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Highways to hell: Mechanism-based management of cytokine storm syndromes

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Since the first textbook devoted to cytokine storm syndromes (CSSs) was published in 2019, the world has changed dramatically and the term's visibility has broadened. Herein, we define CSSs broadly to include life/organ-threatening systemic inflammation and immunopathology regardless of the context in which it occurs, recognizing that the indistinct borders of such a definition limit its utility. Nevertheless, we are focused on the pathomechanisms leading to CSSs, including impairment of granule-mediated cytotoxicity, specific viral infections, excess IL-18, and chimeric antigen receptor T-cell therapy. These mechanisms are often reflected in distinct clinical features, functional tests, and/or biomarker assessments. Moreover, these mechanisms often indicate specific, definitive treatments. This mechanism-focused organization is vital to both advancing the field and understanding the complexities in individual patients. However, increasing evidence suggests that these mechanisms interact and overlap. Likewise, the utility of a broad term such as "cytokine storm" is that it reflects a convergence on a systemic inflammatory phenotype that, regardless of cause or context, may be amenable to "inflammo-stabilization." CSS research must improve our appreciation of its various mechanisms and their interactions and treatments, but it must also identify the signs and interventions that may broadly prevent CSS-induced immunopathology. (J Allergy Clin Immunol 2020;146:949-59.)

Key words: *Cytokine storm, hemophagocytic lymphohistiocytosis, macrophage activation syndrome, cytokine release syndrome*

Abbreviations used

AOSD:	Adult-onset Still's disease
CAR:	Chimeric antigen receptor
COVID-19:	Coronavirus disease 2019
CRS:	Cytokine release syndrome
CSS:	Cytokine storm syndrome
CTL:	Cytotoxic T lymphocyte
FHL:	Familial hemophagocytic lymphohistiocytosis
HLH:	Hemophagocytic lymphohistiocytosis
IL-18BP:	IL-18 binding protein
MAS:	Macrophage activation syndrome
NK:	Natural killer
SIRS:	Systemic inflammatory response syndrome
SJIA:	Systemic juvenile idiopathic arthritis
XLP:	X-linked lymphoproliferative

DEFINITIONS, CRITERIA, AND THE MORASS OF CYTOKINE STORM SYNDROME NOMENCLATURE

It is necessary to introduce the many overlapping and competing terms that make up cytokine storm syndromes (CSSs) (hemophagocytic lymphohisiocytosis [HLH], *familial* hemophagocytic lymphohistiocytosis [FHL], macrophage activation syndrome [MAS], cytokine release syndrome [CRS], ...). It is also critical to appreciate that (like nearly everything in biology) no CSS represents a binary state. Regardless of whether a patient meets a specific set of criteria, our subsequent focus on mechanisms will hopefully enable practitioners to appreciate specific patterns of CSS immunophysiology.

HLH, FHL, and X-linked lymphoproliferative disorder

Perhaps the canonical CSS is HLH. Diagnostic criteria for HLH were most recently updated in 2004[1] to address the utility of biomarkers such as ferritin and soluble IL-2 receptor alpha (aka sCD25), functional assays such as natural killer (NK)-cell killing, and genetic testing. Meeting 5 of 8 such criteria is sufficient to diagnose HLH under these criteria (Table I), but patients can be diagnosed as suffering from HLH on the basis of a molecular diagnosis (usually via genetic testing) alone. These criteria function better for classification than for diagnosis, because some elements do not return quickly whereas others are often not present early in disease (eg, hemophagocytosis). Practitioners frequently need to initiate immunomodulatory treatment while this workup is incomplete.

HLH can occur as *primary* HLH (including *familial* HLH, or FHL), meaning genetically defined, or *secondary* (*or reactive or acquired*) *HLH*, which occurs because of infection, malignancy, or rheumatic disease. Infection is by far the most common cause of secondary HLH. Initially, the genetic perturbations associated with "primary HLH" were restricted to profound defects in genes known or strongly suspected to be associated with granule-mediated cellular cytotoxicity. FHL is a subset of primary HLH restricted to perturbations in 5 distinct genes/loci (Chr9q deletion, PRF1, UNC13D, STX11, and STXBP2). With time, a broader range of genetic causes of HLH have been discovered, many of which appear to cause hyperinflammation without impairing cytotoxicity. Likewise, less severe cytotoxic impairments may

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cause HLH at a later-onset or only with certain triggers.[2,3] Thus, it is not currently clear how much of a genetic attribution a patient must have to be called as suffering from primary HLH, or whether this term is restricted only to genetic defects that impair cytotoxicity.

Often lumped with primary HLH are 2 X-linked lymphoproliferative (XLP) disorders that cause CSSs most often (but not exclusively) in the context of EBV infection. XLP1 is associated with NK T-cell deficiency and several signaling abnormalities. XLP2, however, has less association with EBV, no malignancy risk, and no known cytotoxic impairment, and now appears to be more related to innate immune activation.[3-5]

The HLH criteria do not reflect a more modern appreciation of the utility of each element. For instance, ferritin elevation has become a prerequisite for diagnosis, whereas other features suffer from lack of specificity (NK-cell function), sensitivity (fibrinogen), or both (hemophagocytosis). The HScore was developed to fill this gap, broadening the features contributing to a diagnosis and weighting more important elements.[6] Its utility has been somewhat limited by its complexity (although several online calculators exist, including http://saintantoine.aphp.fr/score/) and its being developed predominantly using data from adult malignancy-associated HLH. When not all variables are available to calculate the HScore, its sensitivity suffers.

