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Making Sense of the Cytokine Storm: a conceptual framework for understanding, diagnosing and treating hemophagocytic syndromes

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SYNOPSIS

Cytokine Storm Syndromes (CSS) are a group of disorders representing a variety of inflammatory etiologies with the final common result of overwhelming systemic inflammation, hemodynamic instability, multiple organ dysfunction, and potentially death. The hemophagocytic syndromes hemophagocytic lymphohistiocytosis (HLH) and macrophage activation syndrome (MAS) represent two clinically similar CSS with an unknown degree of pathoetiologic overlap. The clinical presentations of all CSS can be strikingly similar, creating diagnostic uncertainty. However, clinicians should avoid the temptation to treat all CSS equally, as their inciting inflammatory insults vary widely. Failure to identify and address this underlying trigger will result in delayed, inoptimal, or potentially harmful consequences. This review endeavors to place the hemophagocytic syndromes HLH and MAS within a conceptual model of CSS, and thus provide a logical framework for diagnosis and treatment of CSS of suspected rheumatic origin.

Keywords

Cytokine storm; Macrophage Activation Syndrome; Hemophagocytic Lymphohistiocytosis; Sepsis

THE FINAL COMMON PATHWAY

History

In the days before germ theory, the term “sepsis” (from the Greek *sepo*, “I rot”) was applied to all states of uncontrolled inflammation. Today we reserve the moniker “sepsis” to refer to overwhelming inflammation in the context of a systemic infection (although even this definition can be ambiguous). The term “Cytokine Storm Syndrome” (CSS) was developed to accommodate the observation that multiple inflammatory causes can result in a disease

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that appears very similar to sepsis. The unifying feature of CSS is a clinical and laboratory phenotype suggestive of massive inflammation progressing to multiple organ dysfunction syndrome (MODS) and eventually death, a final common pathway.

Examination and Laboratory Findings

The clinical constituents of this pathway can include fever, tachycardia, tachypnea, hypotension, malaise, generalized swelling, altered mental status, diffuse lymphadenopathy, organomegaly (particularly of the liver and spleen), and often erythematous or purpuric rash. In response to the desire by intensive care practitioners to standardize hemodynamic management of CSS, criteria for Systemic Inflammatory Response Syndrome (SIRS) were proposed in 1992¹ and have been amended a number of times, notably to accommodate pediatric practice² (Table 1).

CSS also have a number of common laboratory abnormalities. Hematologic parameters like leukocytosis or thrombocytosis can indicate the acute phase response. Alternatively, elevated cell counts can drop precipitously as a feature of nearly all CSS, suggesting consumption. Clinicians can also take advantage of a host of non-specific acute phase reactants, including erythrocyte sedimentation rate (ESR), c-reactive protein, procalcitonin, serum amyloid A, ferritin, and fibrinogen among others. Notably, and akin to acute cytopenias, an acute drop in ESR and fibrinogen are most associated with Macrophage Activation Syndrome (MAS), but can be seen in any cytokine storm syndrome and often suggest active disseminated intravascular coagulopathy (DIC). Screens for coagulopathy such as fibrin split products and d-dimer are often elevated in CSS even in the absence of overt DIC, suggesting subclinical endothelial activation. Likewise, hypoalbuminemia is frequently observed and likely represents systemic capillary leak. Routine testing often reflects various organs in distress including the liver, pancreas, and kidneys. Such tests are rarely capable of distinguishing direct inflammatory damage from that induced by insufficient oxygen delivery.

The Elusive Hemophagocyte

Hemophagocytes are activated macrophages seen histologically to have engulfed other hematopoietic elements (erythrocytes, leukocytes, or platelets, see Figure 1). Hemophagocytes are the pathologic hallmark of Hemophagocytic Lymphohistiocytosis (HLH) and Macrophage Activation Syndrome (MAS). However, hemophagocytes are not essential to the diagnosis of HLH³, and can be seen in juvenile arthritis patients without overt MAS⁴. Additionally, hemophagocytes are found commonly in a host of other inflammatory states including sepsis⁵ and following bone marrow transplant⁶. Whether hemophagocytes are inflammatory, anti-inflammatory, or serve different roles in differing diseases is a matter of ongoing study.

