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The Multifaceted Immunology of Cytokine Storm Syndrome

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Abstract

Cytokine storm syndromes (CSS) are potentially fatal hyperinflammatory states that share the underpinnings of persistent immune cell activation and uninhibited cytokine production. CSS can be genetically determined by inborn errors of immunity (i.e. familial hemophagocytic lymphohistiocytosis) or develop as a complication of infections, chronic inflammatory diseases (e.g. Still disease), or malignancies (e.g. T-cell lymphoma). Therapeutic interventions that activate the immune system such as chimeric antigen receptor T cell therapy and immune checkpoint inhibition can also trigger CSS in the setting of cancer treatment. In this review, the biology of different types of CSS are explored, and the current knowledge on the involvement of immune pathways and the contribution of host genetics are discussed. The use of animal models to study CSS is reviewed, and their relevance for human diseases is discussed. Lastly, treatment approaches for CSS are discussed with a focus on interventions that target immune cells and cytokines.

Introduction

There is nothing quite as powerful as a worldwide fatal pandemic to bring increased attention to cytokine storm syndromes (CSS) (1). From a lumper's perspective, CSS have garnered attention from a vast variety of biomedical disciplines. CSS, including hemophagocytic lymphohistiocytosis (HLH), macrophage activation syndrome (MAS), and cytokine release syndrome (CRS), are frequently fatal end common pathways of an overly activated immune response to variety of triggers, from intracellular pathogens to hematologic malignancies to autoimmune and autoinflammatory diseases, and beyond (2). Over the last 2.5 decades, the immunology (3) and genetics (4) of CSS have been better defined using both murine models and human studies. While genetic defects in perforin-mediated cytolysis is perhaps the best studied and most common pathway resulting in CSS, defects in the inflammasome, and other pathways are being explored and better defined (5). Because of its broad clinical implications and highly translational immunology, CSS should

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be considered an integral aspect of even undergraduate immunology education curricula (6). Herein, the current state of the art understanding of CSS immunopathogenesis (studies of mice and humans), and the practical implications for effective therapeutics, will be presented (7). The heroic investigations of basic science immunologists, clinicians, and clinician-scientists over the last half century have championed the appropriate attention and scientific exploration to bare on this fascinating but deadly hyperinflammatory host immune response (8).

Clinical features of CSS

A hyperinflammatory immune response is at the core of CSS. No matter what the trigger or underlying condition, there are shared features among those suffering CSS. Broadly speaking, CSS is an overly exuberant immune response to a trigger frequently in the setting of a prior state of chronic inflammation and/or with an underlying genetic predisposition. Shared among CSS of multiple etiologies are clinical features secondary to the excess pro-inflammatory insults. Patients with CSS typically exhibit prolonged high fevers, cytopenias, coagulopathy, liver dysfunction, and central nervous system derangement (9). Associated laboratory and pathologic findings include hyper-ferritinemia, elevated soluble IL-2 receptor alpha (sCD25), elevated soluble haptoglobin receptor (sCD163), increased markers of inflammation (e.g. C-reactive protein), hyper-cytokinemia (e.g. IFN-γ, IL-1β, IL-6, IL-18), liver inflammation, and hemophagocytosis (5). Many of the clinical and pathologic features are utilized in various CSS diagnostic and classification criteria (10). There is no perfect set of diagnostic criteria for the various CSS of different etiologies, including HLH (11), MAS (12, 13), and even the CSS associated with severe coronavirus disease 2019 (COVID-19) (14). Broader, more disease inclusive, criteria have been proposed, but are dependent on data (e.g. hemophagocytosis) that are not always available (15). In addition, because of the severity and rapid disease progression, simplified criteria based on serum ferritin and erythrocyte sedimentation rate can be employed as timely simple screens for CSS (16).

For the purpose of this review, familial or primary HLH is defined by biallelic pathogenic variants in genes involved in the cytolytic function of T lymphocytes and natural killer cells (see section on Host genetics). MAS, also called secondary HLH by some subspecialities, is broadly defined as CSS associated with external triggers including infections, malignancies, and inflammatory diseases.