Macrophage activation syndrome

MAS is the term traditionally associated with HLH-like features occurring in the context of rheumatic disease. Most studies focus on MAS in the context of systemic juvenile idiopathic arthritis (SJIA), the pediatric equivalent of adult-onset Still's disease (AOSD).[7] Case reports abound of MAS occurring in other rheumatic diseases,[8] particularly systemic lupus erythematosus,[9] in which it is likely the most common cause of adult MAS.[10] Criteria have been developed to distinguish MAS from primary HLH[11] and from SJIA disease flare,[12] but are less widely used than the 2016 criteria classifying SJIA-associated MAS.[13] In addition, these criteria perform less well in patients with SJIA who are already on therapies that block IL-1 or IL-6.[14] Despite its rheumatic origins, MAS is triggered by intercurrent infection in up to half the patients.[15]

Systemic inflammatory response syndrome and sepsis

Infection is likely the most common context for CSSs even in individuals without immunocompromise or known inborn error of immunity. Sepsis is certainly the oldest term still applied to CSSs in the context of infection, and its definition draws on the physiology of systemic inflammatory response syndrome (SIRS), shock, inflammation, and organ dysfunction.[16-18] However, a growing literature demonstrates that immune responses are blunted in most patients with sepsis, as demonstrated by *ex vivo*–stimulated cytokine production from both myeloid and T cells.[19-21] With dramatic improvements in sepsis identification and strategies that support ventilation and organ perfusion, mortality in many patients with sepsis occurs because of nosocomial or opportunistic infections owing to a state of "immunoparalysis." Indeed, bolstering immune responses with inflammatory cytokines (eg, GM-CSF or IFN-γ) or checkpoint inhibitors shows promise in improving outcomes in patients with sepsis with features of immunoparalysis.[21-25]

More recently, sepsis investigators have identified a small but consistent subset of patients with features of HLH and MAS (eg, ferritin, hepatobiliary dysfunction, and coagulopathy). Dubbing these patients MAS-like, they represent 5% to 7% of all patients with sepsis, have greater organ dysfunction/mortality, and may respond to anti-inflammatory treatments that failed in studies of all patients with sepsis.[26,27] The precise definition of this cohort has varied between studies. Where these patients intersect/overlap with definitions of SIRS and multiorgan dysfunction syndrome is also unclear.

Recent events have generated debate about the extent to which severe coronavirus disease 2019 (COVID-19), viral sepsis, and CSSs overlap. The definitions of severe COVID-19 largely center around the degree of respiratory failure, but multiple studies have demonstrated elevation of CSS-related biomarkers.[28]

Cytokine release syndrome

CRS is the systemic inflammatory state that occurs in many patients up to 2 weeks after the infusion of immunotherapies directed against malignant B cells. This was first described with chimeric antigen receptor (CAR) T cells and bispecific T-cell–engaging antibodies directed against CD19,[29-31] and more recently with anti-CD22 CAR T cells.[32] Its definition initially varied between studies, but more recent efforts have largely harmonized definitions of CRS and its severity.[33] These definitions generally include many of the same features used to define HLH, MAS, and SIRS/sepsis.

Kawasaki disease, thromobocytopenic purpura, and other primary vascular causes of CSSs

Although CSSs and sepsis can cause profound endothelial activation and disseminated intravascular coagulation, some patients with primary immune-mediated vasculopathies develop syndromes with substantial overlap with CSSs (fever, disseminated intravascular coagulation, and organ dysfunction). This is perhaps best described in Kawasaki disease-shock syndrome and/or Kawasaki disease-MAS.[34] A substantial proportion of patients with thrombotic thromobocytopenic purpura often develop prominent CSS features such as prolonged fever, hemodynamic instability, central nervous system organ failure, and coagulopathy.[35]

Problems with CSS nomenclature

A patient with lupus who develops CSS in the context of EBV viremia could be simultaneously and accurately called as suffering from "MAS," "viral sepsis," "sepsis-MAS," "EBV-HLH," "secondary HLH," "acquired HLH," or "reactive HLH." Each of these terms carries with it a context and may suggest a specific immunomodulatory treatment. Often these paradigms are at odds with each other.[36] Improving the CSS nomenclature will require an unprecedented collaboration that crosses disciplines and age boundaries, and can accommodate the contributions of multiple factors.

KNOWN MECHANISMS OF CSSs

CSSs are the immunophysiologic end-products of multiple distinct and interacting infectious/environmental and host

TABLE I. Comparison of CSS criteria

Test	HLH-04*	MAS-2016	HScore†
Fever	✓	✓‡	<38.4/38.4-39.4/>39.4
Ferritin (ng/mL)	>500	>684‡	<2000/2000-6000/>6000
Splenomegaly	✓		Y/N
Hepatomegaly			Y/N
Neutrophils (cells/μL)	<1000§		Leukocytes <1000
Hemoglobin (g/dL)	<9§		<9.2
Platelet count (10^9/L)	<100§	<182	<110
Aspartate aminotransferase (U/L)		>48	>30
Triglycerides (mg/dL)	>265‖	>156	<133/133-354/>354¶
Fibrinogen (mg/dL)	<150‖	<361	<250
Hemophagocytosis	✓		✓
Low/absent NK-cell activity	✓		
Soluble IL-2Ra (CD25) (U/L)	>2400		
Known immunosuppression			Y/N

N, No; *Y*, yes.
*Five of 8.
†Point system; see http://saintantoine.aphp.fr/score/.
‡Required, plus any 2 of 4 other parameters.
§As part of cytopenias affecting 2 or more lineages.
‖Part of same criterion.
¶Converted from mmol/L.