ETIOLOGIC FACTORS

An analysis of the underlying pathoetiology of all CSS supports this simple but essential postulate: cytokine storm results from excessive proinflammatory stimuli, inadequate regulation of inflammation, or elements of both. Proinflammatory stimuli can include antigens, superantigens (compounds that trigger non-specific but massive activation of T-cell receptors), adjuvants (such as toll-like receptor (TLR) ligands, for an excellent review of TLRs in infection and autoimmunity see⁷), allergens (antigens triggering an allergic response), and proinflammatory cytokines themselves. Anti-inflammatory mechanisms can be humoral or cellular and seek to dampen or terminate a proinflammatory pathway. Table 2 provides several examples of both pro-inflammatory stimuli and anti-inflammatory

mechanisms. Defects leading to excessive pro- or inadequate anti-inflammatory responses can be host-derived or environmental.

Host Factors

Conventional Immunodeficiencies—Sepsis being quite common among otherwise immunologically normal hosts, it is easy to forget that there are likely hundreds of genetic and epigenetic risk factors for the development of sepsis. Patients with immunodeficiencies should be considered at risk for CSS by virtue of their inability to effectively clear the proinflammatory elements of an infection. Persistent infection provides a rich source of both antigen and adjuvant that, as the infection worsens, can quickly overwhelm the body's ability to regulate inflammation.

The primary immunodeficiencies encompass defects in both innate and adaptive immunity, and include entities such as severe combined immunodeficiency (SCID), X-linked agammaglobulinemia, Common Variable Immunodeficiency, Chronic Granulomatous Disease, and complement component deficiencies, among many others (see www.immunodeficiencysearch.com for a thorough, searchable review of immunodeficiency syndromes⁸). Other host-derived immunodeficiency states such as extremes of age and malnutrition can contribute to the inability to effectively clear inflammatory stimuli.

Familial Hemophagocytic Lymphohistiocytosis (fHLH)—Familial HLH is a cytokine storm syndrome usually occurring in younger children who present with immense inflammation, pancytopenias, and other features of the final common pathway. Such patients' disease is usually triggered by viral infection, and patients are unable to effectively clear the virus. Familial HLH, by definition, occurs in individuals with little to no ability of their Natural Killer (NK) cells and cytotoxic T-cells to kill targeted cells³. The genetic defects associated with fHLH all relate to the packaging, exocytosis, or function of cytotoxic granules (see Table 3)⁹, with perforin gene defects being the most common and best studied. Similar to SCID patients, children with fHLH will eventually succumb to their illness without eventual allogeneic bone marrow transplantation³. Animal models of HLH suggest that uncontrolled activation of cytotoxic T-cells by antigen presenting cells (APCs) drives the disease, with interferon- γ (IFN γ) being the primary pathogenic cytokine^{10–12}. Other inflammatory pathways are also involved in these models, as recently illustrated by the crucial role of MyD88 in disease development¹³. MyD88 is a critical signaling molecule downstream of IL-1 receptor and TLR signaling, and as discussed below, may provide some common inflammatory link to MAS.

The absence of functional cytotoxicity makes it impossible for T-cells to terminate the stimulatory responses they receive from APCs. In this way, HLH may rightly be viewed as a primary immunodeficiency: these patients' genetic defect makes it impossible for them to clear infection and terminate T-cell stimulation by APCs. Similar to sepsis, in fHLH it is the uncontrolled immune response to infection that causes the cytokine storm.

