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Sars-Cov-2 Spike Protein Induces Cellular Changes in Primitive and Mature Hematopoietic Cells

James Ropa, BS, MSc, PhD,[1] Scott Cooper, MS,[*,1]
Maegan L. Capitano, PhD,[1] Hal E. Broxmeyer, PhD[1]

[1]Department of Microbiology & Immunology, Indiana University School of Medicine, Indianapolis, IN

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The devastating effects of SARS-CoV-2 infection (which causes COVID-19) highlight the need for a deeper understanding of the virus, host response, and the tissues affected by infection, including the hematopoietic stem cells (HSC), hematopoietic progenitors (HPC), and more mature blood cells including immune cells. While other coronavirus infections have been associated with hematopoietic or immune cell complications (Chu et al., *J Infect Dis* 2016), the effects of SARS-CoV-2 infection on HSC/HPC and mature immune cells are not yet understood. We performed studies to examine the potential susceptibility of HSC/HPC subpopulations and immune cells to infection and functional studies to explore if exposure to SARS-CoV-2 proteins impacts these cells. The most well-studied receptor for SARS-CoV-2 is ACE2 (Hoffmann et al., *Cell* 2020), a cell surface protein to which SARS-CoV-2 spike protein (S protein) binds to facilitate viral entry to host cells. We explored expression of ACE2 in primitive and mature blood cells. We demonstrated using RT-qPCR as well as western blotting that ACE2 is expressed at both the mRNA and protein levels in pooled low-density peripheral blood cells (PB) from healthy donors (n=5); pooled CD34+ cells from cord blood (CB) enriched for HSC/HPC (n=7); lineage+ (lin+) low density CB cells (n=7), and high density polymorphonuclear leukocytes (PMN) from CB (n=3). We then determined ACE2 expression on specific subpopulations of HSC/HPC and immune cells. Flow cytometry analysis (FACS) demonstrated that ACE2 is expressed on the cell surface of small subpopulations of PB immune cells, including 1-2% of T-cells, 2-4% of B-cells, and <1% of NK cells and monocytes. However, larger populations of HSC/HPC exhibited ACE2 cell surface expression. The percent ACE2+ HSC, multipotent progenitor cells (MPP), and multipotent lymphoid progenitor cells (MLP) were respectively 15-60%, 5-50%, and 5-15% of cells (n=5). We next determined if exposure to viral S protein affects these cell populations. We first tested if SARS-CoV-2 S protein induces responses in HSC/HPC. We isolated CD34+ cells from low density CB and expanded them in the presence of serum and growth factors

(100ng/mL TPO, FLT3L, and SCF) in the presence or absence of recombinant full-length S protein. After 7 days expansion, cells were analyzed for immunophenotype using FACS or were plated in semi-solid methylcellulose in an HPC colony forming unit (CFU) assay and grown for 12 days before scoring for functional CFU-Granulocyte/Erythroid/Macrophage/Megakaryocyte (CFU-GEMM) and CFU-Granulocyte/Macrophage (CFU-GM) progenitor cells. CB CD34+ cells exhibit significantly less expansion when exposed to SARS-CoV-2 S protein, showing 33% less expansion of CD34+ cells. The most significant delay in expansion of phenotyped cells is evident in granulocyte-monocyte progenitors (GMP, 38% less expansion), with a more modest 15-30% decrease in expansion for common myeloid progenitors/megakaryocyte-erythroid progenitors (CMP/MEP), HSC, MPP, and MLP (n=4). S protein lessens functional HPC colony forming cell expansion compared to control, with 12-fold and 2.1-fold expansions compared to 30.8-fold and 6-fold expansions respectively for CFU-GM and CFU-GEMM. These data indicate that exposure to SARS-CoV-2 S protein impacts hematopoiesis and myeloid differentiation *in vitro*. We next tested effects of S protein exposure on PB by isolating low density blood cells from healthy donors and culturing them *ex vivo* for different time periods in the presence or absence of recombinant full-length S protein. After 2hrs incubation, we observed by FACS a modest but consistent 1.2-fold increase in CD14 positive monocytes in cells exposed to S protein compared vehicle control (n=4). After 18hrs incubation with the S protein, the PB monocytes exhibited consistent changes in morphology, becoming smaller and more granular, determined by changes in forward scatter and side scatter by FACS (n=5). This suggests that exposure to SARS-CoV-2 protein may have a physiologic impact on PB cells *ex vivo*, despite their low levels of ACE2 expression. Our data thus has important implications for hematopoiesis and immune response in COVID-19 patients. It demonstrates the need to determine whether CB potentially exposed to SARS-CoV-2 should be banked, as the effects we observed may affect the efficacy of CB transplantations.

Disclosures

No relevant conflicts of interest to declare.

Author notes

* Asterisk with author names denotes non-ASH members.

Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.