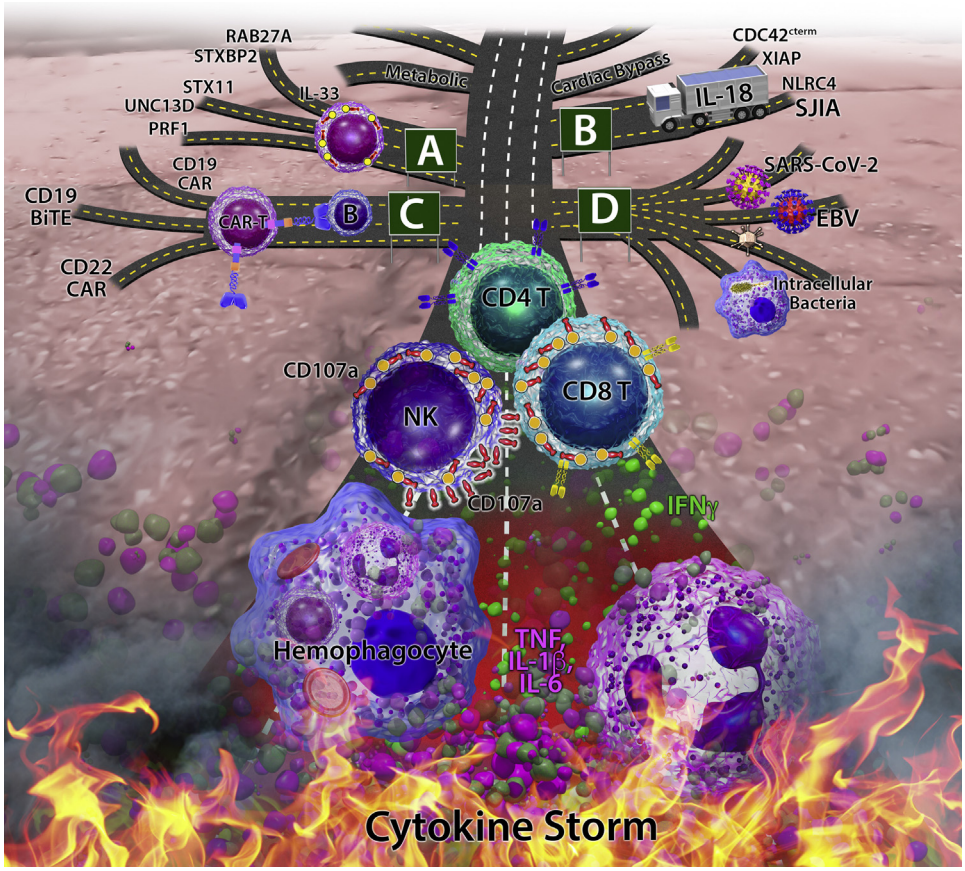

FIG 1. The merging mechanisms that promote cytokine storm. Genetic impairment of cellular cytotoxic (**A**), excess of IL-18 (**B**), latrogenic T-cell hyperactivation via CAR T cells and BiTE antibodies (**C**), and specific infections (particularly EBV and possibly SARS-CoV2, **D**) converge on T- and NK-cell hyperactivation and production of lymphokines such as IFN-γ. This lymphokine surge acts on myeloid cells to promote histiocytosis, cytokine storm, and life-threatening systemic immunopathology. *BiTE*, Bispecific T-cell engaging.