Interestingly, the limited studies to date in patients with HLH have not yet borne out a major role for IFN γ . Microarray of RNA from HLH patients' peripheral blood mononuclear cells (PBMCs) did not show induction or suppression of IFN γ -inducible genes¹⁴. Rather, signaling downstream of various inflammatory cytokines such as IL-6, IL-1, and IL-8 was upregulated. Accordingly, IL-10 (a potent anti-inflammatory cytokine) signaling was highly upregulated. Genes governing NK cell activities, T-cell differentiation, and TLR signaling were also downregulated¹⁴. Such genes downregulated in sick patients supports a possible pathogenic role for these pathways. Of note, only a minority of patients in this study had identified mutations¹⁴, suggesting that our knowledge of all of the genetic defects that lead to fHLH is incomplete.

X-linked lymphoproliferative Syndromes (XLP) and Epstein Barr Virus-related HLH (EBV-HLH)—While in fHLH, it appears that a genetic inability to kill infected, activating APCs results in uncontrolled stimulation of T-cells. Other genetic defects have been associated with HLH as well. The X-linked Lymphoproliferative Syndromes XLP1 & XLP2 are both marked by a tremendous susceptibility to EBV infection, often resulting in an HLH phenotype¹⁵. XLP1 is caused by a mutation in SAP, an intracellular protein important for regulating IFN γ signaling. XLP2 is due to mutations in XIAP, a protein known to inhibit apoptosis. How XIAP mutations contribute to HLH is unknown. Nonetheless, HLH in XLP patients nearly always occurs in the context of EBV infection¹⁵.

Even in the absence of a known mutation, chronic EBV infection is a risk factor for the development of HLH. In EBV-HLH, viral loads correlate with the progression or improvement of disease¹⁶, suggesting persistent pro-inflammatory stimulation is important for disease maintenance. The association of EBV with various other lymphoproliferative diseases and hematologic malignancies suggests this virus has a propensity for uncontrolled immune activation and proliferation¹⁶.

Macrophage Activation Syndrome—The nomenclature among CSS does not lend itself to clear delineations. For the purposes of this review, we will define Macrophage Activation Syndrome (MAS) as a cytokine storm syndrome occurring in the context of a rheumatic disease and not otherwise associated with severe infection. Others have referred to hemophagocytic syndromes not associated with fHLH mutations as secondary or reactive HLH^{3,17}. MAS occurs in between 10 and 50% of patients with systemic juvenile idiopathic arthritis (sJIA) depending on its definition⁴. MAS also complicates systemic lupus erythematosus (SLE), Kawasaki Disease, and more rarely a host of other rheumatic conditions^{18,19}. That MAS appears to occur more commonly in some rheumatic illnesses than others suggests that MAS-prone diseases may share a similar mechanistic propensity for cytokine storm. Additionally, the fact that MAS occurs only very rarely amongst patients with inflammasome disorders (illnesses associated with genetic defects known to cause excessive cytokine activation²⁰) suggests that proinflammatory conditions alone are not sufficient to result in MAS.

Efforts to understand the pathogenesis of MAS have focused on sJIA patients, as this appears to be the most susceptible population. A number of studies have suggested that, like in fHLH, a defect in cytotoxic function plays a role in the development of MAS^{21,22}. More recent studies have even found an increased risk of developing MAS in sJIA patients harboring polymorphisms associated with fHLH genes^{23,24}. However, since many sJIA patients with NK dysfunction do not develop MAS, it is possible that cytotoxic cell dysfunction may be a non-specific finding in sJIA that correlates with MAS.

Like in fHLH, IL-1/TLR signaling may be important for the development of MAS. Studies looking at PBMC RNA microarrays from newly-diagnosed, untreated sJIA patients identified regulation of the IL-1 and TLR pathways in those patients at higher risk for MAS²⁵. Additionally, polymorphisms in a gene critical for TLR signaling (Interferon regulatory factor 5) are associated with a four-fold higher risk of MAS among sJIA patients²⁶.

