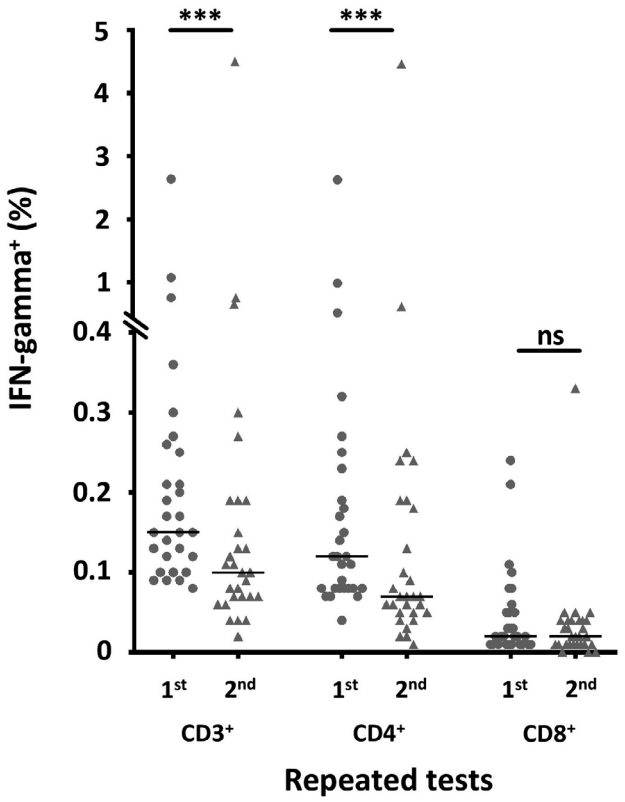

Fig. 3. Repeated tests after five months. Comparison of the percentage of cells secreting IFN-γ in T cells ($CD3^+$), T_H cells ($CD4^+$), and T_C cells ($CD8^+$). Data are shown for the 1st and 2nd test. The interval between testing was 156 days (median; range 142–183 days). Bars represent the median. Asterisks indicate statistical significance according to the Wilcoxon matched-pairs test.

Fig. 4A shows the distribution of the T cells reacting to viral proteins M, N, and S. In five samples (Cov-1, Cov-18, Cov-19, Cov-25, and Cov-33), the highest response was induced by protein M, followed by protein S, and the lowest response was observed for protein N. However, T cells from sample Cov-15 showed a completely reversed order with the highest response for protein N, followed by protein S and lowest for protein M. To explain this mismatch, we analysed the ratio between T_H and T_C subsets secreting IFN-γ upon contact with the virus. As illustrated in Fig. 4B, in all five M-favouring samples, we found that T_H cells were more active than T_C cells (11 times in average). In contrast, the N-favouring Cov-15 sample was the only one with higher T_C-cell activation (2.6 times more than T_H cells). In a larger picture, of 42 samples that exceeded the 0.08 % IFN-γ-positive T cell threshold, only six showed the same or higher activation of T_C cells. In 36 samples, the T_H response to SARS-CoV-2 proteins prevailed.

Interestingly, the one N-favouring sample with preferential T_C-cell activation (Cov-15) has shown a massive decrease in the percentage of SARS-CoV-2-reacting T cells between the two rounds of testing with an under-the-threshold (< 0.08 %) response in the second round of testing after five months (0.27 % in the first round to 0.04 % in the second round). In all of the M-favouring samples (T_H cell mediated), the percentage of SARS-CoV-2-reacting T cells remained above-the-threshold (≥ 0.08 %).