Broad array of CSS associated conditions

The importance of CSS cannot be understated, and it is likely under-recognized in children and adults across the globe (17). Whereas familial HLH is rare (1 in 50,000 live births), secondary or acquired forms of CSS may affect up to 1 in 3,000, or more, children and adults (18). Infectious agents, largely intracellular pathogens, contribute to a large percentage of CSS (Table I). The herpes virus family member is most notorious, particularly Epstein-Barr virus (EBV) and cytomegalovirus (CMV), but over 100 organisms have been reported to trigger CSS (7). Other CSS triggering pathogens include pandemic strains of influenza (19) and hemorrhagic fever viruses like dengue (20). Lassa fever, a hemorrhagic fever syndrome, is caused by an arenavirus, as is lymphocytic choriomeningitis virus

(LCMV), which triggers HLH in susceptible mouse strains (21). Infections often trigger CSS in patients with underlying inflammation (e.g. rheumatic or oncologic illness), immune suppression (e.g. HIV-1/AIDS), or various genetic predispositions (e.g. familial HLH).

Although a large number of autoimmune conditions have been associated with CSS/MAS, systemic lupus erythematosus (SLE) and Still disease (juvenile and adult) are most notorious (Table I). The pathogenesis of CSS in SLE is likely multi-factorial, potentially involving underlying cytopenias, increased type I IFN, and TLR triggering by self-nucleic acids, but it remains largely unknown (22). However, CSS etiology in Still disease is better understood with massive IL-18 (an IL-1 family member activated by caspase following inflammasome activation) levels integral to disease pathophysiology (23). In addition, defects in NK cell cytolysis (24) with contributing HLH associated gene defects (25) reveals some shared pathology with familial HLH. Indeed, a recent murine model combining excess IL-18 production with perforin deficiency were synergistic in causing spontaneous CSS with $CD8^+$ T cell expansion (26). Since IL-18 in combination with IL-12 triggers IFN-γ production, this hyper-inflammatory state was responsive to IFN-γ blockade (26).

IFN-γ excess production is also associated with CSS in the setting of hematologic malignancies (Table I) (27), as is IL-6 over-production. Not only can the underlying malignancy contribute to CSS by excess IL-6 production, iatrogenic cytokine release syndrome (CRS) can result from novel therapeutics (e.g. CAR-T cell therapy and immune checkpoint inhibitors) used to treat refractory leukemias and lymphomas (Table I). CRS is also characterized by excess IL-6 and IL-1 expression, and targeted cytokine approaches in humans and murine models have proven effective in treating CRS (28-30). Solid organ malignancies can also trigger a CSS but much less frequently than malignancies of blood forming tissues (31-33).

Other less common, or perhaps less well documented, triggers of CSS are diverse in origin. Conditions/triggers associated with CSS range from cardiac bypass circuits (34, 35), to pregnancy (36), to drug-induced (37), to graft versus host disease and post-transplantation (35, 38, 39), to Castleman disease (40), to metabolic disorders (41) (Table I). However, the most well studied conditions associated with CSS/HLH are primary or familial forms of HLH (42). Familial HLH is a term often restricted to genetic defects in the perforin pathway of lymphocyte mediated cytolysis (Table I). This includes autosomal recessive mutations in *PRF1, STX11, STXBP2*, and *UNC13D* (4, 5, 42). However, homozygous defects in genes (*RAB27A, LYST, AP3B1*) responsible for syndromes characterized by albinism and neutrophil dysfunction (Griscelli type 2, Chediak-Higashi, Hermansky-Pudlak type 2) that are important for intracellular granule (containing melanin as well as those with perforin) function are also linked to primary HLH (4, 5). Other primary genetic contributions to CSS/HLH are defects in X-linked lymphoproliferative (*XLP1* and *XLP2*) genes, which are associated with EBV triggering (5). Additional primary immunodeficiencies associated with CSS are individually rare, but the list of genes with defects associated with CSS is rapidly evolving (43). This includes genes important for viral control (e.g. *ITK*), lymphocyte activation (e.g. *PIK3CD*), and inflammasome activity (e.g. *NLRC4*, *CDC42*) (4, 5). Finally, gene defects resulting in inborn errors of metabolism (e.g. *SLC7A7*) have been linked

to CSS (44). Thus, ever-expanding genetic contributions to CSS are increasingly being recognized (4, 5).