The only animal model of MAS to date utilizes repeated TLR9 stimulation in otherwise genetically normal mice²⁷. TLR9 is a bacterial and viral DNA sensor implicated in bacterial and EBV infection and SLE^{28,29}. As in HLH models, the TLR9 model supports an important role for IFN γ in the development of disease²⁷. Perhaps most interestingly, IL-10 is critical in this model for protection from severe disease and hemophagocytosis. Further supporting a protective role for IL-10 in MAS, two genomic studies have associated low IL-10

expressing polymorphisms with sJIA^{30,31}. In contrast, IL-10 inhibition in a fHLH model did not appear to hasten disease¹⁰, suggesting that this regulatory mechanism may be more important in some CSS than others.

Measurements of serum cytokines from MAS patients have done little to clarify a single pathogenic pathway. IL-18, macrophage colony stimulating factor (M-CSF), neopterin, and IL-6 have all been shown to be elevated in MAS patients, but variably depending on the underlying disease^{32,33}. This suggests that different rheumatic conditions exploit different pathways to arrive at an MAS phenotype.

Malignancy-Associated Hemophagocytic Syndromes (MAHS)—Hemophagocytic syndromes occur in the context of malignancy under two circumstances: first, MAHS can occur as a masking feature of the presentation of various hematolymphoid malignancies, and second, they may complicate the initial course³⁴. The types of malignancies in each group are different. T-cell leukemias and lymphomas are often masked by a hyperinflammatory MAHS state, while B-cell leukemias and germ cell tumors are often complicated by MAHS³⁴. When MAHS masks a malignancy, it is often the case that neoplastic cells are producing macrophage-activating cytokines such as IFN γ and CD25, whereas HLH complicating a malignancy usually occurs when severe infection complicates an intrinsically or iatrogenically immunocompromised state³⁴.

Environmental Factors

We have described that infection plays a key role in the induction of genetic causes of CSS like fHLH and XLP. However, even in normal hosts environmental agents frequently provide sufficient proinflammatory stimuli (or inhibition of regulatory processes) to drive a CSS. More plainly, anyone can get septic.

Infection—As stated, infection provides (at least) two important proinflammatory stimuli: antigen and adjuvant. Antigenic stimulation is necessary for a specific response as well as generation of memory, while adjuvant provides the first stimulus to cells of the innate immune system. While theoretically any infection can provide sufficient stimulation to generate cytokine storm, this section will focus on infections commonly associated with hemophagocytic syndromes.

Bacteria—It was previously held that bacterial infections were uncommon causes of hemophagocytic syndromes. A review spanning 1979 to 1996 identified only 11 cases of bacterial-induced HLH, but 149 cases of viral-induced HLH³⁴. However, this understanding preceded widespread testing for mutations associated with XLP and fHLH, and thus many viral-associated HLH cases would now be classified as primarily genetic. Additionally, there is an increasing appreciation for the consumption, cytopenias, and hemophagocytosis in severe infection^{5,17}.

Bacterial infection provides a rich source of antigenic as well as TLR stimulation. However, if host defects in cellular cytotoxicity are critical to the development of hemophagocytic syndromes, it may be that most bacteria do not require this mechanism for their efficient clearance. Interestingly, a model of secondary HLH utilizes the intracellular bacteria *salmonella enterica* to drive disease³⁵, suggesting that perhaps viruses and intracellular bacteria exploit a common immunologic weakness in driving hemophagocytic syndromes.

Viruses—It is not entirely clear why viral infections should be especially predisposed to hemophagocytic CSS. IFN γ is made in abundance by a variety of hematopoietic cells in response to viral infection, and may be particularly important in facilitating

hemophagocytosis³⁶. EBV, cytomegalovirus, and other γ -herpesviruses are the infections most commonly associated with HLH³⁴, and this may have to do with their predilection for triggering TLR9, which has been associated in animal models with MAS^{27,28}. Additionally, certain viruses alter the immune response to infection and may predispose to cytokine storm. The EBV genome encodes an IL-10 homologue that may alter the host immune response to infection³⁷. Additionally, there are numerous case reports of HLH complicating both the presentation of and opportunistic infections in human immunodeficiency virus infections³⁸.

