SARS-COV-2

The immune roadmap for understanding multi-system inflammatory syndrome in children: opportunities and challenges

In the spring of 2020, a series of reports from Europe and the USA described clusters of children and adolescents presenting with a life-threatening, hyperinflammatory syndrome — called 'multi-system inflammatory syndrome in children' (MIS-C) — that was seemingly linked to prior exposure to the coronavirus SARS-CoV-2. In June 2020, the US National Institutes of Health convened a workshop of immunologists and clinicians to discuss emerging knowledge and identify key questions surrounding MIS-C, with a focus on innate and adaptive immunity, genetics and epigenetics. This Meeting Report describes the main findings from the workshop.

The emergence of a late-onset COVID-19-associated acute inflammatory syndrome in children, who present with severe abdominal pain, volume-resistant shock and cardiovascular injury, was first described in reports from Europe and the UK in the spring of 2020 (https://www.ecdc.europa.eu/en/publications-data/paediatric-inflammatory-multisystem-syndrome-and-sars-cov2-rapid-risk-assessment) and was subsequently recognized in New York City[1–3]. The name 'multi-system inflammatory syndrome in children (MIS-C) temporally associated with COVID-19' was coined, and diagnostic criteria were established (https://health.ny.gov/press/releases/2020/docs/2020-05-13_health_advisory.pdf; https://emergency.cdc.gov/han/2020/han00432.asp). Two publications described almost 300 US children with MIS-C[4,5]. In those cohorts, the evidence for association with antecedent COVID-19 disease or exposure to SARS-CoV-2 was demonstrated by PCR positivity (40–50%), antibody positivity (31–45%) or clear history of exposure. Affected children had a median age of about 8 years, and children of Black race and/or Hispanic ethnicity were over-represented. At presentation, 80–90% had severe abdominal pain, and almost a third presented in shock, with volume-resistant hypotension and evidence of cardiac injury (elevated troponin and echocardiographic evidence of left ventricular dysfunction) and/or other end-organ damage. Ultimately, 80% required support in an intensive care unit. Coronary-artery ectasia or aneurysms developed in 8–9%. Almost all had laboratory evidence of immune activation, including elevations in C-reactive protein, erythrocyte sedimentation rate, D-dimers, fibrinogen and/or interleukin 6 (IL-6). Approximately half of the children, predominantly those under 13 years of age, had some signs and symptoms overlapping those of toxic shock syndrome and Kawasaki Disease (KD) (fever, rash, conjunctivitis, mucosal symptoms, and swollen hands and feet); however, features that distinguish KD from MIS-C include older age, increased prevalence among Black and Hispanic children, and the severity and ubiquity of shock, ventricular dysfunction, severe abdominal pain and degree of elevation of laboratory indicators of inflammation (C-reactive protein, D-dimers and serum ferritin) in MIS-C. Treatment included intravenous immunoglobulin (IVIG) in about 75% of children, and corticosteroids in half to two thirds. The average hospital stay was 6–7 days and, despite the critical nature of the illness, survival was 98%. The immunological events that lead to the development, clinical course and resolution of MIS-C are not known, nor is it understood why children are uniquely vulnerable, why or how they recover and whether long-term consequences might ensue. Thus, there is an urgent need to study these children. As part of the US National Institutes of Health's response to the COVID-19 pandemic, the Division of Allergy, Immunology and Transplantation of the National Institute of Allergy and Infectious Diseases sponsored a workshop, entitled 'The Immune Roadmap to Understanding MIS-C', on Wednesday 24 June 2020. The goal of the workshop was to inform the National Institutes of Health, immunologists and clinicians of critical questions and scientific approaches to understanding the immunopathology of MIS-C. Experts in B cell immunity, T cell immunity, innate immunity, genetics, epigenetics and other immunological disciplines were invited to address the following questions: how do specific components of the immune system contribute to the development and manifestations of MIS-C? What data currently support the proposal of that role? How would it be possible, in a clinical study of children with MIS-C, to gather information that supports or refutes that hypothesis? And, finally, what are the real-world issues that affect the ability to carry out the recommended studies?