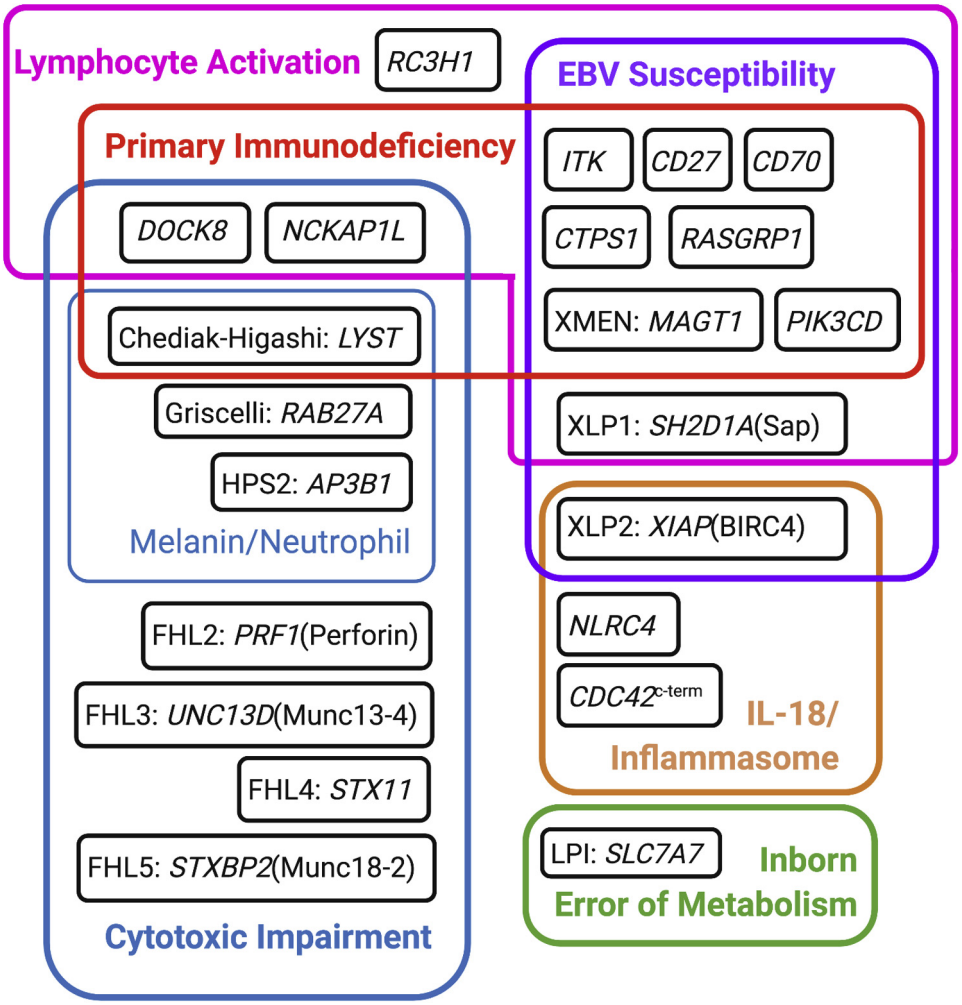

FIG 2. Classification of genetic defects commonly associated with CSSs shows substantial mechanistic overlap. *HPS*, Hermansky-Pudlak syndrome; *LPI*, lysinuric protein intolerance; XMEN, X-linked immunodeficiency with magnesium defect, EBV infection, and neoplasia. Created with biorender.com.

mechanisms that appear to converge on lymphocyte hyperactivation, histiocytosis, and systemic immunopathology (Fig 1).

Impaired cytotoxic function

Many cells produce and secrete granules, but cytotoxic granules containing perforin and granzymes are unique to lymphocytes, and of these, mostly NK cells and cytotoxic ($CD8^+$) T lymphocytes (CTLs). FHL is genetically associated with biallelic mutations resulting in profound deficiency of proteins critical for the formation, migration, fusion, or function of cytotoxic granules.[37,38] Correspondingly, disorders of impaired formation of melanin and neutrophil granules result in phenotypes that include HLH as well as albinism and neutrophil dysfunction (Fig 2).

When NK cells and CTLs interact with other cells, they must integrate an array of signals from both cell-cell contacts (including the T-cell receptor) and soluble mediators such as cytokines, which if strong enough will promote formation of a lytic synapse. Model systems suggest that the IL-1 family member IL-33 may be a particularly important activating signal on T cells.[39,40] Lytic synapse generation involves the dramatic mobilization of various protein complexes to the point of contact between the killer cells and their targets. The results of a robust lytic synapse include NK-cell or CTL proliferation and their production of cytokines such as TNF, GM-CSF, IL-2, and especially IFN-γ. Finally, the killer cells mobilize their cytotoxic granules to the synapse, where they fuse with the plasma membrane and extrude their contents. Now in the synapse, perforin mediates the uptake of granzymes by the target cell, and these internalized granzymes initiate target-cell apoptosis.[41] Termination of the lytic synapse is an active process initiated by the death of the target cell.[42]

Mechanistically, HLH (due to cytotoxic impairment) combines features of both immunodeficiency and immune dysregulation. When granules are dysfunctional or lack perforin, cytotoxic cells lose an important tool in their fight to clear intracellular pathogens. However, it has become clear that granule-mediated cytotoxicity is also a critical mechanism regulating T-cell activation, and thereby inflammation.[43,44] At the cellular level, perforin deficiency results in failed target-cell death, prolongation of the lytic synapse, continuous stimulation of cytotoxic cells, and their resulting expansion and outpouring of inflammatory cytokines.[45,46]

Animal models of HLH require both profound cytotoxic impairment and a viral trigger. Less severe cytotoxic impairments cause less severe HLH in mice[47] and later-onset disease in humans.[2] These models suggest that autoinflammation (not immunodeficiency) is the fatal mechanism, because mice whose CD8 T cells have been removed or blunted fail to clear virus but nevertheless survive with minimal pathology.[48] There may even be a role for cytotoxic killing of other cytotoxic cells—so-called fractricide.[49] More recent evidence suggests that perforin may play a critical immunoregulatory role, even in the absence of infection, in the context of chronic exposure to IL-18.[50]

FHL typically requires biallelic mutations that profoundly impact cytotoxic function. Mutations that cause FHL when homozygous appear to be well tolerated by most heterozygous carriers.[51] Nevertheless, such mutations consistently appear to be 2- to 5-fold enriched in other CSS states: notably MAS and fatal H1N1 influenza.[51-53] One variant in PRF1, Ala91Val, is present in up to 5% of healthy populations, yet sensitive functional assays have shown that it has a detectable effect on cytotoxic function.[54,55]

With rare exception, immunopathology in cytotoxic impairment appears to require the effects of IFN-γ on myeloid cells, predominantly macrophages.[44] In mice, the abundance of memory T cells or complete absence of IFN-γ signaling appears to enable lymphocytic choriomeningitis virus (LCMV)-induced immunopathology to proceed by IFN-γ–independent mechanisms.[56,57] Correspondingly, mycobacteria-associated immunopathology meeting HLH-04 criteria has been reported in people with genetic impairment of IFN-γ signaling.[58]