Other Infectious Agents—Fungal and parasitic infections are also capable of inducing a robust immune response. Again, it may be instructive there are only rare case reports of hemophagocytic disease complicating highly cytokine-driven infections such as *plasmodium falciparum*³⁹. Alternatively, it may be that fungal and malarial sepsis are common enough causes of cytokine storm that a thorough evaluation for defective cytotoxicity or hemophagocytosis is rarely undertaken.

Immunosuppressive therapy—As suggested in relation to MAHS, chemotherapy not only inhibits the ability to clear infections, but may alter the regulatory machinery necessary for modulating the immune response to infection.

Allergens—Allergens are a potent source of antigenic stimulation without infection. Prolonged exposure to high doses of the allergy-associated cytokine IL-4 resulted in hemophagocytosis in mice⁴⁰. However, this model of hemophagocytic disease occurs independent of IFN γ . Data regarding hemophagocytosis in human anaphylaxis or other severe allergic reactions, such as DRESS (Drug Reaction with Eosinophilia and Systemic Symptoms) are lacking.

DISTINGUISHING CYTOKINE STORM SYNDROMES

We and others have supported the notion that the universe of CSS share a similar final phenotype¹⁷. Additionally, we have shown that a variety of host-derived and environmental insults can result in this phenotype. We will later show that optimal treatment varies widely between CSS. Thus, distinguishing among these syndromes is critically important if one wishes to interrupt the final common pathway and restore inflammatory homeostasis.

The workup of any CSS must begin with an assessment of hemodynamic stability and appropriate intensive care intervention. Next, an assessment of organ dysfunction and coagulopathy is critical, but rarely provides specific diagnostic information. Finally, a thorough evaluation for infection or malignancy should preempt a diagnosis of HLH or MAS.

Cytopenias and Macrophage Activation

Diagnostically, the pattern or trend of hematologic and immunologic values can be useful. While sometimes observed in severe sepsis, acute drops in all three cell lines are characteristic of the hemophagocytic syndromes. Pancytopenia occurring prior to the onset of systemic inflammation should raise concern for malignancy. Elevated triglycerides may be a serum marker for HLH and MAS.

As its name implies, MAS is accompanied by markers of macrophage activation. Most notably, ferritin levels in MAS and HLH are often above 10,000 ng/mL, while such values are in the range of a few hundred ng/mL in sepsis^{3,41}. Other markers of macrophage activation, such as neopterin, soluble CD163, and soluble CD25 (aka soluble IL-2 receptor α , or sIL2R α), may show more clinical utility as they become available outside of reference laboratories^{42,43}.

Tests of Cytotoxic Dysfunction

As mentioned previously, fHLH is characterized by the paralysis of cellular cytotoxicity, and this mechanism may be important in MAS as well. Such cytotoxicity is mediated through the exocytosis of preformed lytic granules within cytotoxic cells⁹. Increasing arrays of tests are available to assess this exocytic and cytotoxic function. Many centers are able to perform a standardized assay of NK cell cytotoxic function, which assesses the ability of NK cells to lyse a tumor cell line that lacks major histocompatibility complex (MHC) type I. This *ex vivo* assay is useful in screening for CSS associated with cytotoxic dysfunction, but impaired NK function in this assay is frequently described in sepsis and MODS as well¹⁷.

Other assays of cytotoxic dysfunction are increasingly available in reference laboratories. One can test for the presence of the perforin protein in cytotoxic cells through flow cytometric assays. Additionally, screening for a defect in fusion of cytotoxic vesicles to the cell membrane can be accomplished by evaluating for “mobilization” of CD107a (aka lysosomal associated membrane protein 1, or LAMP1)⁴⁴. Once a defect of cellular cytotoxicity is strongly suspected, genetic testing for fHLH-associated mutations should proceed (Table 3).

