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Commentary In-Press Preview

Multisystem Inflammatory Syndrome in Children (MIS-C) is a rare but deadly new disease in children that rapidly progresses to hyperinflammation, shock, and can lead to multiple organ failure if unrecognized. It has been found to be temporally associated with the COVID-19 pandemic and is often associated with SARS-CoV-2 exposure in children. In this issue of the *JCI*, Porritt, Paschold, and Rivas et al. identify restricted T cell receptor (TCR) β-chain variable domain (Vβ) usage in patients with severe MIS-C indicating a potential role for SARS-CoV-2 as a superantigen. These findings suggest that a blood test that determines the presence of specific TCR beta variable gene segments (TRBV) may identify patients at risk for severe MIS-C.

SARS-CoV-2 as a superantigen in multisystem inflammatory syndrome in children (MIS-C)

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Abstract

Multisystem Inflammatory Syndrome in Children (MIS-C) is a rare but deadly new disease in children that rapidly progresses to hyperinflammation, shock, and can lead to multiple organ failure if unrecognized. It has been found to be temporally associated with the COVID-19 pandemic and is often associated with SARS-CoV-2 exposure in children. In this issue of the *JCI*, Porritt and Paschold et al. identify restricted T cell receptor (TCR) β-chain variable domain (Vβ) usage in patients with severe MIS-C indicating a potential role for SARS-CoV-2 as a superantigen. These findings suggest that a blood test that determines the presence of specific TCR beta variable gene segments (TRBV) may identify patients at risk for severe MIS-C.

Evolution of MIS-C as a new pediatric disease

Over the past year, SARS-CoV-2 has been implicated in a variety of different disease processes. In addition to the respiratory syndrome now known as COVID-19, SARS-CoV-2 has also been implicated in formation of blood clots, rashes, neurological symptoms, and a post-infectious syndrome termed "long COVID." In sharp contrast to other respiratory viruses such as RSV and influenza, which cause a substantial disease burden in the pediatric population every year during the fall and winter months, the burden of disease for COVID-19 has fallen predominantly on adults.

However, in April 2020, several weeks following the peak of the pandemic in Europe, there were reports of an increased incidence of a Kawasaki's Disease (KD)-like syndrome in the UK and Italy (1,2). New York City, the initial US epicenter of the COVID 19 pandemic, followed with a similar rise in May. KD is a rare childhood vasculitis typically seen in children under 5 years of age and is the most common cause of acquired heart disease in children in developed countries due to the development of coronary artery aneurysms (3). Classically, it is clinically diagnosed based on the presence of five or more days of fever, and four or more of the following: mucosal changes, conjunctival injection, swelling of hands and feet, and cervical lymphadenopathy greater or equal to 1.5 cm.

In contrast to KD, this new syndrome termed multisystem inflammatory syndrome in children (MIS-C), which appears to be temporally associated with COVID-19, more commonly affects older children and adolescents, is associated with gastrointestinal complaints, and progresses much more rapidly to severe illness and multi-organ dysfunction. Cytokine profiling and immunophenotyping data from several studies demonstrate that KD and MIS-C are likely two distinct clinical entities (4).

So dire are the consequences of missing a diagnosis of MIS-C due to the rapid progression to multi-organ dysfunction that the CDC released a Health Alert Network (HAN) advisory in May 2020 defining MIS-C in overly broad clinical terms to capture as many affected patients as possible. Unfortunately, this broad approach has also led to overly aggressive medical workups and hospital admissions for children with otherwise benign viral syndromes. Thus, a critical need exists to identify diagnostic biomarkers of MIS-C.

Current trends in MIS-C research have focused on profiling the immune perturbations caused by the disease via cytokine analysis, high-dimensional flow cytometry, and RNA-Seq (5). These big data approaches have generated a wealth of knowledge and certainly paint a picture of a highly dysregulated immune response to SARS-CoV-2. However, these techniques are not easily translatable to widespread clinical use.