We conclude that the T_H subset exerts the cellular response against SARS-CoV-2 in the majority of cases (86 %) and its main activation trigger is viral protein M. The T_C subset prevails only in a minority of patients

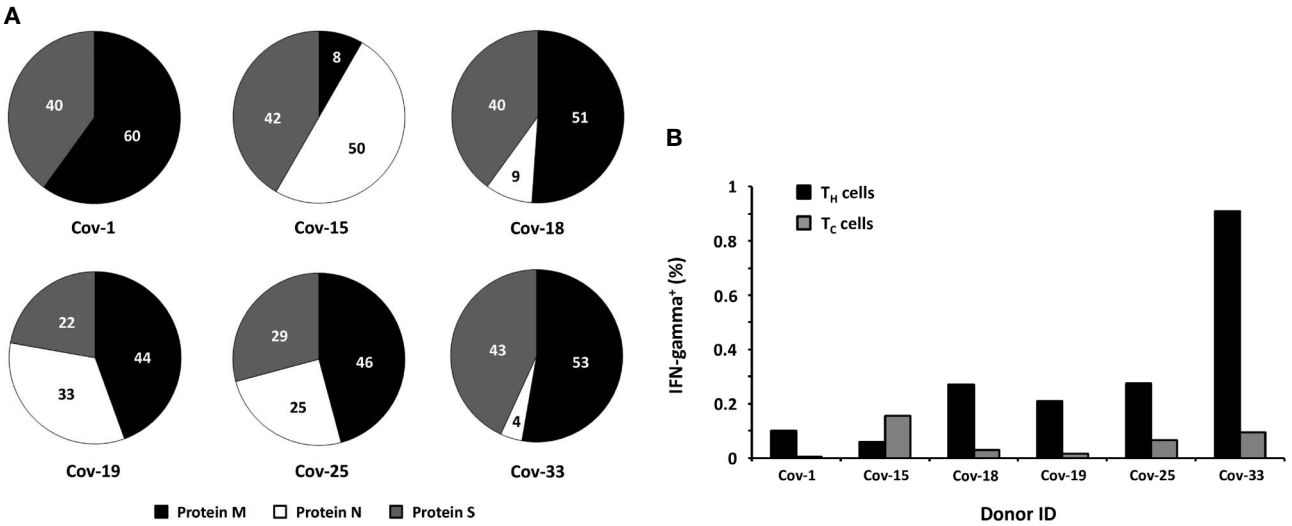

Fig. 4. (**A**) Response of T cells to SARS-CoV-2 proteins. Pie charts illustrate the proportion of T cells activated to secrete IFN-γ upon contact with coronaviral protein M (black), N (white), or S (grey) in six COVID-19 samples (Cov-1, Cov-15, Cov-18, Cov-19, Cov-25, Cov-33). The number indicates the percentage. (**B**) Ratio between T_H and T_C cells. Visualization of the percentage of T_H cells ($CD4^+$; black column) and T_C cells ($CD8^+$; grey column) secreting IFN-γ in six COVID-19 samples.

(14 %) and is triggered preferentially by SARS-CoV-2 protein N. M-favouring T_H cells may provide longer protection against COVID-19 reinfection than N-favouring T_C cells.

Correlation between the severity of COVID-19 and cellular immunity

Next, we investigated whether there is any correlation between the severity of COVID-19 and cellular immunity. According to the survey, COVID-19 patients were divided into two groups: (i) mild symptoms, and (ii) severe symptoms. The first group included patients who were treated at home and reported none or mild symptoms (N = 28). The second group included patients who were either treated at home with medium or severe symptoms (N = 15) or were hospitalized (N = 3).

We found a difference in the cellular immunity response between patients with mild and severe symptoms in T cells and T_H cells (Fig. 5). The difference was not observed for the T_C subtype. The median value of IFN-γ-positive T cells in the "Mild" group was 0.12 % and in the "Severe" group 0.18 %. In the case of T_H cells, 0.08 % positivity was detected in the "Mild" group and 0.14 % in the "Severe" group. Finally, for T_C, we recorded 0.02 % positivity in the "Mild" group and 0.04 % in the "Severe" group. However, it has to be strongly emphasized here that seven samples from the "Mild" group were suspected of being RT-PCR false-positive, as mentioned previously. If these seven samples were excluded from the analysis, there would be no statistically significant difference between the two groups.