The presentations and discussions outlined a wide range of possible mechanisms underlying MIS-C: a consequence of a person's initial innate immune response to infection with SARS-CoV-2, which sets the stage for all that follows; an autoimmune response resulting from cross-reactivity between viral antigens and host antigens; a response to ongoing viral replication in unrecognized viral reservoirs; a response to a viral superantigen; and/or the influence of genetic or epigenetic susceptibilities. This list is not exhaustive, and the hypotheses are not mutually exclusive. What follows is a summary of the key points and recommendations from the meeting.

B cell immunity

MIS-C usually presents with sudden symptom onset without a preceding respiratory illness. In patients with recorded COVID-19-compatible symptoms preceding MIS-C (7–24%), the interval between the two manifestations of COVID-19 is typically 21–25 days[4,5]. Accordingly, most of these children display antibodies to SARS-CoV-2. The development of neutralizing antibodies that target the receptor-binding domain of the spike (S) protein of SARS-CoV-2

is considered a sign of protection and represents a major goal of ongoing vaccine trials. Betty Diamond (Feinstein Institute) opened the B cell immunity session of the workshop by highlighting the enigma surrounding the role of B cells and antibody-secreting cells (ASCs) in SARS-CoV-2-related disorders. Circulating anti-viral antibodies, for example, may be short-lived, especially in patients with mild disease[6], and the therapeutic role of convalescent serum remains to be firmly established in randomized trials. Understanding the durability and quality of anti-SARS-CoV-2 B cell responses in patients with different clinical presentations and disease severity remains an important task. Ignacio Sanz (Emory University) reported robust expansion of oligoclonal ASCs spanning immunoglobulin M (IgM), IgG and IgA isotypes in adult patients with COVID-19 who required admission to an intensive care unit despite the presence of high titers of neutralizing antibodies[7]. The impact of ASC expansion, which has also been described by others[8], on protective immunity or disease pathogenesis remains to be determined. Notably, the expansion of ASCs is correlated with activation of the extrafollicular activation pathway with increased numbers of naive and DN2 effector B cells. This profile separates severe COVID-19 disease from milder disease[7], and preliminary analysis suggests its presence in childhood MIS-C as well (M. Woodruff, K. Cashman and I. Sanz, personal communication). Of interest, similar B cell responses are found in severe systemic lupus erythematosus, especially in African Americans[9], who also suffer from a higher incidence of MIS-C, suggestive of a pathogenic role for these responses in severe COVID-19.

Searching for clues to understand why MIS-C affects predominantly children, Patrick Wilson (University of Chicago) suggested that autoantibody cross-reactivity with SARS-CoV-2 epitopes or bystander activation of autoreactive B cells could induce organ damage. Using SARS-CoV-2 multi-antigen panels, his group selected virus-specific B cells from adult patients with COVID-19 to study their phenotype, gene-expression profile and binding affinity at the single-cell level[10]. Scott Boyd (Stanford University) agreed that the delayed peak of presentation of MIS-C relative to the symptomatic viral infection is compatible with antibody- and/or immunocomplex-mediated disease. In adults with classic COVID-19, he reported the expansion of polyclonal IgM and isotype-switched antibodies characterized by low rates of somatic mutations[11], a finding recently reported by others[12].

In rare circumstances, anti-viral antibodies can cause antibody-dependent enhancement, which might result from cross-reactivity to conserved proteins from related viral strains, as has been well documented for dengue. Such antibodies would bind, but not neutralize, and would eventually enhance disease severity, possibly by triggering Fcγ receptor–mediated uptake of virus by myeloid cells and subsequent cytokine storm[13]. While data in support of such mechanisms in COVID-19 are missing[14], conflicting experimental data have been presented for antibodies to the S proteins of the coronaviruses MERS-CoV or SARS-CoV[15]. In this context, Jeff Ravetch (Rockefeller University) highlighted the importance of interactions between the immunoglobulin Fc fragment and its receptor FcR in modulating innate and adaptive immune responses to viruses. These interactions are heavily dependent on post-translational modifications of Fc, especially glycosylation (fucosylation or sialylation)[16]. Notably, these modifications vary tremendously among humans and might explain differential responses to viral infections and vaccination, as well as unique susceptibility to inflammatory diseases. In fact, diminished fucosylation of IgG, which enhances interaction with the activating receptor FcγRIIIa, has been observed in adult patients with COVID-19 but not in SARS-CoV-2-seropositive asymptomatic children[17]. While no data are available yet on children with MIS-C, these patients respond to IVIG, an immunomodulatory therapy that relies on the display of a specific glycan, α2,6-sialylated, on the Fc C_H2 domain of a small proportion of antibodies[18]. Whether differential glycosylation of Fc contributes to the pathogenesis of MIS-C can be studied in children, as small volumes of plasma yield enough IgG to carry out these assays. These mechanistic studies not only would help elucidate MIS-C but also would support the continued development of vaccines and/or passive antibody interventions in COVID-19.

