

## Spotlight

## Unraveling the mechanisms of MIG immunotherapy in MIS-C

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In *The Journal of Clinical Investigation*, Zhu et al.<sup>1</sup> report that intravenous immunoglobulin (IVIG) targets IL-1 $\beta$ <sup>+</sup> neutrophils to exert anti-inflammatory effects in multisystem inflammatory syndrome in children (MIS-C), a post-infectious inflammatory condition associated with COVID-19.

Though SARS-CoV-2 infection in children is either mild or asymptomatic, in some children it is associated with the development of a post-infectious, serious inflammatory condition called multisystem inflammatory syndrome in children (MIS-C).<sup>2</sup> MIS-C is characterized by immune dysregulation and cytokine storm, and the disease has overlapping features of myocarditis, toxic shock syndrome, and Kawasaki disease (KD), a common acute febrile childhood vasculitis. While there is considerable acute morbidity with MIS-C—nearly two-thirds of patients need intensive care treatment—mortality is low (1.9%) and short-term outcomes are favorable.<sup>2</sup> Due to the novelty of the syndrome and its similarity to KD, most centers have extrapolated KD guidelines for the clinical management of MIS-C.<sup>3</sup>

Our understanding of molecular mechanism(s) that trigger MIS-C is still evolving; however, some studies have suggested a possible role of IL-1 family signaling intermediates in triggering a hyper-inflammatory response. To understand the pathogenesis of MIS-C and the source of IL-1 $\beta$ , Zhu et al. compared the expression of IL-1 $\beta$  in various immune cells of MIS-C patients, KD patients, and febrile controls.<sup>1</sup> Intracellular flow cytometry using human IL-1 $\beta$ -specific monoclonal antibodies indicated that IL-1 $\beta$ <sup>+</sup> leukocytes were highly predominant in KD patients, followed by MIS-C patients. Further dissection of the signature of the immune cells identified neutrophils as a major source of IL-1 $\beta$ . Analyses of RNA in MIS-C neutrophils also confirmed high expression of IL-1 $\beta$  but not IL-1 $\alpha$ . It

should be noted that the increased percentage of IL-1 $\beta$ <sup>+</sup> neutrophils was not specific for either KD or MIS-C and was also observed in febrile controls. Therefore, it appears that enhancement of IL-1 $\beta$ <sup>+</sup> neutrophils is a common feature of pediatric inflammatory conditions.

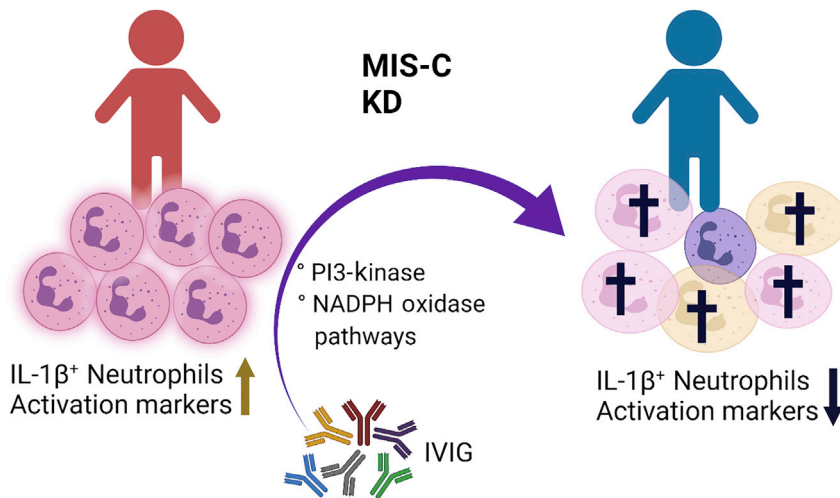
Zhu et al. used mass cytometry to further analyze the phenotype of neutrophil lineage cells and noted that neutrophils from MIS-C and KD display shared as well as distinct expression patterns of adhesion and activation markers.<sup>1</sup> While neutrophils from both sets of patients had similar levels of CD44, CD54, CD63, HLA-DR, and high-affinity Fc $\gamma$  receptor CD64, the expression of CD62L, CD66b, CD11b, and low-affinity Fc $\gamma$  receptors CD16 and CD32 was high in MIS-C. On the other hand, markers of maturation including CD49d, CD101, and CD10 were more prominent in the febrile control group, suggestive of a relatively higher number of immature neutrophils in KD and MIS-C. Similar findings were also recorded by Syrimi et al., who noted neutrophilia with decreased expression of maturation markers in MIS-C patients.<sup>4</sup> The signals that trigger activation of neutrophils in MIS-C are not yet clear. MIS-C develops 3–6 weeks post-SARS-CoV-2 exposure, and these patients have been reported to maintain highly inflammatory anti-SARS-CoV-2 IgG that might trigger neutrophil activation.<sup>5</sup> In fact, Zhu et al. reported that the expression of both CD32 and CD16 was high on MIS-C neutrophils compared to KD.<sup>1</sup> In addition, various inflammatory cytokines that were shown to be enhanced in

MIS-C patients could also induce neutrophil activation. Neutrophils play an important role in pathogenesis of KD and have been demonstrated in the early coronary artery lesions characteristic of the disease.<sup>6</sup> Current evidence also suggests that neutrophils are activated in MIS-C.<sup>2</sup> The data from Zhu et al. thus shed new light on the role of neutrophils in triggering inflammatory responses in MIS-C.