Host genetics

While clinical descriptions of CSS/HLH date back almost over a century (42), and therapeutic protocols are in place (11), the immunology and genetic contributions were really only best understood over the last 2-and-a-half decades. This was sparked by the identification of homozygous defects in *PRF1* as the first gene contributing to a subset of infants with primary or familial HLH (45, 46). This was later modeled in mice in Pippa Marrack's lab (47). This landmark report demonstrated that *PRF1* deficient mice uniformly succumbed to LCMV infection within 2 weeks of exposure, but it was the host immune response that yielded fatal outcomes (47). Specifically, removal of cytolytic $CD8^+$ T lymphocytes (CTL) or blockade of interferon-gamma (IFN-γ) largely reversed mortality (47). It was later shown in both murine and human lytic lymphocytes (CTL or natural killer (NK) cells) that perforin or granzyme deficiency not only disrupted cytolysis of the target antigen presenting cell (APC), but this resulted in prolonged (5-times longer) engagement between the lytic lymphocyte and its associated APC (48, 49). It was elegantly shown that the disrupted interaction is dependent on caspase activity within the target cell (49). When this was disturbed, the prolonged interaction led to increased pro-inflammatory cytokines such as IFN-γ and tumor necrosis factor (TNF) believed to be responsible for the hyperinflammatory state (48, 49). Thus, lack of killing via the perforin pathway was directly responsible for excess immune cell cross talk resulting in inappropriately elevated pro-inflammatory cytokines believed to be responsible for the clinical features of CSS/HLH.

In addition to defects in *PRF1*, other homozygous mutations in genes required for perforin-mediated cytolysis were identified among infants with familial HLH (Fig. 1A). In order for preformed perforin and granzyme containing cytolytic granules to traffic along the actin cytoskeleton to the immunological synapse, dock and fuse with the cell membrane, and release perforin to form a channel into the target cell, a number of intact non-redundant gene products are necessary. Soon after the identification of homozygous *PRF1* defects were identified as responsible for subsets of infants with primary HLH (46), homozygous mutations in genes required for perforin delivery to the target cell, *STX11*, *STXBP2*, *UNC13D*, *RAB27A*, *LYST*, and *AP3B1*, were linked to familial cases of HLH (4, 5, 42). Clinically, these different genetic etiologies do not all present identically in terms of the CSS/HLH severity. Graded defects in cytotoxicity determines the severity of disease observed in humans and corresponding murine models of CSS/HLH (50, 51). Moreover, CSS can develop with polygenic combinations of heterozygous defects in different genes shared in the perforin cytolytic pathway (52-54). Thus, there are multiple potential genetic contributions to primary HLH via disruption of perforin-mediated cytolysis by CTL and NK cells.

The distinction between primary and secondary HLH, however, is becoming blurred, as heterozygous mutations in familial HLH genes have been shown to contribute to CSS beyond infancy via hypomorphic or dominant-negative effects on perforin-mediated cytolysis (4, 25, 55). This has been most clearly demonstrated by CSS patient derived

complete dominant-negative mutations in *STXBP2* inhibiting fusion of perforin containing cytolytic granules to the cell membrane at the immunologic synapse (56). However, even partial dominant-negative mutations in *STXBP2* can contribute to CSS in a threshold model of disease where infectious triggers (57) and/or an underlying hyper-inflammatory disease state (55) may push the pro-inflammatory environment to a point that immune regulatory mechanisms can no longer maintain a state of inflammatory homeostasis (58-60). Similarly, common heterozygous missense mutations of *PRF1* that act as partial-dominant-negatives diminishing NK cell lysis (19, 61, 62) have been associated with late-onset CSS/HLH/MAS triggered by infection (e.g. H1N1 influenza) (19) or hyper-inflammatory states (e.g. Still disease) (55, 63, 64). Heterozygous missense mutations in *UNC13D* have also been associated with CSS in children and adults (55, 64). Interestingly, non-exonic mutations in *UNC13D* have also been reported in individuals with CSS (65-67), such that whole exome sequencing may overlook potential genetic defects contributing to decreased NK cell and CTL perforin mediated cytolysis (4). Intriguingly, a missense mutation in *RAB27A* identified in 2 unrelated individuals with CSS was shown to disrupt the interaction with MUNC13-4 (*UNC13D* protein), thus diminishing NK cell lytic function by delaying cytolytic granule polarization to the immunologic synapse (68). Similar to homozygous defects in perforin pathway genes (48, 49), this heterozygous HLH gene defect resulted in increased IFN-γ production likely contributing to CSS disease manifestations (68).

Notably, heterozygous defects in several different perforin pathway HLH genes have been shown to contribute to CSS pathophysiology, likely via a threshold model of disease potentially explaining why some individuals fare worse and develop a CSS when infected with the same pathogen as others (19). Therefore, heterozygous gene mutations that partially disrupt NK cell or CTL lytic function can contribute to CSS in select hyper-inflammatory states. Along these lines, mutations in familial HLH genes have been noted in those with severe COVID-19 (69) and in children with the SARS-CoV-2 post-infectious CSS, multisystem inflammatory syndrome in children (MIS-C) (70).