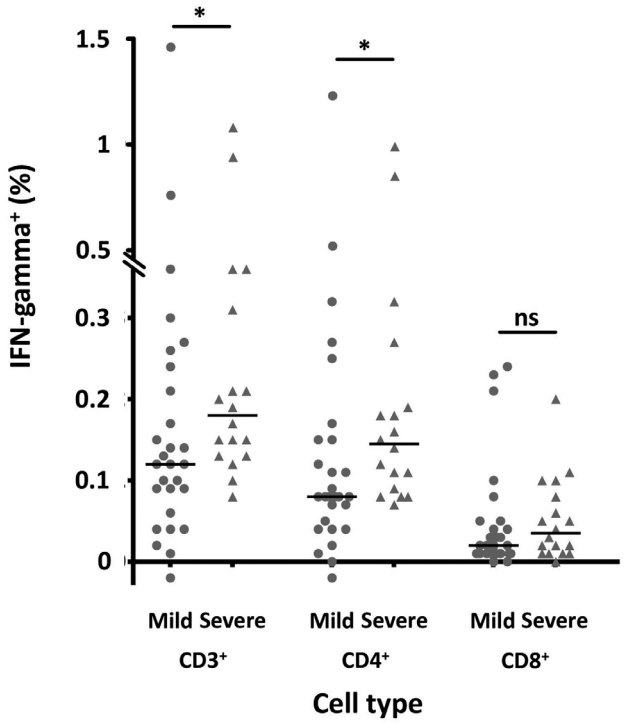

Fig. 5. Correlation between the severity of COVID-19 symptoms and cellular immunity. Visualization of the percentage of cells secreting IFN-γ in T cells ($CD3^+$), T_H cells ($CD4^+$), and T_C cells ($CD8^+$) in COVID-19 donor samples. Data are shown for the group of patients with mild symptoms and with severe symptoms. The bars in the middle of dots represent the median. Asterisks indicate statistical significance according to the Mann-Whitney test.

Discussion

Using the PepTivator®-based method, we were able to quantify the intensity of the SARS-CoV-2-specific immune response of T cells *in vitro*. We analysed samples both from donors who recovered from COVID-19 and donors who were not infected. We have proved that this method reliably identifies the group of donors who were previously tested positively for SARS-CoV-2 by RT-PCR. Based on our results, we propose the $CD3^+$ T cell population with coronavirus-specific IFN-γ secretion ≥ 0.08 % as a diagnostic tool that identifies patients who underwent COVID-19. Using this threshold, there was 85% match between RT-PCR positivity and activated T cells. Fifteen % of the RT-PCR positive samples dropped under the threshold. This can be explained by the false-positive results of RT-PCR. On the other hand, 8 % of donors with no history of COVID-19 achieved levels of activated T cells high above the threshold, indicating that they either underwent the disease unnoticed, or the RT-PCR test was not performed in the acute phase or it was false-negative. Cross-reactivity between circulating ''common cold'' coronaviruses and SARS-CoV-2 should also be taken into account (Kellam and Barclay, 2020).

False-positivity or false-negativity is an important issue in RT-PCR testing (Kiecker et al., 2004; Leung et al., 2020). This method works nearly flawlessly in ideal laboratory conditions; however, in real situations, it may not work so well. The result depends greatly on the laboratory practice standard and personnel skill. In the peak of the COVID-19 crisis in the Czech Republic, most of the samples for RT-PCR were collected in medical tents, with the help of volunteers. Taking into account the supersensitivity of the RT-PCR method and the number of tested people, a certain proportion of false-positive/negative results cannot be excluded. It should also be noted that testing of a broader sample needs to be performed to confirm the threshold of IFN-γ-secreting T cells that precisely identifies SARS-CoV-2-positive donors.