Diagnostic Criteria

In 2004 the Histiocyte Society revised criteria for the diagnosis of both familial and reactive HLH (HLH-04, Table 4)³. While the performance of these criteria against other CSS has not been formally evaluated, increasing data would suggest that they do not offer a high degree of specificity for fHLH¹⁷.

In recognition that distinction of MAS from a flare of its underlying disease presented a diagnostic challenge, *Ravelli et al* have created criteria for the distinction of MAS from a flare of sJIA (Table 5)^{41,45}. These criteria were based on retrospective evidence of features that may distinguish sJIA from MAS and have yet to be prospectively validated.

ETIOLOGY-BASED TREATMENT

As with diagnosis, treatment of CSS must begin by addressing the final common pathway: intensive care of hemodynamic instability, support of specific organ dysfunction, and correction of coagulopathy. However, such supportive measures are unlikely to be sufficient to re-establish homeostasis without also addressing the factors driving cytokine production and effect.

Altering the Environment

For CSS of primarily environmental etiology, elimination of the offending microbe, allergen, or cytotoxic agent when possible may be all that is needed to allow restoration of homeostasis. An assessment for underlying immunodeficiency should help guide empiric treatment of suspected sepsis. MAHS, while of intrinsic origin, require a similar strategy: clear the source of inflammatory stimulation, in this case neoplastic cells.

While supportive care and antimicrobials may be sufficient to treat sepsis, antimicrobial use is indicated in any CSS where a contributing treatable pathogen is suspected. For example, a patient with a known defect in cytotoxicity infected with a herpesvirus would likely benefit from appropriate antiviral therapy.

In addition to clearing pro-inflammatory stimuli, treatments that alter immune response to such stimuli are emerging as potentially beneficial. Agents such as hydroxychloroquine, that are purported to act through inhibition of TLR signaling, are useful in treating SLE. More

specific inhibitors of TLR signaling are now being considered for use in autoinflammatory disorders⁴⁶.

Managing the Host Defect

Conventional Immunodeficiency—While elimination of the opportunistic pathogen is critical, supplementation of immune responses may be beneficial. In conventional immunodeficiency, this is best achieved preventatively with IV Immune globulin, GM-CSF, and/or appropriate vaccination. Acute supplementation of the immune response may also be beneficial, and it is unclear to what extent IVIg treatment successes in hemophagocytic syndromes are attributable to clearance of infection versus direct anti-inflammatory effects^{17,39}.

Hemophagocytic Lymphohistiocytosis—HLH-04 provided treatment recommendations for both fHLH and reactive HLH that utilize corticosteroids, cyclosporine, and etoposide as a bridge to transplant³. The goals of acute treatment of fHLH are to manage the immune response and preserve organ function in anticipation of allogeneic bone marrow transplantation. While some alterations in this protocol have been proposed⁴⁷, HLH-04 remains the foundation of fHLH treatment.

However, it is increasingly recognized that most disorders meeting HLH-04 criteria will not require such aggressive immunosuppression¹⁷. Additionally, chemotherapeutic strategies similar to HLH-04 carry a high risk for iatrogenic infection and hematologic malignancy^{17,48}. This concern for excessive toxicity has led to the use of more specific cytotoxic treatments in diseases with more discrete target populations. For example, rituximab has been shown to be effective in EBV-HLH, potentially due to cytotoxic effects on infected cells⁴⁹, and IVIg may be effective in secondary HLH and MAS^{17,50}.

Macrophage Activation Syndrome—The management of MAS is far from standardized and remains controversial. Most agree that high doses of corticosteroids are useful, but the mechanisms by which corticosteroids exert this beneficial effect are unclear. One postulated mechanism is that they help skew macrophages away from an M1, or proinflammatory differentiation state, toward a more regulatory or scavenger state⁵¹. In this case, one may expect corticosteroids to be effective in the treatment of any CSS exhibiting significant macrophage activation.

