Supplemental Information

Patients

All patients included in this study were admitted to the University Hospital in Motol, Prague, Czech Republic, between March and May 2020 and tested positive for the presence of SARS-CoV-2 RNA in nasopharyngeal swab using reverse real time polymerase chain reaction (rtPCR). The median age of 19 COVID-19 patients (10 female) was 73.6±29.2 (range 13.7-96.7). The median age of the 28 healthy donors (16 female) was 38.5±10.4 (range 21.4 – 62.8). Patients with moderate course of the disease had clinical signs of pneumonia (cough, auscultation) and verified infiltration on chest X-ray or computed tomography; patients with severe course of the disease required mechanical ventilation; patients with mild course of the disease did not fulfil any of the criteria above but had positive SARS-CoV-2 nasopharyngeal swab rtPCR; and patients with fatal course of the disease died during the course of the study.

Media and reagents

Cells were cultured in RPMI 1640 (Invitrogen, Carlsbad, USA) medium supplemented with 10% FBS, 1% penicillin and 1% glutamax (ThermoFisher Scientific, Waltham, MA, USA). Cells were stimulated with 1µg/ml LPS (Sigma Aldrich, St. Luis, USA). NOS inhibitor L-NMMA and arginase-1 inhibitor L-NHA (Sigma Aldrich) was used in concentration 5µg/ml. ROS production was inhibited by using catalase (2000U/ml) and superoxide dismutase (400U/ml) (both from Sigma Aldrich). NOS activity in human serum and cell culture supernatants was detected as nitrite formed by NO oxidation detected by Gries reagent kit (Sigma Aldrich). Arginase activity was detected by Arginase activity assay according to manufacturer instructions (Sigma Aldrich). CD3/CD28 stimulation of T cells was accomplished by human T cell activator DynaBeads (Invitrogen). Th1 and Th17 specific cytokines were detected upon 50ng/ml PMA and 750ng/ml ionomycine stimulation.

Isolation of peripheral neutrophils and PBMCs

Peripheral blood was collected into EDTA-coated tubes. First, peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque (GE Healthcare BioSciences, Uppsala, Sweden). Neutrophils (polymorphonuclear leukocytes, PMNs) were further isolated using the Dextran sedimentation method; remaining red blood cells were hypotonically lysed and granulocytes were washed twice in PBS without EDTA. Neutrophil purity was <90%, the major contaminants were eosinophils. T cells were isolated from PBMC by positive selection using CD3+ T cell isolation kit (Stemcell Technologies, Vancouver, Canada).

T cell subpopulation induction and proliferation

Isolated CD3+ T lymphocytes were cocultures with granulocytes in ratio 1:2, 1:5 and 1:10 T cells to granulocytes overnight in concentration 1x10⁶ Tly/ml. Then the cells were stimulated with PMA and ionomycin for 3 hours, with 2 hours in presence of Brefeldin A and then cells were stained according to a previously published protocol (Parackova et al., 2019). Briefly, the cells were stained with CD3-A700, CD4-PC7 and CD8-PEDy594, fixed and permeabilized with Fixation/Permeabilization solutions (ThermoFisher Scientific) and stained with IL-17-A647 and IFNgamma-FITC. Samples were acquired on Fortessa and analysed using FlowJo software. Gating strategy is shown at Supplementary Figure 2. When indicated, NOS, arginase1 and ROS inhibitors were added 30 minutes to granulocytes before T cells were added to the cocultures. For proliferation, T cells were cocultured with granulocytes for 5 days in presence of DynaBeads. Then the cells were stained with anti CD3-A700, fixed, permeabilized with Fixation/Permeabilization solutions (ThermoFisher Scientific) and stained with anti-Ki-67-PE antibody (Exbio). Proliferating cells were considered Ki-67+CD3+ T cells. Samples were acquired on Fortessa and analysed using FlowJo software.

TaqMan analysis of mRNA expression
Basal Arg-1 mRNA expression was analyzed in neutrophils after isolation and the following assay was used Hs00163660_m1(ThermoFisher Scientific). RNA isolation, reverse transcription and RT-PCR were performed according to a previously published protocol (Zentsova et al., 2019). TaqMan primer/probe sets were used (ThermoFisher Scientific). The sample data were matched to a standard curve generated by amplifying serially diluted products using the same PCR and normalized to GAPDH (ThermoFisher Scientific) to obtain the relative expression value. Real-time assays were run on an FX96 cycler (Bio-Rad, CA, USA). The primer/probe sets are available from the authors upon request.

**Statistical analysis**

The results obtained from at least four independent experiments are given as the median. Not all patients were involved in all experiments due to the limited amount of blood available per sample. Statistical analysis was performed using non-parametric one-way analysis of variance (ANOVA) with multiple comparisons Dunn’s post-test where applicable. A two-tailed paired Wilcoxon or unpaired Mann-Whitney t-test was also applied for data analysis using GraphPad Prism 8. Values of p<0.05 (*), p<0.01 (**) p<0.001 (***) and p<0.0001 (****) were considered statistically significant.

**References**

Parackova, Z. et al. (2019) ‘Mutual alteration of NOD2-associated Blau syndrome and IFNγR1 deficiency’, *Journal of Clinical Immunology*. doi: 10.1007/s10875-019-00720-6.

Zentsova, I. et al. (2019) ‘Monocytes contribute to DNA sensing through the TBK1 signaling pathway in type 1 diabetes patients’, *Journal of Autoimmunity*. doi: 10.1016/j.jaut.2019.06.005.
Supplementary Figure 1: Gating strategy of granulocyte myeloid-derived suppressor cells (G-MDSC). G-MDSC were detected in whole blood and defined as CD3-CD19-CD20-CD56-HLA-DR+CD14-CD15+CD11b+CD33+cells.
Supplementary Figure 2: Gating strategy of T cell subpopulations. PBMCs or isolated CD3 T cells were stained with CD3-A700, CD4-PC7 and CD8-PEDy594, fixed and stained with IL-17-A647 and IFNgamma-FITC. Th1 were defined as IFN\(\gamma\)+CD4+ and Th17 as IL-17+CD4+.
Supplementary Figure 3: Representative dot plots of patient’s T cell/neutrophil cocultures. Analysis of Th1 (IFNγ+CD4+), IFNγ+CD8+ and Th17 (IL-17+CD4+) in autologous co-cultures of T cells and neutrophils in various ratios. When indicated, cells were co-cultured in the presence of arginase I, ROS and NOS inhibitors.