Superantigen theory of disease

MIS-C shares many clinical similarities with toxic shock syndrome (TSS) in that both are rapidly progressive, involve multiple organ systems, and appear to develop because of an overwhelming hyperinflammatory immune response. Thus, a superantigen (SAg) effect for SARS-CoV-2 has previously been hypothesized (6,7).

Superantigens (SAgs) are a group of antigens from bacteria or viruses that activate T cells in a non-specific manner by binding to the β-chain variable (Vβ) region of the T cell receptor (TCR). In contrast, conventional antigens are processed and presented by antigen presenting cells (APCs) and require a very specific interaction with the TCR known as the conventional peptide/MHC complex. A massive release of proinflammatory cytokines such as Tumor Necrosis Factor α (TNFα), Interleukins (IL-2) and Interferon γ (IFNγ) ensues following polyclonal non-specific activation of T cells by SAgs leading to what is referred to as a "cytokine storm" (8). The most well-studied bacterial SAgs are the exotoxins produced by *Streptococcus aureus*, which are responsible for TSS.

In addition to TSS, SAgs have also been implicated in a variety of acute and chronic autoimmune diseases such as KD, mentioned previously, acute rheumatic fever, chronic rheumatic heart disease and psoriasis (8). While TCR repertoire studies in each of these cases have found restricted Vβ amplification, which is a hallmark of SAg activity, a causative link has yet to be established. In addition to the direct effects of cytokine release, downstream mechanisms of disease include tissue damage leading to abnormal presentation of self-peptides by APCs, activation of autoreactive T cells, and skewing of the balance between $CD4^+$ and $CD8^+$ T cell populations.

Identification of Vβ restriction in children with MIS-C

In this issue of the *JCI*, Porritt and Paschold et al. examined the TCR repertoires of children with mild and severe MIS-C compared with age-matched febrile control patients. In contrast to febrile control patients without MIS-C and patients with mild MIS-C, TCR-sequencing analysis revealed enrichment of TRBV11-2, TRBV24-1, and TRBV11-3 gene segments in patients with severe MIS-C, and that TRBV11-2 enrichment, in particular, was associated with the development of a "cytokine storm" characterized by elevated levels of TNF-α, IFN-γ, IL-6, and IL-10 (9).

Importantly, the authors went on to demonstrate that diversity in CDR3 and J gene usage was maintained and did not differ substantially amongst the patient groups (9). Because the CDR3 loop is the major determinant of antigen specificity for conventional peptide/MHC interactions, the observation that CDR3 diversity is maintained supports the authors' hypothesis that T cell expansion is being driven by a superantigen rather than a specific TCR/peptide/MHC interaction. Further, because SAgs bind predominantly to the V segment of the TCR, diversity in J gene usage also supports this hypothesis.

To date, the majority of children affected by MIS-C appear to be of Hispanic Latino or Non-Hispanic Black ethnicity while Asian children have been relatively unaffected (10). This observation suggests the presence of an underlying genetic contribution to disease pathogenesis. In this study, HLA typing revealed that all four patients with severe MIS-C that had TRBV11-2 expansions also used HLA class I

A02, B35 and C04 alleles. In contrast, only 10% of febrile controls and 0% of MIS-C patients without TRBV11-2 expansions utilized all three alleles. Interestingly, compared to the Asian population, these alleles are found in relatively higher frequencies in the Non-Hispanic Black, Hispanic Latino, and Caucasian populations in the United States (11).

Although the authors do propose an in silico model for how SARS-CoV-2 may interact with the TCR and MHC molecule in a SAg fashion, in vitro and in vivo studies demonstrating a direct causal relationship still need to be performed (9).

Concluding Thoughts

Enrichment of TCRBV11-2 in peripheral blood samples of patients with suspected MIS-C has the potential to be used as a biomarker to help physicians identify patients at risk for severe disease, and thus spare many children from an extensive medical work-up or unnecessary hospital admissions.