While many practitioners treat MAS with an HLH-04-like approach, less toxic strategies targeted at specific inflammatory defects have emerged. Accumulating evidence suggests that anakinra, a recombinant IL-1 receptor antagonist, may be beneficial in MAS. The successful treatment of sJIA with anakinra^{52,53}, coupled with evidence that IL-1 receptor signaling may be implicated in MAS²⁵, support IL-1 inhibition as a viable strategy. Indeed, several practitioners have reported successful treatment of MAS with only corticosteroids and anakinra^{54,55}. It is unclear if IL-1 inhibition is a viable strategy for MAS in general, or whether it is uniquely suited to a potentially IL-1 mediated disease such as sJIA.

SUMMARY

The hemophagocytic syndromes MAS and HLH overlap considerably with other CSS, and share a final common pathway with them. Laboratory findings such as dropping cell counts, dropping ESR, elevated ferritin, NK dysfunction, and hemophagocytosis that were once felt to be unique to hemophagocytic disorders, are increasingly recognized in a host of infectious and possible even allergic CSS. However, since addressing the primary defect(s) driving CSS is critical to restoring immunologic balance, it is critical to be able to understand and differentiate the mechanisms by which various CSS arrive at their final common pathway.

We propose a framework for understanding CSS wherein a careful assessment for the presence of host and environmental contributors to CSS will guide a rational therapeutic approach (Figure 2). A greater understanding of the mechanisms by which host and environmental factors contribute to CSS, as well as more precise knowledge of how our therapeutics alter those mechanisms, will allow even more precise direction of treatment.

ABBREVIATIONS

APC	Antigen Presenting Cell
CSS	Cytokine Storm Syndrome(s)
DIC	Disseminated Intravascular Coagulation
EBV	Epstein-Barr Virus
ESR	Erythrocyte Sedimentation Rate
HLH	Hemophagocytic Lymphohistiocytosis
MAHS	Malignancy-associated Hemophagocytic Syndrome
MODS	Multiple Organ Dysfunction Syndrome
NK	Natural Killer
SIRS	Systemic Inflammatory Response Syndrome
sJIA	Systemic Juvenile Idiopathic Arthritis
SLE	Systemic Lupus Erythematosus
TLR	Toll-like Receptor
XLP	X-linked Lymphoproliferative Disorder

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OBJECTIVES

After completion of this article the read should be able to:

1. Identify clinical similarities and differences between the variety of Cytokine Storm Syndromes.
2. Describe the clinical and immunologic hallmarks of Macrophage Activation Syndrome and Hemophagocytic Lymphohistiocytosis.
3. Describe a pathoetiologic framework for understanding Cytokine Storm Syndromes.

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Figure 1. Hemophagocyte seen in the spleen of a WT mice treated with TLR9 stimulation and IL-10 blockade. *=engulfed RBC (so-called ghost cell); #=engulfed leukocyte.

Figure 2. Conceptual model of various cytokine storm syndromes on a spectrum based on the nature of their mechanism of inflammation.

Table 1**Pediatric SIRS Criteria**

Systemic Inflammatory Response Syndrome (SIRS) *	
Presence of at least 2 of the following 4 criteria Must include abnormal temperature or leukocyte count	
Core Temperature	>38.5°C or <36°C
Abnormal Heart Rate	>2SD above normal for age [#] , OR
	Unexplained persistent elevation over 0.5-to 4-hr time period, OR
	For children <1 year old: heart rate <10 th percentile for age [#] , OR
	Unexplained persistent heart rate depression over a 0.5-hr period
Respiratory Rate	>2SD above normal for age, OR
	Acute requirement for mechanical ventilation
Leukocyte Count	Elevated or depressed [^] for age, OR
	>10% immature neutrophils

