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*J Clin Invest.* 2021. [https://doi.org/10.1172/JCI151467](https://doi.org/10.1172/JCI151467).

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Zonulin as a biomarker and potential therapeutic target in multisystem inflammatory syndrome in children

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COI statement: The authors declare no conflicts of interest.

Abstract

Multisystem inflammatory syndrome in children (MIS-C) occurs during, or recently following SARS-CoV-2 infection and is characterized by persistent fever, inflammation and severe illness requiring hospitalization. The majority of MIS-C cases also present with gastrointestinal (GI) symptoms, including abdominal pain, vomiting and diarrhea. In a recent issue of the JCI, Yonker and Gilboa et al., identify zonulin as a biomarker of GI permeability in children with MIS-C, and present the results of an intriguing proof-of-concept study which suggests that zonulin may represent a potential therapeutic target for MIS-C treatment and prevention. Together, these findings suggest that intestinal mucosal dysfunction and epithelial barrier breakdown may represent a biological mechanism underlying the development of MIS-C in SARS-CoV-2-infected children.

Identification of MIS-C in SARS-CoV-2 infected children

Since the first reported cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19) (1), the World Health Organization has reported over 173.5 million confirmed cases of COVID-19 worldwide and over 3.7 million deaths (2). Throughout the pandemic, the COVID-19 situation for children has differed from that of adults. Indeed, most reports indicate that while children are equally likely to become infected with SARS-CoV-2, the disease is mild for a large majority of pediatric cases (3). As such, the COVID-19 mortality rate in the United States is 0.34 per 100,000 population and 0.16 per 100,000 for children aged 0-4 and 5-14 years, respectively (4). In stark contrast, the overall COVID-19 mortality rate is 91.5 per 100,000 population in adults, with the highest rate for those aged 85 years and older (1,797.8 per 100,000) (5).

In April 2020, reports of children with severe complications that developed days to weeks after SARS-CoV-2 infection or exposure began to emerge (6). In these cases, otherwise healthy children were hospitalized with cardiogenic shock or Kawasaki disease-like symptoms, including persistent fever, abdominal pain, vomiting, diarrhea, skin rash, mucocutaneous lesions and, in severe cases, hypotension and shock (7). Based on these observations, the CDC issued a health advisory including a case definition for Multisystem Inflammatory Syndrome in Children (MIS-C) Associated with Coronavirus Disease 2019 (COVID-19) (7). The CDC guidance describes MIS-C as an individual younger than 21 years of age presenting with fever, laboratory evidence of inflammation (including elevated CRP, ESR, d-dimer, IL-6, etc.), and severe illness requiring hospitalization and multisystem organ involvement. In addition, to meet the case definition, patients need to demonstrate evidence of current or recent SARS-CoV-2 infection by RT-PCR, antigen test, or serology; or have exposure to a suspected or confirmed case of COVID-19 within four weeks of symptom onset (7). As of June 2, 2021, the CDC has reported 4,018 cases of individuals meeting this criteria for MIS-C and 36 MIS-C-related deaths in the United States (8). Multiple immunological mechanisms for MIS-C have been proposed including an inflammatory response to SARS-CoV-2 superantigen (9), delayed interferon response resulting in the distinct MIS-C cytokine
storm (10), and production of pathologic autoantibodies (11). Notably, most children with MIS-C test positive for antibody to SARS-CoV-2 rather than SARS-CoV-2 by RT-PCR (10, 12), supporting the proposed mechanism of dysregulated immune responses to infection rather than pathology driven directly by the virus. However, an autopsy report of a child who died of cardiac failure related to MIS-C showed evidence of SARS-CoV-2 RNA and viral particles in cardiac tissue, suggesting that a second wave or low-level ongoing viral replication may contribute to fatal outcomes (13).

**Gastrointestinal dysfunction as a mechanism underlying MIS-C**

Interestingly, more than 80% of MIS-C cases present with gastrointestinal (GI) symptoms (12), as compared with the 10-15% of adult COVID-19 infections that report GI manifestations (14, 15). These differences in the prevalence of GI symptoms, combined with the delay between infection and clinical presentation of MIS-C, suggests that GI pathology in MIS-C may occur via a mechanism distinct from that which causes GI disruption in adults with active COVID-19. Recent reports have suggested that microbial dysbiosis and GI barrier breakdown may drive inflammation and immune activation in severe COVID-19 infection of adults (16, 17); however, little is known about GI dysfunction in MIS-C. In a recent study in the *JCI*, Yonker and Gilboa et al. sought to address the potential role of GI disruption in MIS-C by measuring zonulin, a proposed biomarker of intestinal permeability (18).