Clinical correlates of impaired cytotoxic function

FHL is a devastating disease, with mortality still approximately 40% despite decades of heroic basic and clinical investigation.[59] Early organ failure, particularly central nervous system involvement, portend a particularly grim prognosis. Early identification and treatment of FHL clearly improve outcomes, supporting the argument for newborn screening. As such, various functional tests have become clinically available:

- *Biomarker testing* is an important part of the workup of CSSs, but no biomarkers specific to cytotoxic impairment have yet been identified. Assessment of IFN-γ levels makes logical sense given its central role, but IFN-γ is a poor peripheral blood biomarker due to its low dynamic range. As such, clinical trials of the IFN-γ–blocking antibody emapalumab have largely followed levels of the IFN-γ–induced chemokine CXCL9. However (as discussed below), no blood biomarker is unique to cytotoxic impairment, and IFN-γ also plays a central role in CSSs due to other mechanisms.
- *The NK-cell killing assay* was among the first developed, but with time this assay's lack of specificity (it is easily affected by low NK-cell numbers, higher proportions of immature NK cells, glucocorticoid exposure, etc) has limited its utility. It is not known whether transient/acquired killing defects may also contribute to CSSs. Because functional testing requires a substantial amount of blood, often from anemic and lymphopenic infants, this test has become less prioritized.[60]
- *Perforin protein content* is assessed by flow cytometry of patients' NK and CD8 T cells. Although perforin deficiency is the most common cause of FHL, flow cytometry for perforin will be normal or elevated in patients with FHL due to other causes. These other causes all result in defective fusion of cytotoxic granules with the plasma membrane.
- *CD107a mobilization* may detect such defects. Intracellularly, cytotoxic granules are coated with the membrane protein CD107a (aka LAMP1). When incubated with target cells, normal NK cells will degranulate and CD107a will become detectable on the cell's surface. When incubated with target cells, some FHL patients' NK cells and CTLs will show impairment in their ability to "mobilize" CD107a onto their cell surface. Defective CD107a mobilization is to FHL3-5 as absent perforin expression is to FHL2, and both will profoundly affect cytolytic capacity.

Currently, allogeneic hematopoietic stem cell transplantation is the only available method of restoring cytotoxic function, offering some patients a full recovery. As such, the treatment strategy is to blunt hyperinflammation and prevent immunopathology until hematopoietic stem cell transplantation can be performed, and the HLH-94 protocol using dexamethasone, etoposide, and intrathecal methotrexate (if indicated for central nervous system involvement) is the current standard of care.[1,59,61] Other therapies such as alemtuzumab and the IFN-γ–neutralizing antibody emapalumab are effective for refractory HLH,[62] although their use earlier in the course of FHL is being investigated. Animal studies suggest that restoration of cytotoxic function in as few as 20% of cells may be sufficient to treat FHL,[63] making gene therapy a viable future therapeutic possibility.

EBV

Although HLH has been associated with viral, bacterial, protozoal, and fungal infections of nearly every kind, EBV stands out. The spectrum of EBV-infection phenotypes spans mild upper respiratory tract infections, infectious mononucleosis, chronic active EBV infection, and EBV-HLH. In less severe infections, the primary cellular targets of EBV are B cells and oropharyngeal cells, and these cells are the most common targets of EBV-related malignancies. In contrast, chronic active EBV infection is also associated with infection of NK and CD4 T cells, and EBV-HLH is also associated with infection of CD8 T cells.[64]

EBV is a complex DNA virus with a large genome, encoding many immunomodulatory and prosurvival genes. Elements from its latent genome (eg, microRNAs and the BCL2-homolog BHRF1) support proliferation and inhibit apoptosis.[65,66] As such, the circumstantial evidence indicates that (as in FHL) the proclivity for EBV to cause HLH may relate to its impairing target-cell and CTL apoptosis.

Extrinsic to EBV, several monogenic defects have been associated with EBV-HLH (Fig 2). Most of these promote HLH via impairing control of EBV. The connection between SAP deficiency (XLP1) and EBV persistence has been best studied and relates to both cytotoxic impairment (via invariant NK T-cell deficiency[67] and impaired signaling through the activating receptor 2B4) and defective lymphocyte apoptosis. SAP deficiency also promotes CTL survival by interfering with cell death induced by T-cell restimulation.[67-69]

Clinical correlates of EBV-HLH. With rare exception, evaluation for both active EBV viremia and EBV seropositivity

is part of the core, early workup for uncharacterized HLH. Similar evaluations for Herpesvirus family members (eg, cytomegalovirus, herpes simplex virus (HSV)6/8, HSV1/2) and adenovirus are also indicated.[60] Assessing which cell types have been EBV-infected is not yet clinically possible. No biomarkers reliably distinguish EBV-HLH from other HLH causes. Testing for SAP and XIAP protein expression by flow cytometry is available clinically.

Therapeutically, a wide variety of antiviral medications (acyclovir, ganciclovir, cidofivir, …) have shown anti-EBV activity *in vitro* but limited utility in clinical trials.[70] Instead, beneficial effects have been reported using B-cell–depleting strategies such as rituximab.[71-73] Such therapies eliminate the virus' main cellular reservoir and thereby limit antigen presentation. However, rituximab works best in treating HLH associated with XLP.[73]

Therapeutic T-cell activation in cancer

Recent years have witnessed dramatic success treating refractory B-cell leukemias with CAR T-cell constructs. These constructs contain internal signaling moieties borrowed from costimulatory molecules such as CD28 and 4-1BB (aka CD137),[29,30,32] but require systemic hyperinflammation to exert their full effect. The life-threatening responses following the administration of TGN1412, a CD28 superagonistic antibody, provided an early clue to the CSSs and shock that such costimulation can induce.[74] However, bispecific T-cell–engaging antibodies, which tether B-cell antigens to T-cell receptors, have demonstrated that CRS does not require artificial signaling through T-cell costimulatory receptors.

