Inflammasome activation and pyroptosis in lymphopenic liver patients with COVID-19

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Supplementary methods

SARS-CoV-2 Specimen Collection and Testing

Clinical specimens of the nasopharynx for SARS-CoV-2 testing were obtained, handled, and processed via real-time polymerase chain reaction assay as previously reported by our hospital group[1, 2]. Briefly, viral nucleic acid was extracted by the MagNA Pure 96 IVD automated instrument (ROCHE Diagnostics) from nasopharyngeal swab specimen. Real-Time PCR was performed using the COBAS ® Z480 Real-Time PCR System (ROCHE Diagnostics).

Blood Sample Acquisition, Cell Preparation and Immunofluorescence Staining

Patient samples for clinical immunophenotyping were obtained during patients' hospitalizations. Samples were processed by Amerimmune in accordance with Amerimmune's clinical safety SOPs. Appropriate PPE were used when processing samples.

Peripheral blood from venipuncture was drawn into EDTA and heparin coated vacutainer tubes (BD Bioscience) for clinical immunophenotyping. Whole blood collected in EDTA tubes was immunostained per the clinical standard immunophenotyping protocol (Amerimmune LLC, Fairfax, VA). The samples were stained with the antibody combinations as indicated below for 30 minutes at 4˚C. Red blood cells were lysed using BD FACS lysis solution (BD Bioscience, Jan Jose, CA) as per manufacture directions.

Peripheral blood mononuclear cells (PBMC) were separated from 2 mL of whole blood diluted 1:1 with phosphate buffered saline pH 7.2 (PBS) (Thermo Fisher Scientific,

Carlsbad, CA) using Lymphoprep (Stem cell Technologies, Cambridge, MA) and Accuspin tubes (Sigma-Aldrich, St. Louis, MO) as per manufactures directions. PBMCs' were washed in PBS and resuspended in 0.4 mL PBS. 100 µL of the PBMCs were immunostained with a mixture of antibodies as indicated below at 4℃ for 1 hour. Cells were washed and resuspended in PBS prior to acquisition.

The antibodies utilized from Thermo Fisher Scientific were CD45 eF506 [HI130], CD3 FITC [SK7], CD8 PerCP-eF710 [SK1], CD4 APC [SK-3], CD3 PE-CY7 [SK7], CD8 APC-eF780 [SK1], CD45RA FITC [HI100], CD45RO PerCP-eF710 [UCHL1], CD4 AF700 [RPA-T4], CD4 SB600 [SK-3], CD45RA FITC [HI100], CD8 AF700 [SK1], CD25 APC [BC96].

The antibodies utilized from BD Bioscience were HLA-DR BV480 [G46-6], CD38 PerCP-CY5.5 [HIT2], CD45 APC H7 [2D1], CXCR5 PerCP-CY5.5 [RF8B2], CD278 BV421 [DX29], CD127 BV480 [HIL-7R-M21], CD45RO PerCP-CY5.5 [UCHL1].

Pyroptosis Measurement by Flow Cytometry

Pyroptosis was measured by Amerimmune via flow cytometry using fluorescent-labeled inhibitors of caspase probe assay, FLICA, as per manufactures protocol (Immunochemistry Technologies, Minneapolis, MN). FAM-FLICA probes specific for active Caspase 1 were directly added to 100 µl PBMC, incubated for 1 hour at 37℃. Cells were washed 3 times with wash buffer to remove unbound FLICA probes. Cells were stained for CD45 PE-CY7 [HI30], CD3 AF700 [UCHT1], CD4 PE [RPA-T4] and Viability Dye 780 (Thermo Fisher Scientific, Carlsbad, CA) to identify viable cell subsets. Lymphocytes were identified using a gating strategy that identified lymphocytes on a

FSC/SSC plot, then viable cells on a Viability 780 vs SSC and singlets on a FSC-A/FSC-H plot. CD45+ cells were identified on a CD45 vs SSC plot. CD3+ and CD3+CD4+ cells were identified on a CD3 vs CD4 plot gated on CD45+.

Instrumentation and Flow Cytometric Acquisition and Software

The samples were acquired by Amerimmune on a 3 laser BD FACS Canto 10. CS&T beads (BD Bioscience, San Jose, CA) were acquired daily to ensure consistent performance of the Canto10. The BD FACS Canto 10 was cleaned with 10 minutes of 10% bleach and water following acquisition of samples. The CANTO10 utilized for this study has been validated for T, B, NK and Dendritic cell immunophenotyping clinical diagnostic testing. Denovo FCS Express v6 clinical edition (De Novo Software, Pasadena, CA) was used for flow cytometric analyses.

IL-18 ELISA

IL-18 was determined by Amerimmune in plasma obtained from peripheral blood utilizing the Human IL-18 ELISA Kit (Abcam, ab215539) as per manufacturer's directions. Samples were examined minimally in duplicate and triplicate when sufficient sample was available. Analysis was performed using GraphPad Prism v8. A four parameter curve fit (4PL) was performed and IL-18 concentration was interpolated using Prism Interpolate function.

Table S1. Normal Reference Ranges

Item *(unit)*	MedStar Georgetown Transplant Institute	SUNY Downstate Medical Center
Aspartate Aminotransferase (AST) (*u/L)*	3-34	13-39
Alanine Aminotransferase (ALT) *(u/L)*	15-41	7-52
Total Bilirubin *(mg/dL)*	0.2-1.3	0.3-1.0
Alkaline Phosphatase *(u/L)*	45-117	34-104
Lactate Dehydrogenase (LDH) *(u/L)*	84-246	14-271
C-reactive Protein (CRP) *(mg/L)*	0.0-3.0	<10
Ferritin *(ng/mL)*	5.0-148.0	14-233
D-dimer VTE *(mcg/mL FEU)*	<0.65	<0.4
White Blood Cell Count (WBC) *(K/µL)*	4.0-10.8	3.5-10.8
Lymphocytes (*K/µL)*	0.6-4.9	0.9-2.9
CD3+ *(/µL)*	510-2607	742-2750
CD3+CD4+ *(/µL)*	302-1779	404-1612
CD3+CD8+ (*/µL)*	101-951	220-1129

Table Legend: Table showing reference ranges for Liver Function Tests, Inflammatory Markers, Immunomonitoring, and T-Cell Counts for MedStar Georgetown Transplant Institute and SUNY Downstate Medical Center.

References

[1] Iqbal SN, Overcash R, Mokhtari N, Saeed H, Gold S, Auguste T, et al. An Uncomplicated Delivery in a Patient with Covid-19 in the United States. N Engl J Med 2020.
[2] Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020;25.