Two important questions remain: 1. if SARS-CoV-2 functions as a SAg, why is the inflammatory cascade delayed by up to two to four weeks post-infection rather than coinciding with massive T cell activation? and 2. What is the trigger? The delayed response would indicate a mechanism that is separate from large scale T cell activation and the associated cytokine release that is classically seen in TSS. The authors hypothesize that this trigger may be a second exposure to SARS-CoV-2 in the absence of neutralizing antibody titers or residual viral particles in the intestinal tract (9). Another possibility follows a two-hit model whereby SARS-CoV-2 infection is the first-hit that non-specifically expands a polyclonal population of T cells. A second-hit with a different virus, already endemic in humans, two to four weeks later might activate a subpopulation of T cells initially expanded from the first-hit with cross-reactivity to self-antigens.

While the study is limited by small sample size and lack of in vitro or in vivo data to demonstrate mechanism, it should also be remembered that obtaining adequate blood volumes from children to perform robust T cell studies can be challenging, MIS-C is a rare but catastrophic disease, and we are in the midst of a global pandemic that has taxed the healthcare and scientific infrastructure. Thus, the work done by Porritt, Paschold and colleagues (9) that has shed light on this mysterious and lethal childhood disease is commendable.

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References

1. Whittaker E, Bamford A, Kenny J, et al. Clinical Characteristics of 58 Children with a Pediatric Inflammatory Multisystem Syndrome Temporally Associated with SARS-CoV-2. *JAMA - J Am Med Assoc*. 2020;324(3):259-269. doi:10.1001/jama.2020.10369

2. Verdoni L, Mazza A, Gervasoni A, et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *Lancet*. 2020;395(10239):1771-1778. doi:10.1016/S0140-6736(20)31103-X

3. McCrindle BW, Rowley AH, Newburger JW, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: A scientific statement for health professionals from the American Heart Association. *Circulation*. 2017;135(17):e927-e999. doi:10.1161/CIR.0000000000000484

4. Rubens JH, Akindele NP, Tschudy MM, Sick-Samuels AC. Acute covid-19 and multisystem inflammatory syndrome in children. *BMJ*. 2021;372. doi:10.1136/bmj.n385

5. Brodsky NN, Ramaswamy A, Lucas CL. The Mystery of MIS-C Post-SARS-CoV-2 Infection. *Trends Microbiol*. 2020;28(12):956-958. doi:10.1016/j.tim.2020.10.004

6. Cheng MH, Zhang S, Porritt RA, et al. Superantigenic character of an insert unique to SARS-CoV-2 spike supported by skewed TCR repertoire in patients with hyperinflammation. *Proc Natl Acad Sci U S A*. 2020;117(41):25254-25262. doi:10.1073/pnas.2010722117

7. Lee K-Y, Rivas MN, Toplak N, et al. Pediatric Inflammatory Multisystem Syndrome Temporally Related With SARS-CoV-2: Immunological Similarities With Acute Rheumatic Fever and Toxic Shock Syndrome. *Front Pediatr | www.frontiersin.org*. 2020;8:574. doi:10.3389/fped.2020.00574

8. Commons RJ, Smeesters PR, Proft T, Fraser JD, Robins-Browne R, Curtis N. Streptococcal superantigens: Categorization and clinical associations. *Trends Mol Med*. 2014;20(1):48-62. doi:10.1016/j.molmed.2013.10.004

9. Porritt RA, Paschold L, Noval Rivas M, et al. HLA class I-associated expansion of TRBV11-2 T cells in Multisystem Inflammatory Syndrome in Children. *J Clin Invest*. Published online March 11, 2021. doi:10.1172/JCI146614

10. Health Department-Reported Cases of Multisystem Inflammatory Syndrome in Children (MIS-C) in the United States | CDC. Accessed March 10, 2021. https://www.cdc.gov/mis-c/cases/index.html

11. Cao K, Hollenbach J, Shi X, Shi W, Chopek M, Fernández-Viña MA. Analysis of the frequencies of HLA-A, B, and C alleles and haplotypes in the five major ethnic groups of the United States reveals high levels of diversity in these loci and contrasting distribution patterns in these populations. In: *Human Immunology*. Vol 62. Elsevier Inc.; 2001:1009-1030. doi:10.1016/S0198-8859(01)00298-1