SUPPLEMENTARY APPENDIX

Complement dysregulation is associated with severe COVID-19 illness

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Supplemental Methods

The modified Ham test

The modified Ham test was used to assess complement activation in patient serum as described previously.[1–4] Briefly, TF1*PIGA*null cells were maintained at a density of 500,000 cells/mL daily. The cells were washed with phosphate-buffered saline (PBS) and seeded in a round-bottom 96-well plate at a density of 6700 cells/well in 80 µL GVB^{++} buffer (Cat. B102, Complement Technology, Inc) in triplicate. 20 µL of serum was added to the cells and incubated at 37°C for 45 minutes with constant shaking. Normal human serum (Cat. NHS, Complement Technology) was heated at 56°C for 30 minutes and used as a negative control (NHS(H)). As a positive control for the mHam, NHS was first incubated with Shiga toxin 1 (stx1, Cat. SML0562, Sigma-Aldrich) for 15 minutes on ice before exposure to the cells. As a positive control for APC activation, the cells were treated with 50 units of Sialidase per sample (Cat. P0720L, New England Bio Labs) at 37°C for 30 minutes, followed by addition of NHS.

After incubation of the cells and serum, the cells were washed with PBS and resuspended in 100 µL 10% WST-1 proliferation solution (WST-1: RPMI 1640 without phenol red at a ratio of 1:9, WST-1 Cat. 11644807001, Roche, Switzerland) and incubated for 2 hours at 37°C. The absorbance of the chromogenic metabolized product was measured with a plate reader (ELX808, BioTeK, Winooski, VT) at 450 nm with a reference wavelength at 630 nm. The sample absorbance was normalized by subtracting the absorbance of a blank control, which contained WST-1 solution only.

The percentage of live cells was calculated using [(A_{sample}-A_{blank})/$A_{sample(H)}$ -A_{blank}) x 100]. The percentage of non-viable cells (100- percentage of live cells) was used as a measure of complement-mediated cell killing. All experiments were performed in triplicate.

Detection of complement activity by flow cytometry

Cell surface deposition of C5b-9 and C3c on TF1*PIGA*null cells was measured by flow cytometry as previously described.[3–5] TF1*PIGA*null cells were washed with PBS and seeded in a V-bottom 96-well plate (1.2 x 10^5 cells/well) in 80 µL of either GVB^{++} buffer or GVB^0·MgEGTA buffer (pH 6.4) (GVB^0 Cat. B103, Complement Technology, Inc). GVB^{++} allows for activation of all complement pathways while GVB^0·MgEGTA only allows for alternative pathway activation. 20 µL patient serum was added to the cells and incubated at 37°C for 15 minutes with constant shaking. For alternative pathway activation, NHS was acidified to pH 6.4 with 0.2 M HCl. 5 mM of ethylenediaminetetraacetic acid (EDTA) was added to NHS to inhibit complement activation and used as a negative control.

After incubation, the reaction was stopped by addition of PBS supplemented with 1% BSA and 15 mM EDTA. The cells were centrifuged at 600 g for 3 minutes and the cell pellet was collected. The cells were stained with anti-C5b-9 monoclonal antibody (Cat. Sc-58935, Santa Cruz Biotechnology, Inc, dilution at 1:100) followed by Alexa 647 conjugated secondary antibody (Cat. Ab 172325, Abcam, dilution at 1:500). The cells were also stained with Alexa 488 conjugated anti-C3c antibody (Cat.4212, Abcam, dilution at 1:150). Ten thousand events per sample were recorded using BD

FACSCalibur, and data were analyzed using FlowJo Software version 10.5.3 (FlowJo Inc).

Supplemental References

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