T cell immunity

The temporal relationship between exposure to SARS-Cov-2 and the onset of MIS-C, as well as the central role of T cells in viral immunity, suggests that altered or dysregulated T cell function may contribute to the immunopathology of MIS-C. One challenge in testing this hypothesis is that the characteristics of effective T cell responses to SARS-CoV-2 have not been established. Shane Crotty (La Jolla Institute of Immunology), in collaboration with Alessandro Sette (La Jolla Institute of Immunology), analyzed the T cell response to SARS-CoV-2 in non-hospitalized, convalescing adult patients with COVID-19[19]. Stimulation of T cells with biocomputationally predicted peptide epitopes of the virus and assessment of cytokine production indicated that all patients were able to generate antigen-specific $CD4^+$ T cell responses to SARS-CoV-2, while $CD8^+$ T cell responses were detected in more than two thirds of the patients. The $CD4^+$ T cell response was robust and broad in specificity, and the proportion of S-specific $CD4^+$ T cells correlated well with IgG antibodies specific for the receptor-binding domain within the S protein, indicative of productive T cell–B cell collaboration. Overall, the T cell response to SARS-CoV-2 in this cohort is consistent, in terms of its magnitude and cytokine production, with a canonical response to a respiratory virus. Interestingly, when T cells obtained in the pre-COVID era from healthy people were tested against the same SARS-CoV-2 peptide panel, $CD4^+$ T cell responses and $CD8^+$ T cell responses were observed in half and one fifth of the samples, respectively, possibly representative of recall responses to cross-reactive antigens previously encountered from 'common cold' coronaviruses. It was recently confirmed that these are true memory cells[20]. It will be important to determine if these cross-reactive T cells contribute to immunoprotection against SARS-CoV-2 or, conversely, to immunopathology. Antigen-specific T cell assays in conjunction with single-cell RNA sequencing could be informative in assessing the magnitude, breadth and functional attributes of T cell immunity to SARS-CoV-2 in MIS-C.

In contrast to the aforementioned convalescing patients with COVID-19 who had mild disease, Laura Vella (Children's Hospital of Philadelphia and University of Pennsylvania) described profound immunological differences, including perturbations in the T cell compartment, revealed through deep immunoprofiling of peripheral blood mononuclear cells obtained from hospitalized adult patients with COVID-19[8]. While a range of illness was exhibited by the COVID-19 cohort, three distinct 'immunotypes' were identified, including one marked by increased activation of $CD4^+$ T cells and $CD8^+$ T cells that correlated best with disease severity. Vella suggested that it will be important to follow the dynamics of T cell signatures over time to fully appreciate the importance of distinct immune phenotypes. In the setting of MIS-C, longitudinal analysis could be especially relevant, as the onset of the inflammatory syndrome occurs several weeks after infection, and some children

with MIS-C still harbor viral RNA in the nasopharynx at presentation. It is possible that ongoing, chronic viral exposure in the respiratory system or other tissue reservoirs provides a persistent source of antigen for T cells, with clinical consequences.

It will be important to compare peripheral T cell responses versus tissue-specific T cell responses. Donna Farber (Columbia University) used high-dimensional flow cytometry to demonstrate that the airways of intubated patients with COVID-19 had a distribution of major T cell subsets markedly different from that in the blood, with tissue-resident memory T cells present in airway samples but not in paired blood samples; the association of tissue-resident memory T cells with disease severity is being studied. Measures of the association of anti-SARS-CoV-2 antibody response with clinical manifestations of infection, including a cohort with MIS-C, and adult patients with mild and severe COVID-19 disease, were explored. Examination of antibody specificity, isotype and neutralizing activity revealed distinct responses in the group with MIS-C, including lower neutralizing activity and fewer antibodies to the nucleocapsid protein than that of adult patients[21]. Studies are ongoing to compare the antibody response in MIS-C to that in a pediatric group with COVID-19. Ideally, future studies of MIS-C will combine cellular analyses and humoral analyses to evaluate coordination between different arms of the immune response.