T-cell activation testing has a capacity to assess the intensity of the T-cell response. In some of the donors, the level of IFN-γ was significantly higher. While the median value of IFN-γ-secreting $CD3^+$ T cells was 0.01 % for the control samples and 0.15 % for the COVID-19 samples, five samples surpassed 0.5 %. However, we have not found conclusive evidence that the level of T-cell activation correlates with the severity of the disease. This is illustrated by the fact that the

highest level of IFN-γ-secreting $CD3^+$ T cells (2.64 %) was recorded for the Cov-64 sample from the control group. This donor was tested RT-PCR negative twice and declared only mild putative symptoms such as fatigue and muscular/joint pain. Additionally, we detected lower levels of secreted IFN-γ in the second round of testing after five months in the majority of donors (79 %). Considering that the threshold for COVID-19 positivity was set to ≥ 0.08 % IFN-γ-secreting $CD3^+$ T cells, 34 % of the originally positive patients fell into the "negative" group after five months. This finding implies that several months after infection, the cellular immunity protection against SARS-CoV-2 re-infection might be compromised. Interestingly, 17 % of the donors revealed higher levels of anti-virus T cells. We speculate that these patients may have been re-exposed to the virus in between the two rounds of the testing, although they did not report any symptoms nor were positively tested with PCR. It remains to be investigated whether the intensive reaction may indicate stronger or longer-lasting protection against possible second infection and how anti-SARS-CoV-2 cellular immunity evolves in time.

Both $CD4^+$ and $CD8^+$ cells were activated to secrete IFN-γ upon contact with SARS-CoV-2 proteins M, N, and S. In our experiments, $CD4^+$ cells were more active than $CD8^+$ cells in the majority of patients and were triggered predominantly by proteins M and S. In one recorded sample in which $CD8^+$ cells took the major part, these were triggered mostly by N and S proteins. In a similar study, Grifoni et al. (2020) reported that both $CD4^+$ and $CD8^+$ T-cell response in COVID-19 cases was directed predominantly against proteins M and S, while only a small part of the cells reacted against the N protein (Kellam and Barclay, 2020).

Antibodies against SARS-CoV-2 are commonly monitored to assess the infection (Freeman et al., 2020). While virus-specific IgG and IgM antibodies become detectable by ELISA during the first three weeks after the symptom onset (Long et al., 2020), it is unclear how long the antibody protection lasts. During time, antibody titres slowly decline. Based on the studies of other coronavirus species, the antibodies are estimated to be maintained for up to several years (Sawicki et al., 2007; Tahamtan and Ardebili, 2020; van Kasteren et al., 2020). Cellular immunity mediated by T cells represents another part of the immune defence against SARS-CoV-2. Investigation of the cellular immune response can give us clearer information on how the body copes with the virus and a tool to monitor the spread of the disease across the population. Our data showed that T-cell activation could be used as an alternative method to ELISA to assess whether an individual underwent COVID-19. Our study was originally designed to compare RT-PCR results with T-cell activation testing, and ELISA testing was not available for all the donors. The ability to match the T-cell activation with antibody production would provide valuable information for validating the presented method as a diagnostic tool.

Besides a diagnostic tool, SARS-CoV-2-specific T cells represent a promising approach to the cellular therapy of COVID-19. Adoptive transfer of virus-specific T cells has been successfully used to treat various types of viral infections, such as cytomegalovirus, Epstein-Barr virus, and adenovirus, refractory to antiviral chemotherapy in immunocompromised hosts (reviewed previously by Wrapp et al., 2020). As shown recently by Leung et al. (2020), SARS-CoV-2-specific T cells can be isolated in clinical grade from convalescent donors for urgent clinical use (Wu et al., 2007). An obvious hurdle for transfer of this technology to clinical practice seems to be the risk of graft-versus-host disease (GVHD) development. However, the GVHD risk is limited by depletion of naïve T cells in the product and relatively low clinically effective dose.