Zonulin modulates epithelial barrier integrity by triggering a signaling cascade that results in phosphorylation and displacement of tight junction proteins (19). Increased GI permeability through a zonulin-dependent mechanism allows paracellular passage of antigenic triggers from the gut lumen into the mucosa and eventually systemic circulation. Zonulin-mediated disruption in the GI tract has been implicated as a pathological mechanism underlying multiple chronic autoimmune and hyperinflammatory diseases (20). Notably, some studies have proposed that zonulin can be viewed as a marker of enterocyte function, suggesting decreased plasma levels of zonulin could indicate epithelial cell death or dysfunction (21, 22). Indeed, lower plasma zonulin concentrations in antiretroviral therapy treated people living with HIV predicted mortality in one cohort study (21). While it is possible that reduced zonulin may represent epithelial cell death or dysfunction, multiple studies in which inhibition of zonulin prevented immune-mediated disease support a role for zonulin in pathological intestinal permeability (19).

**Zonulin-dependent intestinal permeability in MIS-C**

Yonker and Gilboa et al. (18) demonstrated that children with MIS-C had elevated plasma zonulin levels and detectable SARS-CoV-2 stool viral loads, suggesting ongoing intestinal viral replication. These findings led the authors to hypothesize that intestinal barrier breakdown could allow for SARS-CoV-2 antigenemia, thus inciting development of MIS-C. In support of this, the authors demonstrated that children with MIS-C exhibited elevated levels of SARS-CoV-2 Spike proteins in plasma, specifically the S1 component, as compared with SARS-CoV-2-infected children without MIS-C or healthy controls. Finally, the authors conducted an intriguing proof-of-concept experiment in which they treated a child with MIS-C with the zonulin antagonist larazotide (23). Following larazotide treatment, the child exhibited clinical improvement, including reduced plasma levels of CRP, inflammatory cytokines, and Spike antigen. These findings presented by Yonker and Gilboa et al. suggest that therapies focused on rescuing intestinal barrier integrity may be a viable option for the treatment or prevention of SARS-CoV-2-associated MIS-C (18).

**Concluding thoughts**

Yonker and Gilboa et al. (18) acknowledge that more work remains to be done to conclusively identify the gut as the source of the elevated levels of circulating SARS-CoV-2 viral particles that may trigger development of MIS-C. In particular, studies are needed to assess the ability of SARS-CoV-2 antigens to traverse the intestinal mucosal epithelial barrier, and to verify that zonulin disruption is the major mechanism mediating translocation. Additionally, it will be important to determine how simultaneous translocation of SARS-CoV-2 proteins and additional microbial components may facilitate the risk for MIS-C. Indeed, Yonker and Gilboa et al. (18) demonstrate that plasma levels of the microbial translocation markers, lipopolysaccharide binding protein (LBP) and soluble CD14 (sCD14), are elevated in children with MIS-C as compared with controls or children with SARS-CoV-2 without MIS-C. Furthermore, the role of microbial dysbiosis in intestinal barrier permeability and risk for MIS-C remains unclear. Previous studies have demonstrated that microbial dysbiosis occurs during SARS-CoV-2 infection in adults and identified a potential link between disrupted intestinal microbial profiles and risk of severe COVID-19 disease (24, 25). Given these findings and the importance of the commensal microbiome in maintaining intestinal immunity and barrier integrity (26), it will be critical to define (a) the impact
of SARS-CoV-2 infection on microbial community structure in children and (b) how shifts in microbial profiles due to COVID-19 infection may promote disruption of mucosal epithelial barriers and enhance risk for MIS-C. Finally, it is interesting to speculate whether a method of microbial manipulation to restore intestinal microbial communities, such as probiotics, prebiotics, or fecal microbial transplant, could synergize with larazotide to reduce mucosal permeability and limit the risk for and pathology of MIS-C.

Taken together, the importance of identifying viable treatment options to prevent or mitigate MIS-C is underscored by the increasing prevalence of SARS-CoV-2 infections in children and adolescents and the current unavailability of COVID-19 vaccines for children under 12. The work presented by Yonker and Gilboa et al. (18) provides insight into a potential mechanism in which zonulin-dependent loss of intestinal epithelial barrier integrity precipitates translocation of SARS-CoV-2 proteins from the gut, thereby inducing development of MIS-C. The proof-of-concept data demonstrating that larazotide treatment resulted in clinical improvement of a child with MIS-C warrants further study of potential therapeutic strategies focused on restoring epithelial barrier integrity in SARS-CoV-2-infected children. Moreover, these findings may have wider implications, as this approach could be explored in the pediatric setting for a variety of conditions in which loss of mucosal homeostasis is thought to play a role, such as intestinal bowel diseases or other viral infections, like HIV.

Acknowledgements: The authors would like to thank Jennifer Dubin for assistance with editing and proofreading.

References