The distinction between CRS and CAR T-cell HLH appears largely a difference in timing: CRS typically rises and falls within 2 weeks of administration, whereas CAR T-cell HLH arises after CRS and can persist for weeks afterward. Both are characterized by classical features of CSSs, including shock and potential for organ dysfunction. Biomarkers such as ferritin, IL-6, and sCD25 (particularly in CAR T-cell HLH) can be astronomically elevated.[32] The precise mechanisms linking therapeutic T-cell activation and CSSs remain unknown, but the activation and recruitment of myeloid cells appears critical.[75]

Clinical correlates of therapeutic T-cell activation. Importantly, early efforts to prevent CRS, for instance with prophylactic glucocorticoids, prevented sufficient CAR T-cell activation and they failed to effectively control leukemia.[29] This led to anti-inflammatory therapies that could prevent immunopathology but allow robust and durable antitumor responses. Tocilizumab targets both membrane-bound and soluble IL-6 receptors. Despite the protean effects of IL-6, tocilizumab has dramatically improved CRS without substantial diminution of the antitumor response. CAR T-cell HLH typically occurs after patients have already received tocilizumab. Still wishing to minimize glucocorticoid exposure, such patients have been effectively treated with high doses of the recombinant IL-1 receptor antagonist anakinra.[32]

MAS, the inflammasome, and IL-18

MAS, and its unique association with SJIA and AOSD, has always stood apart from HLH and other causes of CSSs. MAS likely represents the severe end of an immunopathologic spectrum for many patients with SJIA, and the cutoff between SJIA and MAS in such patients is necessarily somewhat arbitrary.[13,76] Indeed, most MAS flares are reported to be triggered by active disease, although about a third have an infectious trigger.[15]

High levels of the inflammasome-activated IL-1 superfamily member cytokine IL-18 were first observed in AOSD in 2001.[77] Since then, multiple groups have confirmed this strong biomarker association, and added the nuance that high IL-18 seems to define nearly all patients with histories or features of MAS.[78-82] IL-18 is naturally antagonized by a soluble, high-affinity neutralizing antagonist called IL-18 binding protein (IL-18BP), which is itself induced by inflammatory cytokines, particularly IFN-γ.[83] High levels of serum total IL-18 are not always associated with bioactive "free" IL-18, as evident in viral infections and FHL where IL-18BP levels are extremely high.[81,84] Free IL-18 has been associated with SJIA, AOSD, and MAS.[81,85,86]

Some recent observations now suggest that IL-18 may be the fundamental mechanism driving CSSs in patients historically called MAS. To become bioactive, IL-18 requires activation typically via the inflammasome. Monogenic diseases of NLRP3 and pyrin inflammasome activation were not associated with particularly high levels of IL-18 or MAS.[81] However, multiple independent reports show that gain-of-function NLRC4 inflammasome mutations are associated with both.[81,87-89] Notably, such patients' IL-18 remains profoundly elevated both during and between flares. Mice generated with one such Nlrc4 mutation (NLRC4-GOF mice) demonstrated elevated IL-18 in the neonatal period.[81] This is dramatically illustrated in the MAS-like symptoms and high IL-18 levels reported in infants born to mothers with AOSD.[90] Some patients with NLRC4-MAS also developed severe enterocolitis as infants, which may relate to intestinal epithelial cells as the source of IL-18 in NLRC4-GOF mice.

Subsequently, profound elevation of IL-18 and CSS has been reported in other monogenic diseases including XIAP deficiency and specific C-terminal mutations in CDC42.[5,91,92] Notably, both clinical improvement and normalization of IL-18 have been reported with hematopoietic stem cell transplantation in these disorders, supporting a hematopoietic source in these diseases. Likewise, IL-18 levels in SJIA-MAS slowly normalize with prolonged disease control.

IL-18 canonically acts in synergy with other T_H1-skewing cytokines, such as IL-12 and IL-15, to augment IFN-γ production. However, NK cells from patients with SJIA fail to respond to *ex vivo* IL-18.[93] Nevertheless, animal models support amplification of type 1 inflammatory responses as IL-18's pathomechanism in MAS, and suggest that the interaction of IL-18 and cytotoxic impairment may be particularly dangerous.[50,81,94]

Clinical correlates of excess IL-18. Serum total IL-18 is available clinically and has demonstrated utility in distinguishing SJIA and MAS from other CSSs such as Kawasaki disease and FHL.[79,81] IL-18 levels are independently associated with severity in sepsis and COVID-19,[27,95] but this could reflect an increase in bioactive IL-18 and/or an increase in IL-18/IL-18BP complexes. A phase II trial of recombinant IL-18BP (tadekinig) in AOSD demonstrated modest efficacy in a cohort not selected for high IL-18,[96] and case reports of efficacy of IL-18BP in MAS prompted an ongoing clinical trial.[97,98] Aiming just downstream of IL-18, a clinical trial of IFN-γ blockade (emapalumab) in MAS is also ongoing. Patients with NLRC4-MAS and $CDC42^{c\text{-}term}$ mutation have been successfully treated with emapalumab.[91] Patients