Conflict of interest statement

None declared.

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Supplement 1/A

ID	Date of sampling	PCR result	Gender	Age	Count of PBMCs isolated from 1 ml of blood (10^6)	Days of the 1st positive PCR	Days between the 1st positive PCR and sampling	Days between the 1st positive and the last negative PCR	IFN-γ above threshold 0.08%	Suspected source of infection
COV-A	06. 05. 2020	negative	F	46	0,54					
COV-B	06. 05. 2020	negative	F	46	0,86					
COV-C	06. 05. 2020	negative	F	40	1,15					
COV-D	06. 05. 2020	positive	M	26	0,82	24. 03. 2020	43	33	X	room-mate
COV-E	06. 05. 2020	positive	F	22	0,54	24. 03. 2020	43	30	X	room-mate
COV-F	06. 05. 2020	negative	F	37	0,77					
COV-G	06. 05. 2020	negative	M	37	1,42					
COV-H	06. 05. 2020	negative	M	26	0,83					
COV-I	06. 05. 2020	positive	F	27	0,54	17. 03. 2020	50	24	X	unknown
COV-J	06. 05. 2020	positive	M	28	1,12	21. 03. 2020	46	20	X	family member
COV-K	06. 05. 2020	positive	M	29	0,79	20. 03. 2020	47	27	X	unknown
COV-1	25. 05. 2020	negative	F	27	1,14				X	
COV-2	25. 05. 2020	negative	F	45	1,25					
COV-3	25. 05. 2020	negative	F	25	0,43					
COV-4	25. 05. 2020	positive	F	30	0,72	25. 03. 2020	61	16	X	unknown
COV-5	25. 05. 2020	positive	M	32	0,59	20. 03. 2020	66	43	X	unknown
COV-6	25. 05. 2020	positive	M	54	0,87	31. 03. 2020	55	17	X	unknown
COV-7	25. 05. 2020	negative	F	27	0,46					
COV-8	25. 05. 2020	negative	F	32	0,55					
COV-9	27. 05. 2020	negative	M	26	0,41					
COV-10	27. 05. 2020	negative	F	25	0,78					
COV-11	27. 05. 2020	negative	M	18	0,74					
COV-12	27. 05. 2020	negative	M	46	0,73					
COV-13	27. 05. 2020	negative	F	40	0,53					
COV-14	27. 05. 2020	negative	F	42	0,53					
COV-15	28. 05. 2020	positive	M	29	0,90	07. 04. 2020	51	17	X	at work
COV-16	28. 05. 2020	positive	F	52	0,98	07. 04. 2020	51	17	X	family member
COV-17	28. 05. 2020	positive	F	40	1,05	17. 03. 2020	72	15	X	family member
COV-18	28. 05. 2020	positive	M	34	0,86	13. 03. 2020	76	27	X	unknown
COV-19	28. 05. 2020	positive	F	26	0,69	21. 03. 2020	68	47	X	room-mate
COV-20	28. 05. 2020	positive	M	26	0,72	22. 03. 2020	67	42	X	room-mate
COV-21	28. 05. 2020	positive	F	21	0,72	16. 03. 2020	73	23	X	room-mate
COV-22	28. 05. 2020	positive	F	34	0,71	16. 03. 2020	73	22	X	room-mate
COV-23	01. 06. 2020	negative	M	40	0,48					
COV-24	01. 06. 2020	negative	M	39	0,94					
COV-25	01. 06. 2020	positive	M	48	0,39	30. 03. 2020	63	17	X	family member
COV-26	01. 06. 2020	negative	F	53	0,88					
COV-27	01. 06. 2020	positive	M	42	0,99	17. 03. 2020	76	27	X	at work
COV-28	02. 06. 2020	positive	M	68	0,81	10. 04. 2020	53	45	X	friend
COV-29	03. 06. 2020	positive	F	51	0,59	03. 04. 2020	61	16		unknown
COV-30	03. 06. 2020	positive	F	53	0,76	03. 04. 2020	61	16		unknown
COV-31	03. 06. 2020	negative	F	52	0,90					
COV-32	03. 06. 2020	negative	F	46	0,67					
COV-33	03. 06. 2020	positive	M	40	1,74	13. 03. 2020	82	14	X	at work
COV-34	03. 06. 2020	negative	M	25	1,18					
COV-35	03. 06. 2020	negative	F	26	0,98					
COV-36	03. 06. 2020	negative	F	26	1,18					

