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CD28 Expression Distinguishes Plasma Cell Fate in Pediatric Patients Suffering from COVID-19/Kawasaki’s Disease Vs MIS-C

Adam Utley, PhD, Kelvin P. Lee, MD, Mark Hicar, MD*

1 Roswell Park Cancer Institute, Buffalo, NY
2 University at Buffalo, Buffalo, NY

Blood (2020) 136 (Supplement 1) : 8.

The COVID-19 pandemic has ravaged the global community and highlighted the importance of antibody-mediated antiviral immune responses. The SARS-CoV-2 virus is highly pathogenic, but is unique from other viral infections in that pediatric patients are largely spared from severe symptoms. However, a small number of pediatric patients present autoimmune-like symptoms after COVID-19 infection, termed Multisystem Inflammatory Syndrome in Children, or MIS-C. Symptomatically, it shares some similarity to that of Kawasaki’s Disease (KD), an autoimmune disorder linked to coronavirus infection thought to be driven by autoantibody production. Understanding the immunological mechanisms that facilitate clearance of SARS-CoV-2 or drive the development of life-threatening autoimmune symptoms in MIS-C or KD is critical for our ability to design successful vaccines that do not elicit autoimmunity in children.

Antibodies are produced by terminally differentiated B lymphocytes known as Plasma Cells (PC). Because successful immune responses/vaccination strategies against SARS-CoV-2 are dependent upon effective PC production, the dysregulation of which may lead to the development of Kawasaki’s Disease or MIS-C, it is critical to understand the immunological mechanisms that define the pediatric PC response leading to these individual outcomes.

We therefore sought to characterize the B cell-PC immune response in pediatric patients that have successfully cleared SARS-CoV-2 and compare the B cell immunological landscape to children who develop either KD or MIS-C. We used the 10X Genomics Platform to interrogate at a single cell level the CD19+ B/PC populations in the peripheral blood of pediatric patients at the transcriptional level as well as with VDJ deep sequencing.
We began by clustering the CD19⁺ populations based on transcriptional similarity and found that both COVID-19 and KD exhibited 12 distinct clusters, but that MIS-C only had 6 clusters. Intriguingly, in evaluating clonal diversity, KD presented a broad spectrum of clonotypes while the COVID-19 and MIS-C patients were much more limited. This suggested that although KD and MIS-C both present with similar autoimmune-like symptoms, the mechanistic basis for their respective etiology may be distinct. We therefore sought to more deeply probe the subset of cells responsible for antibody production by evaluating the PC subsets.

High BLIMP1 expression (PC lineage-defining transcription factor) was present in 2 distinct clusters in COVID-19/KD patients, but was broadly distributed at lower levels in MIS-C. We then looked at genes which were significantly upregulated in the most terminally differentiated cells from each patient. 50 genes were significantly higher in the COVID-19 and KD populations, and 54 in MIS-C. Using gene ontology analysis in COVID-19 and KD, we saw increased expression of transcripts involved in protein trafficking, redox responses, and respiratory metabolism. In comparison, the MIS-C patient demonstrated significance for programs involved in immature B cell development and inflammation. Taken together, this suggests that in COVID-19 and KD there is a program of terminal PC differentiation which is absent in MIS-C. To understand the mechanistic basis for the terminal differentiation in COVID-19 and KD, we sought to probe possible regulators of PC fate.

We have recently published that CD28, the canonical T cell costimulatory molecule, is expressed by PC, and CD28 signaling through the adaptor protein SLP-76 leads to increased BLIMP1 expression and an IRF4-mediated metabolic program necessary for PC survival. Interestingly, SLP-76 was expressed at high levels in COVID-19 and KD patients, but was low in MIS-C. Similarly, CD28 was expressed in both COVID-19 and KD patients and correlated with higher IRF4 levels and metabolic genes, but was entirely absent in MIS-C.

Taken together, these findings suggest that CD28 signaling may facilitate PC fate in COVID-19 pediatric patients and the development of KD arises from broad antibody specificity, possibly explaining how vascular antigens become targets. Furthermore, MIS-C, although similar in symptomatic presentation to KD, has an etiology driven by antigen-independent inflammation arising from immature B cells due to a lack of CD28-mediated PC differentiation and survival, which can be evaluated diagnostically by simple flow cytometry in a vaccination setting.

**Disclosures**

No relevant conflicts of interest to declare.
Author notes

* Asterisk with author names denotes non-ASH members.

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