Mark Davis (Stanford University School of Medicine) has taken a broad approach to identify T cell specificities that might be clinically relevant in the response to SARS-CoV-2. T cell receptor (TCR) sequence analysis with the GLIPH2 algorithm[22,23] identified large numbers of TCRs from different people on the basis of sequence and shared motifs and organized them into clusters indicative of peptide–major histocompatibility complex specificities. The analysis of millions of TCR sequences from patients with a range of COVID-19 disease severities allows investigation of the repertoire for unique TCR motifs shared by people with mild illness that could be important in controlling the disease. The next step is to establish whether these TCRs are indeed SARS-CoV-2 specific. Similar approaches could be applied to patients with MIS-C to determine whether their TCR repertoire usage is skewed or is deficient in 'protective' TCRs, or whether dominant pathogenic TCR specificities are present. Pairing sequencing results with single-cell transcriptomic profiles will link TCR usage to functional and phenotypic features and will provide a comprehensive signature of T cells in MIS-C.

Innate immunity

Innate immunity has a fundamental role in initiating and shaping adaptive immune responses to viruses and underlies inflammatory manifestations that correlate with adverse outcomes in COVID-19 in adults and in MIS-C. A striking difference between these two groups, however, is the targeted organ(s). Children with COVID-19, particularly those with MIS-C, tend to be spared severe pulmonary involvement but display a range of gastrointestinal and cardiovascular manifestations, including myocarditis and coronary-artery dilatation that resemble KD[3]. Respiratory complications, on the other hand, are the leading cause of morbidity and mortality in adults with COVID-19. Gwendalyn Randolph (Washington University) proposed that GM-CSF (granulocyte–macrophage colony-stimulating factor), a myelopoietic growth factor and pro-inflammatory cytokine, might have a role in MIS-C. GM-CSF drives cardiovascular pathology in mouse models of KD[24] while promoting alveolar macrophage–dependent innate host defense and lung protection, especially in the youngest age range[25]. GM-CSF-activated myeloid cells express pro-inflammatory cytokines described in adult patients with severe COVID-19 and in MIS-C. A distinct subset of $CD4^+$ helper T cells produce GM-CSF[26], which in turn enhances the ability of dendritic cells to prime T cells during antigen-specific immune responses. Thus, GM-CSF might link lymphoid cell and myeloid cell crosstalk to the pathogenesis of MIS-C. Endothelial cells are also an important component of the COVID-19 and MIS-C puzzles. They express the SARS-CoV-2 receptor ACE-2 and SARS-CoV-2 co-receptors, and damaged endothelium promotes leukocyte influx and coagulation, two major contributors to the pathogenesis of COVID-19[27]. While endothelial tissue is not readily accessible, protein markers of endothelial damage and coagulation could be studied in soluble and/or exosomal fractions of serum from patients with MIS-C through the use of targeted or unbiased proteomic assays.

Thirumala-Devi Kanneganti (St. Jude's Children's Research Hospital) reminded us of the central role of the inflammasome and inflammatory cell death in COVID-19 and MIS-C. IL-1β and IL-18 are prototypic cytokines at the center of monogenic and sporadic inflammasome-related diseases and macrophage-activation syndrome, both of which clinically overlap severe COVID-19 and MIS-C. IL-1β has been linked to the pathogenesis of KD and is upstream of IL-6. Clinical trials targeting IL-1 or IL-6 are underway in COVID-19, and blocking these cytokines is a therapeutic approach in MIS-C[28,29]. Furthermore, inflammatory-cell death linked to inflammasome activation and pyroptotic and PANoptotic cell death may contribute to tissue damage and the release of inflammatory cytokines, not only IL-1β but also TNF, IFN-γ and others[30,31]. The levels of cytokines, caspase activity and metabolites known to trigger inflammasome activity in serum from patients, together with blood transcriptional signatures, may shed light on the relevance of this pathway in the pathogenesis of MIS-C. Genetic and/or epigenetic approaches might reveal important upstream links between inflammasome activation and MIS-C.