Severity of symptoms					Symptoms							Repeated sampling	Days between the two samplings	Other diseases
None	Mild	Medium	Severe	Hospital	Days of symptoms	Days of body temperature rise	Body temperature (°C)	Cough	Headache	Ageusia	Anosmia			
	X				7	2	38.5					X	181	
	X				6	3	38.5							
	X				12	2	38.5							
	X				9	4	38.5							
	X				8	4	38					X	183	
												X	162	Hyperthyroidism
														Atopic dermatitis
	X				17	1	37.2		X			X	162	
	X				34	4	39	X	X	X	X	X	162	
	X				3	3	37.4					X	162	Allergies – nickel. cadmium. solvents
														Asthma
														Pneumonia 5 years ago
	X				10	3	37.5		X	X	X	X	163	
		X			10	3	37.5		X	X	X	X	163	Colorectal cancer – chemotherapy
		X			14	2	38.5		X		X	X	159	
	X				21			X	X		X	X	161	
		X			16	7	37.5		X	X	X	X	161	
	X				7	1	37.8		X		X	X	163	
	X				2			X				X	161	
	X				22	2	37	X			X	X	161	
														Asthma
			X		15	6	40					X	156	
														Hypertension, varices
			X		31	3	40		X	X	X	X	156	Allergies – pollen
				X	53									Lung cancer, lymphoma
	X				14			X						
X					0									
														Allergies, hyperthyroidism
														Asthma, hyperthyroidism
		X			14	3	38.5			X	X	X	155	Hypertension