* adapted from Goldstein et al²

[#] not explained by external stimuli or drugs,

[^] not due to chemotherapy-induced leukopenia

Table 2

Common examples of pro- and anti-inflammatory contributors to CSS

Proinflammatory Agents	
Antigens	Antibody or B-cell receptor epitopes Peptides presented on MHC
Adjuvants	TLR Ligands LPS, bacterial DNA, dsRNA, flagellin, etc Other PRR ligands microbial nucleic acids Inflammasome triggers Uric acid crystals, hyperglycemia, ATP
Cytokines/Chemokines	IL-1 β , IL-6, IL-12, IL-18, TNF α , IFN γ ...
Anti-inflammatory Agents	
Cells	Regulatory T-cells Alternatively activated macrophages B10 B-cells
Cytokines	IL-10, TGF β , IL-1ra
Molecules	Anti-microbial peptides

MHC-Major Histocompatibility Complex; LPS-lipopolysaccharide; PRR-pattern recognition receptors; ATP-adenosine triphosphate; TGF β -Transforming Growth Factor β ; IL-1ra-IL-1 receptor antagonist

Genetic disorders associated with the occurrence of HLH^{*}

Table 3

	FHL2	FHL3	FHL4	FHL5	GS2	CHS	HPSII
Gene	PRF1	UNC13D	STX11	STXBP2	RAB27A	CHSI/LYST	AP3BI
Protein/Function	Perforin/pore-forming protein	Munc12-14/priming factor	Syntaxin11/membrane fusion	Munc18-2/membrane fusion	Rab27a/tethering	Lyst/lysosomal fission-protein sorting	Ap3β1/sorting of lysosomal protein
Murine	Prf1 ^{-/-}	Jinx	None	None	Ashen	Beige	Pearl
Model							
HLH	+	+	+	+	+	+	1 case report
Cytotoxic activity	–	–	±	±	–	–	–

FHL-familial hemophagocytic lymphohistiocytosis; GS-Griscelli Syndrome; CHS-Chediak-Higashi Syndrome; HPS-Hermansky-Pudlak Syndrome.

^{*} Adapted from Pachlopnik-Schmid et al⁸

Table 4**Diagnostic Guidelines for HLH**

The diagnosis of HLH is suggested by one of either 1 or 2 below	
1) A molecular diagnosis consistent with HLH (see Table 3)	
2) Five out of eight of the below criteria	
	Fever
	Splenomegaly
	Cytopenias affecting at least 2 lineages
	Hemoglobin <90g/L (in infants <4 weeks: hemoglobin < 100g/L)
	Platelets <100 × 10 ⁹ /L
	Neutrophils <1.0 × 10 ⁹ /L
	Hypertriglyceridemia and/or hypofibrinogenemia:
	Fasting triglycerides ≥ 3.0 mmol/L (≥ 265 mg/dL)
	Fibrinogen ≤ 1.5 g/L
	Hemophagocytosis in spleen, lymph node, or bone marrow
	Low or absent NK cell activity (per performing laboratory range)
	Ferritin ≥ 500 µg/L
	Soluble CD25 (IL-2 receptor α) > 2400 U/mL

Guidelines assume no evidence of malignancy. Adapted from Henter et al³.

Table 5

Preliminary diagnostic criteria for MAS complicating SJIA (11)

Laboratory criteria
1. Decreased platelet count ($262 \times 10^9/L$)
2. Elevated levels of aspartate aminotransferase (>59 U/L)
3. Decreased white blood cell count ($4.0 \times 10^9/L$)
4. Hypofibrinogenemia (2.5 g/L)
Clinical criteria
1. Central nervous system dysfunction (irritability, disorientation, lethargy, headache, seizures, coma)
2. Hemorrhages (purpura, easy bruising, mucosal bleeding)
3. Hepatomegaly (3 cm below the costal arch)
Histopathological criterion
1. Evidence of macrophage hemophagocytosis in the bone marrow aspirate

The diagnosis of MAS requires the presence of any 2 or more laboratory criteria or of any 2 or 3 or more clinical and/or laboratory criteria. A bone marrow aspirate for the demonstration of hemophagocytosis may be required only in doubtful cases. These criteria are likely more valuable for classification rather than diagnosis, given many items occur late in the course of disease. Adapted from Ravelli et al⁴⁰.