Wayne Yokoyama (Washington University) addressed the fundamental role of natural killer cells in anti-viral immunity, reviewing how genetic defects in cytolytic granule exocytosis–related pathways lead to viral persistence, relentless macrophage activation and a cytokine storm characterized by high levels of IL-6, similar to what is observed in adults with severe COVID-19 and in MIS-C[32]. Assessing the phenotype and function of natural killer cells, and genetic studies to identify potential genetic defects in these pathways, are warranted.

Dusan Bogunovic (Mount Sinai School of Medicine) discussed a multi-layered approach to studying MIS-C with genetics, single-cell transcriptomics, cytokine and chemokine serum profiling, high definition immunophenotyping of blood cells, and anti-virus and autoantibody profiling[33]. Most patients in this series had clear-cut post-viral syndrome presentation, with negative PCR results for SARS-CoV-2, low or negative anti-viral titers of IgM and positive anti-viral titers of IgG and IgA. These patients all had normal anti-SARS-CoV-2-neutralizing activity relative to that of convalescent adults. Alterations in serum cytokine and chemokine profiles and contraction of unique monocyte and dendritic cell subsets were observed in most patients. Extensive autoantigen arrays showed responses to autoantigens commonly observed in both systemic autoimmune diseases and organ-specific autoimmune diseases that could explain some clinical manifestations of MIS-C. These data support the feasibility and informative yield of a systems-biology approach to studying MIS-C. Most of the children included in this study had received systemic therapy before enrollment, although samples were obtained during

Table 1 | Key questions to address about the immune response in MIS-C

Topic	Question
B cells	• How does the anti-SARS-CoV-2 antibody response in children with MIS-C compare with that of children with asymptomatic infection or mild disease, as well as to adults with COVID-19-related ARDS? • Can features of the antibody response in patients with MIS-C, including kinetics, variable-region repertoire, degree of somatic variation, anti-viral fine specificity and potential autoantigen cross-reactivity, provide insight into disease pathogenesis? • Is the extrafollicular B cell–differentiation pathway predominantly contributing to the antibody response in patients with MIS-C? • Do anti-SARS-CoV-2 IgA responses in mucosal sites contribute to GI and extra-GI manifestations in MIS-C? • Does Fc glycosylation status have a role in MIS-C?
T cells	• Do the $CD4^+$ and $CD8^+$ T cell responses in children with MIS-C resemble conventional anti-viral immunity, or are there deficits in phenotype, T cell effector or regulatory function, or antigen specificity? • What is the viral fingerprint on pediatric immune subsets, and can deep immunoprofiling be used to identify T cell signatures that are predictive of disease progression or outcomes? • How effective are T cells in supporting the development of a broad antibody response with neutralizing activity? Do extrafollicular helper T cells have a role in MIS-C? • Do T cells from children with MIS-C show cross-reactivity with common cold–related coronaviruses, and do these 'memory' cells have a role in immunoprotection or immunopathology? • How does TCR repertoire usage in children with MIS-C compare with that of children convalescing from COVID-19? Are there TCR specificities associated with immunoprotection or immunopathology? • Does viral persistence either in the respiratory tract or in other tissues affect the T cell responses, and is this clinically relevant?
Innate immunity	• Is the innate immune landscape of children with MIS-C, including anti-viral responses involving the interferon family, different from that of children with asymptomatic or mild SARS-CoV-2 infection? • Are cytokines such as GM-CSF responsible for cardiovascular pathology while protecting the respiratory tract of children exposed to SARS-CoV-2? • Is there a role for inflammasome activation and/or inflammatory-cell death (PANoptosis) in MIS-C? • Does NK cell dysfunction have a role in prolonging virus-related pathogenesis and inducing cytokine storm in MIS-C? • Do myeloid cells, including dendritic cells, have a role in MIS-C pathogenesis?
Genetics, epigenetics and other considerations	• Is MIS-C the result of an inborn error of immunity to infection? • Are characteristic epigenetic changes associated with MIS-C? If so, are they present before SARS-CoV-2 exposure or MIS-C onset? If not, do they arise as a result of an initial inflammatory or altered immune response to SARS-CoV-2? • What is the transcriptional consequence of the epigenetic remodeling in terms of disease pathology and severity? • Can epigenetic profiling be informative for clinical management? • What is the status of the virus in MIS-C? Is there inadequate clearance of the virus after the initial infection or after a second exposure to the virus, or is the virus sequestered at sites other than the respiratory tract in these patients? • Does the novel insertion motif seen in the S protein of SARS-CoV-2 mimic a superantigen to induce nonspecific T cell activation and drive the release of proinflammatory cytokines?