TABLE II. Tests useful for the diagnosis and monitoring of CSSs

Test	Utility	Biology	In CSSs	Caveats
Standard acute-phase reactants				
Neutrophil count	All	Bone marrow response to G-CSF/GM-CSF	↓ in most CSS	Steroids artificially ↑
ESR	All	Reflects fibrinogen and IgG levels	Nonspecifically ↑ in inflammation. ↓ in advanced CSSs due to consumption	Anemia, intravenous immunoglobulin may artificially ↑
C-reactive protein	All	Hepatic release in response to IL-6	Nonspecific, useful for monitoring	Blunted by IL-6– blocking therapy
Ferritin	All	Macrophage activation, hepatic injury	Integral part of CSS diagnosis, predicts sepsis mortality	Blunted by IL-6–blocking therapy
Ferritin/ESR ratio	MAS, All?	ESR ↓ with fibrinogen consumption	Higher specificity than ferritin alone	
Procalcitonin	All	Adipokine	Useful for bacteremia screening, useful for monitoring	Not specific to bacterial infection
Cellular/tissue injury markers				
AST, ALT	All	Hepatocyte injury	Indicative of liver injury; can be ↑ by severe myositis/ hemolysis	Can indicate drug reaction
LDH	All	Nonspecific cell death	Nonspecific, useful for monitoring	
Platelet count	All	↑ with inflammation	↓/downtrending levels indicate consumption or entrapment by hepatosplenomegaly	Can be ↓ due to bone marrow failure
Fibrinogen	All	↑ by liver in response to inflammation	↓/downtrending levels indicate DIC	↓ with hemodilution
D-dimer	All	Fibrin degradation product	Reflects DIC, very sensitive	
Protein/functional tests				
NK-cell killing	1^0 HLH	Co Incubation of PBMCs and target cells — Specific target-cell lysis	Profoundly ↓/absent in FHL2	Nonspecific ↓ in acute illness or w/ glucocorticoids
CD107a mobilization	1^0 HLH	Fusion of granule lysosome with cytotoxic cell membrane	Profoundly ↓/absent in FHL3-5 and other causes of primary HLH	
Perforin expression	FHL2	Flow cytometry in NK and CTL cells	Profoundly ↓/absent in FHL2	Often elevated in other causes of FHL/CSSs
SAP/XIAP expression	XLP	Flow cytometry of PBMCs	Profoundly ↓/absent in XLP1 and 2, respectively	Depends on antibody binding as correlate of protein function
iNK T-cell enumeration	See Fig 2	Flow cytometry of PBMCs	Profoundly ↓/absent in XLP1 and PID diseases in Fig 2	
Specialized biomarker testing				
Soluble IL-2Ra (sCD25)	All	Cleaved from activated T cells	Part of HLH-04 and HScore, useful for monitoring	May be higher in HLH than in MAS
IL-6	CRS, all?	Pleiotropic inflammatory cytokine	↑, nonspecific	
sCD163	?	Cleaved from tissue macrophages	↑ in active SJIA and MAS	Likely nonspecific
Neopterin	All?	Metabolite of GTP induced by IFN-γ	↑ in blood and CSF	
IFN-γ	?	Classic type $1/T_H1$ cytokine	↑, but poor dynamic range	
CXCL9	All	Chemokine induced by IFN-γ	↑ in most CSSs, useful for monitoring	May be higher in HLH than in MAS
IL-1β	?	Inflammasome-activated	↑, but poor dynamic range	
IL-18	MAS	Inflammasome-activated, IFN-γ–inducing	Test measures total IL-18 ↑↑ levels unique to active SJIA and MAS	Poor CSS disease activity marker, slow to normalize
IL-18–binding protein	All	Induced by IFN-γ	↑ in most CSSs, useful for monitoring	Research only
Free IL-18	MAS	Circulating IL-18 unbound by IL-18BP	↑ levels unique to active SJIA and MAS	Poor CSS disease activity marker, research only

(Continued)

TABLE II. *(Continued)*

Test	Utility	Biology	In CSSs	Caveats
IL-18/CXCL9	MAS		May improve specificity for MAS	
ADA2	All	Released by IFN-γ–activated monocytes	↑ in HLH/MAS, useful for monitoring	Research only

ADA2, Adenosine deaminase 2; *ALT*, alanine aminotransferase; *AST*, aspartate aminotransferase; *CSF*, cerebrospinal fluid; *DIC*, disseminated intravascular coagulation; *ESR*, erythrocyte sedimentation rate; *LDH*, lactate dehydrogenase; *PID*, primary immunodeficiency.

with severe XIAP deficiency or CDC42$^{c\text{-}term}$ mutations have benefited from hematopoietic stem cell transplantation.

Undiscovered mechanisms

Undoubtedly, the mechanisms described above are not exhaustive, and CSSs have been described in a great number of infectious, metabolic, autoimmune, and vasculopathic contexts.