Intravenous immunoglobulin (IVIG), a pooled normal IgG, has been widely used as immunotherapy for a variety of autoimmune and inflammatory conditions, including KD.<sup>7</sup> More recently, The American College of Rheumatology (ACR) published guidelines that recommend IVIG as a first-line therapy in MIS-C patients.<sup>3</sup> After treatment with IVIG, Zhu et al. noted more than 50% reduction in the neutrophil counts and more than 90% reduction in IL-1 $\beta$ -expressing neutrophils in both KD and MIS-C groups.<sup>1</sup> This remarkable effect persisted even when the analysis was confined to patients who received IVIG as the only immunomodulating medication in either group. Additionally, there was a reduced expression of neutrophil activation and adhesion markers, indicating that IVIG targets IL-1 $\beta$ <sup>+</sup> neutrophils to ameliorate the inflammation in these two inflammatory conditions (Figure 1). Thus, by demonstrating the vital role of IL-1 $\beta$  in the pathogenesis of KD and MIS-C, this study adds evidence for the use of anakinra, an IL-1 receptor antagonist, in patients refractory to therapy with IVIG or steroids.<sup>3</sup>

Neutrophil death is crucial for the resolution of granulocyte-mediated inflammation.





**Figure 1. IVIG targets IL-1 $\beta$ <sup>+</sup> neutrophils to exert anti-inflammatory effects in MIS-C and Kawasaki disease (KD)**

MIS-C and KD patients display enhanced frequency of IL-1 $\beta$ <sup>+</sup> neutrophils with an upregulated activation and adhesion molecules. Zhu et al. report that IVIG therapy reduces IL-1 $\beta$ <sup>+</sup> neutrophils and their activation markers. Mechanistically, IVIG induced neutrophil death by phosphatidylinositol 3-kinase and NADPH oxidase pathways. The figure was created with the support of <https://biorender.com>.

The authors aimed to identify the mechanisms of IVIG-induced neutrophil death using *in vitro* chemical inhibitors and live-cell imaging. They found that IVIG-induced neutrophil cell death was blocked by phosphatidylinositol 3-kinase (PI3K) and NADPH oxidase inhibitors, but not with pan-caspase inhibitors, indicating that IVIG-induced cell death does not occur via apoptosis and caspase-1-dependent pyroptosis, necroptosis, and ferroptosis.<sup>1</sup> This report thus identifies that IVIG and its F(ab)<sub>2</sub> fragments exert cytotoxic effects on neutrophils from patients with inflammatory disorders via PI3K- and NADPH oxidase-dependent pathways. Previous *in vitro* experiments based on the cells isolated from healthy donors have shown that IVIG induces neutrophil death via both caspase-dependent and -independent pathways, mediated via antibodies to Fas and Siglec-9, respectively.<sup>8</sup> Under the influence of inflammatory cytokines, IVIG induced a caspase-independent neutrophil death via the NADPH oxidase pathway.<sup>8</sup> Both KD and MIS-C patients display enhanced levels of various pro-inflammatory cytokines that explain observed caspase-independent cytotoxic effects of IVIG on neutrophils. Of note, the morphology of neutrophils that underwent caspase-independent cell death resembled that of autophagolysosomal death,<sup>8</sup> and recently IVIG has been shown to induce autophagy in

the peripheral blood mononuclear cells by F(ab)<sub>2</sub>- and PI3K-dependent pathways.<sup>9</sup> As IVIG-induced neutrophil cell death in KD and MIS-C patients was also dependent on PI3K, future investigation should aim at further dissection of this pathway.

Does this report explain all the possible mechanisms of IVIG in MIS-C? As correctly pointed out by the authors, the report provides one of multiple IVIG mechanisms that might be responsible for the amelioration of inflammation and pathology in MIS-C. Current evidence shows that various markers of inflammation are decreased in MIS-C patients following IVIG therapy.<sup>4</sup> In fact, IVIG has been shown to suppress the activation of T cells, monocytes, dendritic cells, and endothelial cells<sup>7</sup> that are activated in MIS-C. Also, nonspecific activation of B cells with autoimmune signatures was observed in MIS-C,<sup>5</sup> and IVIG could check B cell activation.<sup>7</sup> Although IVIG has been reported to suppress superantigen-mediated lymphocyte activation, that mechanism might not be responsible for the efficacy of IVIG in these MIS-C patients, as pre-COVID-19 pandemic IVIG preparations do not have anti-SARS-CoV-2 antibodies.<sup>10</sup>

There are limitations of the study, most notably the choice of a small sample size between groups, chosen for convenience rather than powered for statistical anal-

ysis. The authors must be commended for the extensive immune analysis performed; however, there is inconsistency in the number of subjects between groups. Only two KD subjects underwent mass cytometry. Lastly, “febrile control” was not well defined. While the reader is asked to assume that this group consists of common childhood febrile illnesses, this group could include anyone with a common cold to a malignancy, potentially confounding the study’s findings. Pediatric COVID-19 patients without MIS-C would have made an ideal comparison.

The big picture of molecular mechanisms that lead to MIS-C in select SARS-CoV-2-exposed children remains unknown. This article suggests that neutrophils and IL-1 $\beta$  are the important players of pathogenesis of MIS-C. A randomized clinical trial in MIS-C would be useful to validate the findings, but with uncertainty over the COVID-19 pandemic, it might be difficult to recruit sufficient patients at single centers, and international collaboration might be required. Further work on identifying the specific pathways IVIG utilizes to suppress inflammation in MIS-C is needed and may allow us to edit dosages specific to clinical conditions being treated and effectively use adjunct therapies, such as steroids and other biological immunomodulators.

#### DECLARATION OF INTERESTS

J.B. (at Institut National de la Santé et de la Recherche Médicale, Centre de Recherche des Cordeliers, Paris, France) has received research grants from CSL Behring, France, for investigation on the mechanisms of action of IVIG.

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