ARDS, acute respiratory distress syndrome; GI, gastrointestinal; NK cell, natural killer cell.

nearly the peak of clinically documented inflammation. Obtaining samples before the initiation of therapy represents a major challenge, and every effort should be made to avoid the confounding effect of potent immunomodulation on immune pathways relevant to disease pathogenesis.

Genetics, epigenetics and other considerations in MIS-C

Steven Josefowicz (Weill-Cornell Medicine) advanced the thesis that epigenetic 'poising' in myeloid cells could enable the rapid transcription of key genes encoding molecules that promote the over-exuberant inflammatory response in MIS-C. This poising could arise due to aberrant inflammation or distinct features of the immune response during the initial SARS-CoV-2 infection that create durable epigenetic changes. Preliminary data (from Steven Josefowicz and Virginia Pascual) indicate that epigenetic features of myeloid cells and their precursors can distinguish adult patients with COVID-19, according to severity, from children with another inflammatory condition: systemic juvenile idiopathic arthritis. It will be of interest to evaluate the epigenetic state of children with MIS-C for the presence and duration of inflammatory gene poising, as well as its association with specific characteristics of SARS-CoV-2 infection or pre-existing epigenetic signatures. Epigenetic signatures in MIS-C may also be exploitable to advance diagnostics or risk stratification. Kathleen Barnes (University of Colorado), in conjunction with Illumina, has created a COVID-19-customized chip to obtain large-scale host epigenome data that can be combined with machine learning to build disease classifiers and, ultimately, a predictive model for accurately classifying samples for COVID-19 diagnosis and outcomes. This high-throughput strategy could be readily modified to characterize broad epigenetic signatures in MIS-C and could potentially provide insight into its pathogenesis and clinical trajectory. In addition to aiding in COVID-19, this strategy could aid in the diagnosis and prognosis of future viral epidemics, since unique epigenetic signatures are expected for new viral pathogens.

Pairing epigenetics with single-cell transcriptomics might elucidate genome-wide transcription-factor activity and transcriptional networks in MIS-C. Luigi Notarangelo (National Institute of Allergy and Infectious Diseases) discussed the use of this approach as part of an integrated effort by an international consortium that will include whole-genome and/or whole-exome sequencing of people who develop serious illness or those with underlying risk factors (e.g., obesity, hypertension or cardiovascular disease) who develop mild disease after SARS-CoV-2 infection, to determine whether genetic factors underlie immune-based susceptibility or resistance to the virus.

Finally, Moshe Arditi (Cedars-Sinai Medical Center) described a collaborative project that, using structural modeling, revealed a superantigen-like motif within the S protein of SARS-CoV-2[34]. This domain appears capable of high-affinity binding to the α-chain and β-chain variable region of the TCR, and this interaction may thereby