Supplement 1/B

ID	Date of sampling	PCR result	Gender	Age	Count of PBMCs isolated from 1 ml of blood (10^6)	Days of the 1st positive PCR	Days between the 1st positive PCR and sampling	Days between the 1st positive and the last negative PCR	IFN-γ above threshold 0.08%	Suspected source of infection
COV-37	04. 06. 2020	positive	M	56	0,57	10. 03. 2020	86	79	X	unknown
COV-38	10. 06. 2020	positive	F	55	0,53	22. 04. 2020	49	29	X	friend
COV-39	10. 06. 2020	positive	M	53	0,54	23. 04. 2020	48	12	X	public transport
COV-40	10. 06. 2020	negative	F	40	0,48					
COV-41	15. 06. 2020	positive	F	53	0,65	04. 05. 2020	42	24	X	family member
COV-42	15. 06. 2020	positive	M	30	0,57	19. 02. 2020	117	53	X	at work
COV-43	15. 06. 2020	negative	F	66	1,41					
COV-44	15. 06. 2020	negative	F	45	0,37				X	
COV-45	15. 06. 2020	negative	F	47	0,40					
COV-46	16. 06. 2020	negative	F	42	0,68					
COV-47	16. 06. 2020	positive	F	48	0,43	11. 04. 2020	66	24	X	at work
COV-48	16. 06. 2020	positive	M	52	1,04	11. 04. 2020	66	15	X	at work
COV-49	16. 06. 2020	negative	F	35	0,86					
COV-50	16. 06. 2020	positive	F	30	0,50	16. 03. 2020	92	16	X	at work
COV-51	16. 06. 2020	negative	F	35	0,79					
COV-52	16. 06. 2020	positive	M	43	1,01	16. 03. 2020	92	32	X	trip to New York
COV-53	17. 06. 2020	negative	F	31	0,84					
COV-54	17. 06. 2020	negative	F	26	0,42					
COV-55	17. 06. 2020	negative	F	73	2,00					
COV-56	17. 06. 2020	positive	F	51	1,18	27. 03. 2020	82	76	X	at work
COV-57	17. 06. 2020	positive	F	51	0,62	06. 04. 2020	72	27	X	unknown
COV-58	17. 06. 2020	positive	F	21	0,90	30. 04. 2020	48	15	X	friend
COV-59	18. 06. 2020	positive	F	65	1,25	15. 05. 2020	34	14	X	unknown
COV-60	18. 06. 2020	positive	M	65	0,57	18. 05. 2020	31	15	X	unknown
COV-61	18. 06. 2020	positive	M	35	0,14	26. 03. 2020	84	56		family member
COV-62	18. 06. 2020	positive	F	33	0,65	24. 04. 2020	55	13	X	family member
COV-63	18. 06. 2020	positive	F	27	0,30	20. 04. 2020	59	46	X	family member
COV-64	18. 06. 2020	negative	F	48	0,46				X	
COV-65	22. 06. 2020	positive	M	32	0,68	02. 04. 2020	81	15		unknown
COV-66	22. 06. 2020	positive	F	49	0,36	28. 04. 2020	55	17	X	unknown
COV-67	23. 06. 2020	positive	M	36	0,36	14. 03. 2020	101	29		at work
COV-68	18. 06. 2020	positive	F	60	0,52	10. 04. 2020	69	49	X	unknown
COV-69	23. 06. 2020	negative	F	60	1,52					
COV-70	23. 06. 2020	positive	M	54	1,56	02. 06. 2020	21	6		family member
COV-71	23. 06. 2020	positive	F	46	0,67	04. 06. 2020	19	12		unknown
COV-72	23. 06. 2020	positive	F	60	1,60	28. 04. 2020	56	23	X	at work
COV-73	23. 06. 2020	negative	F	18	0,97					

Grey collumns indicate donors positive for SARS-CoV-2 RT-PCR

Severity of symptoms					Symptoms							Repeated sampling	Days between the two samplings	Other diseases
None	Mild	Medium	Severe	Hospital	Days of symptoms	Days of body temperature rise	Body temperature (°C)	Cough	Headache	Ageusia	Anosmia			
				X										Oncological disorder
		X			8	10	38.5	X	X	X	X			Asthma, hyperthyroidism
	X				5	4	38				X	X	150	Hypertension
	X									X	X	X	145	Chronic myelogenous leukaemia, Crohn's disease
	X				3	2	38.5			X	X			
														Asthma, allergies, hypertension
												X	149	
														Atopic dermatitis
			X		11	7	38.8	X	X	X	X	X	148	
			X		10	10	40	X	X	X	X	X	148	After myocardial infarction
														Chronic migraine
		X			9	2	37.5	X	X		X	X	147	
		X			7	5	39	X				X	147	Hypertension
														Coeliac disease
														Diabetes mellitus
			X		92			X	X	X	X	X	146	Cervical cancer, lymph node removal, nephrolithiasis
		X			30	2	37.5		X	X	X	X	146	
	X				94					X	X			
			X		17	14	38			X	X			
	X				2	2	38							
	X				10	1	37.4	X		X	X			Crohn's disease
	X				4			X				X	146	Allergies
	X				21			X	X					
												X	146	
	X				14					X	X			
		X			10	5	38		X					Asthma
	X				5	1	37.8	X	X	X	X			
				X	14	7	38.8	X	X	X	X			
														Hypertension
X					0									Allergies – nuts, birch pollen
	X							X						Allergies, diabetes mellitus
		X			7	4	39		X			X	142	
														Pneumonia 6 months ago, allergies – food, pollen, mould