Infection and immunodeficiency. Because overwhelming infection routinely causes sepsis and in many patients results in the "sepsis-MAS" phenotype, primary immunodeficiency is broadly associated with CSSs. Although any infection can result in CSSs, viruses (perhaps particularly DNA viruses such as EBV, cytomegalovirus, and adenovirus) and intracellular bacteria (eg, ehrlichica and salmonella) are more commonly associated with HLH-like responses.[60] Hemorrhagic fever viruses, including dengue, also routinely trigger CSSs.[99] However, patients with primary immunodeficiencies with susceptibility to intracellular bacteria, such as chronic granulomatous disease or deficiency of *STAT1*, *TYK2*, or *IKBKG* (NEMO), only rarely develop HLH.[69] Interestingly, IL-18 elevation has been associated with susceptibility to the T-cell hyperactivation disorder known as tuberculosis-associated immune reconstitution inflammatory syndrome, which occurs when untreated tuberculosis blossoms with treatment of HIV and the return of CD4 T-cell function.[100]

Inborn errors of metabolism. CSSs have been reported in various metabolic diseases in which infection was not a clear trigger.[69] Foremost among these is lysinuric protein intolerance, a urea cycle defect with protean metabolic and hematologic abnormalities wherein some patients develop repeated HLH-like episodes responsive to treatments such as cyclosporine.[101] How metabolic defects promote CSSs remains an area of active investigation.

Primary vasculopathy. Given their penchant for systemic inflammation, coagulopathy, and organ failure, vasculopathies such as Kawasaki disease and thromobocytopenic purpura may resemble HLH/MAS. The systemic inflammation and vasculopathy in severe COVID-19, and particularly in the post–severe acute respiratory syndrome coronavirus 2 multisystem inflammatory syndrome of children (MIS-C), have generated comparisons to MAS and HLH.[102,103] In addition, the physical forces associated with cardiac bypass can trigger CSSs.[104] These disorders rarely develop the profound hyperferritinemia characteristic of other CSS disorders, and thus it is unclear whether they represent vasculopathy-and-CSSs or vasculopathy-causing-CSSs.

RECOGNIZING CSSs AND "INFLAMMATORY STABILIZATION"

Clinicians are eager to identify and treat the root cause of CSSs from the moment these are identified. However, as we have discussed, such efforts can be protracted and fruitless, or identify multiple contributors. In the meantime, immunopathology can accumulate quickly. Clinicians can draw upon a wide variety of acute-phase reactants, cell death markers, read-outs of specific organ dysfunction, and biomarkers with variable specificity for CSSs (Table II) to help determine the location and severity of immunopathology while the search for a root cause is ongoing.

Because life-threatening CSSs may occur in nearly any context, we recommend a low threshold for screening inpatients with fever and/or unexplained inflammatory features with serum ferritin upon admission and with changes in clinical condition. A ferritin to erythrocyte sedimentation rate ratio may improve the specificity of ferritin for CSSs, particularly MAS.[105,106]

Intensivists have made great strides in the ability to define and prevent organ dysfunction in SIRS, sepsis, and CSSs using "stabilizing" interventions such as fluids, pressors, anticoagulation, and support for specific organ failure. Recognizing that inflammation is a core component of all CSSs, "inflammatory stabilization" refers to the concept that certain anti-inflammatory interventions may prove beneficial (albeit rarely curative) in a wide variety of CSSs. The field of anti-inflammatory therapy for sepsis was largely abandoned after the failure of several strategies in the late 1990s. However, the use of MAS features to reanalyze some of those trials suggested that, at least for inhibition of IL-1, the sepsis-MAS subset may benefit.[26] The COVID-19 pandemic dramatically accelerated the pace at which anti-inflammatory therapies have been developed for treating immunopathology during infection. Dexamethasone has now become standard of care in the early stages of severe COVID,[107] and dozens of other more targeted anti-inflammatories (eg, IL-1, IL-6, and GM-CSF) have shown promise in case series and are in clinical trials.[102]

Although we have discussed treatment strategies indicated by specific mechanisms, a detailed discussion of the anti-inflammatory, immunomodulatory, and immunosuppressive treatments applicable to all CSSs is beyond the scope of this review and discussed in reviews specific to sepsis, MAS, HLH, CRS, and so forth[7,10,21,34,36,37,51,59,60,69,102,108] However, a few strategic points are worth noting:

1. Empiric treatment and efforts at "inflammatory stabilization" should match the patient's current status, likelihood of intercurrent infection, most likely diagnosis, anticipated trajectory, and pace of drug onset. Anakinra has a rapid onset and good safety profile, and may be efficacious beyond MAS,[109,110] but may not be sufficient to slow the progression of severe HLH. Although etoposide has a slower onset and causes substantial immunosuppression, its early use may prevent immunopathology in patients with likely FHL.
2. Relatedly, prophylactic antimicrobials should be considered early in the course of treatment and in consultation

with infectious disease specialists, particularly if the patient is already or likely to become immunocompromised.
3. Some treatments substantially alter the presentation of CSSs. Immunosuppressive therapy may make it challenging to identify hospital-acquired infections. The IL-6 inhibitor tocilizumab's effect on C-reactive protein and ferritin levels may mask ongoing MAS.[14,111]

SUMMARY AND CONCLUSIONS

CSSs encompass a broadening array of hyperinflammatory states of various genetic, infectious, rheumatologic, oncologic, and therapeutic etiologies. They all share an overly active immune response that, if not recognized and treated early, is frequently fatal. Diagnosis by various criteria and biomarkers remains problematic, but early intervention targeting both the infectious insult, when present, and the exacerbated immune response is critical for saving lives worldwide. Although wrestling with life-threatening immunopathology is ancient, the COVID-19 pandemic has brought new light to the topic and many new options for testing and treatment that, if wielded wisely, may quell the storm and save the patient.

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