Table 2 | Opportunities and challenges in addressing key questions in MIS-C

Sample	Question	Opportunities	Challenges
DNA	• Is there a genetic basis for MIS-C?	Samples from patients are easy to obtain during either the acute episode or convalescent period.	Disease incidence is low. Obtaining DNA from parents or siblings exposed to the virus might be challenging.
Serum or plasma	• Are antibody responses from children with MIS-C different from those of children with asymptomatic and/or mild SARS-CoV-2 infection? • Is cross-self reactivity responsible for some of the pathogenic events characteristic of MIS-C?	These studies require small serum volumes applicable to children across age groups.	
	• Is there a specific cytokine and/or proteomic profile that explains the involvement of a number of organs, including the cardiovascular system, while the respiratory tract is spared?		
	• Do post-translational modifications in the Fc region of antibodies elicited by SARS-CoV-2 have a role in the pathogenesis of MIS-C?		
	• Do exosomes have a role in endothelial inflammation in children with MIS-C?		Serum and plasma volumes required for exosome purification might not be possible to obtain from younger children.
Whole blood or PBMCs	• Are there unique transcriptional profiles at the time of MIS-C?	The blood transcriptome can provide information on both anti-viral responses and pro-inflammatory responses relevant to the pathogenesis of MIS-C. Small blood volumes can be obtained from children across age groups.	Immunomodulatory agents used to treat MIS-C (IVIG, IV steroids and/or anti-cytokine therapies) have profound effects on blood transcriptional profiles, cell populations and activation states. Obtaining samples from patients with MIS-C before the initiation of therapy is challenging.
	• Which cell subsets and/or activation pathways are involved in the pathogenesis of MIS-C?	Deep immunophenotyping of whole blood and/or frozen PBMCs (flow or mass cytometry; scRNA-seq; T cell activation with viral peptides) requires limited blood volumes easy to obtain from children across age groups.	
	• Are the antigen-receptor repertoires of virus-specific B cells and T cells from patients with MIS-C different from those of children with asymptomatic and/or mild SARS-CoV-2 infection?	Specific anti-SARS-CoV-2 B cells, ASCs and T cells from patients with MIS-C can be cloned from PBMCs that were frozen and can be used to phenotype the cells and/or to characterize their antigen receptors at the bulk and/or single-cell level(s).	These studies require blood volumes that might be difficult to obtain from younger children.
	• Do T cells from patients with MIS-C recognize a unique set of viral peptides?	T cell–activation assays reveal history of exposure to SARS-CoV-2 and other common cold–related coronaviruses.	
	• Is there an epigenetic imprint on innate and/or adaptive immune cells or their progenitors that explains a predisposition to MIS-C?	Epigenetic assays can be applied at the bulk and/or single-cell level(s).	Sufficient cell numbers (i.e., progenitors) might be difficult to obtain from younger children.
NP swab and stool	• Is there inadequate clearance of the virus after the initial infection or a second exposure to the virus, or is the virus sequestered at a site other than the respiratory tract in these patients?	NP swabs and stool samples are easy to obtain from children across age groups.	
Urine	• Are there urinary biomarkers specific to patients with MIS-C?	Urine samples are easy to collect across age groups.	

PBMCs, peripheral blood mononuclear cells; scRNA-seq, single-cell RNA sequencing; NP, nasopharyngeal.

initiate an inflammatory cascade in MIS-C much like the cytokine storm in COVID-19[5]. The SARS-CoV-2 superantigen-like motif has notable structural similarity to the *Staphylococcus* enterotoxin B (SEB) superantigen, which indicates functional implications but also raises the possibility that children with pre-existing antibodies to SEB may be less susceptible to development of MIS-C, and that the anti-SEB activity present in IVIG preparations contributes to the efficacy of IVIG in treatment of patients with MIS-C. Experiments that are underway will test this intriguing hypothesis: does the putative SARS-CoV-2 superantigen directly activate T cells, and is TCR skewing of specific variable-region families, a hallmark of superantigen responses, found in patients

with MIS-C as well as in adults who develop cytokine storm? Recently obtained data support the hypothesis that such β-chain-variable-region skewing of the TCR may occur in adults with severe COVID-19.

Concluding remarks

The unprecedented mobilization of the scientific and medical communities in response to the COVID-19 pandemic has already yielded important insights into the immune response to SARS-CoV-2. However, it is evident both that the mechanisms responsible for the different clinical manifestations of COVID-19 according to age remain a mystery and that the study of MIS-C poses unique challenges. Less research has been done directly in the population with MIS-C, and many of the presentations in the workshop reflected data from adult studies. Although there is an assumption that the findings reported will be relevant to MIS-C and will provide a roadmap to understanding this syndrome, only the study of affected children will address these points. In addition to the fundamental issue of why children and adolescents comprise the population susceptible to MIS-C, several key questions emerged from the workshop (Table 1) and will form the basis of future studies to elucidate the underlying viral and host immune mechanisms that culminate in the immunopathology of MIS-C. Approaches and challenges to answer these questions in the pediatric age group are also outlined here (Table 2). ❐

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Competing interests

The authors declare no competing interests.