In addition, genes indirectly involved in trafficking cytolytic granules via their regulation of movement along the actin cytoskeleton have recently been linked to CSS, namely *CDC42* (71, 72) and *DOCK8* (70, 73) (Fig. 1A). Besides a putative role in perforin-mediated cytolysis, dominant mutations in *CDC42* may contribute to CSS via activation of the inflammasome resulting in increased production of IL-1 and IL-18 (71, 72, 74). Dominant activating mutations in the inflammasome component gene *NLRC4* have also been newly associated with CSS (75, 76) and excessive levels of IL-1 and IL-18 (77) (Fig. 1B). Hence, two established and unique immunologic pathways, perforin mediated cytolysis and inflammasome activation, contribute to the multifaceted immunology of CSS (Fig. 1) with multiple immune cell types involved in CSS development. The roles of the multiple immune cell types involved in various CSS have been illuminated using murine models of disease.

Murine models of cytokine storm syndrome

The biology of CSS is complex and the pathogenic cytokine(s) may vary depending on the underlying cause of inflammation. While profiling of human samples at various stages of CSS provides valuable insight, these studies are often limited by the availability of

samples and the significant clinical heterogeneity among patients. Moreover, the results are often descriptive in nature, and a causal role of specific proinflammatory mediators remains difficult to establish. The mechanistic understanding of CSS is aided by the availability of mouse models, which also serve as important tools for preclinical evaluation of therapeutic agents. In this section, we review the use of murine models to study HLH and MAS (Fig. 2).

Murine models of primary HLH

Primary or familial HLH is caused by genetic defects that impair the exocytosis of cytotoxic granules, which result in defective $CD8^+$ T cell- and NK cell-directed cytotoxicity (78, 79). Perforin (encoded by *PRF1*) is a glycoprotein that forms channels on the target cell membrane to allow entry of granzyme and other cytotoxic proteins. Biallelic mutations in *PRF1* represent the most common cause of familial HLH in humans, and, fittingly, perforin deficiency in mice is the first described model of HLH (47).

Perforin-deficient mice develop normally and features of HLH occur only after infection with lymphocytic choriomeningitic virus (LCMV) (47). This is congruent with the observations in patients with HLH as disease onset is often precipitated by infections. Following LCMV infection, perforin-deficient mice develop the hallmarks of HLH including splenomegaly, pancytopenia, hypertriglyceridemia, hypofibrinogenemia, and cytokine storm. Fulminant hemophagocytosis is observed in tissues including the liver, spleen, and bone marrow (47). In the absence of perforin, activated $CD8^+$ T cells fail to eliminate antigen-presenting cells (APC) and thereby creates a vicious cycle of reciprocal immune activation (80). The pathologic features of this model are largely prevented by depletion of $CD8^+$ T lymphocytes or neutralization of IFN-γ (47). These findings strongly support the rationale of targeting T lymphocytes and IFN-γ in the treatment of HLH.

Murine models of *UNC13D* deficiency (Jinx mice), *RAB27A* deficiency (ashen mice), *LYST* deficiency (souris mice) and *STX11* deficiency are also used to study the biology of familial HLH (51, 81-83) (Fig. 2). Similar to perforin-deficient mice, these strains show normal growth and development at baseline. In the setting of LCMV infection, however, they exhibit impaired viral clearance and develop features of HLH including hypothermia, splenomegaly, pancytopenia, hypertriglyceridemia, and cytokine storm. The severity of disease in *RAB27A*-deficient mice and *STX11*-deficient mice is milder compared to *PRF1*-deficient mice, which is in line with the later disease onset in patients with the corresponding mutations (51).

The aforementioned mouse strains are typically healthy until immune cell activation is triggered by LCMV. Spontaneous disease onset is noted in perforin-deficient mice with dendritic cell-specific deficiency of Fas (84). The interaction between Fas and Fas-ligand induces apoptotic cell death of activated immune cells. These data further show that timely elimination of APC by CTL is essential to prevent the development of hyperinflammation and cytokine storm.

CpG-DNA induced model of MAS

MAS is a CSS induced by intrinsic factors (i.e. Still disease and malignancy) or extrinsic factors (i.e. infections and drugs). To model MAS in mice, researchers often use Toll-like

receptor (TLR) ligands to elicit systemic inflammation and cytokine storm. Behrens et al. described a model of MAS using repeated injection of the TLR9 ligand CpG DNA (85). Recurrent activation of TLR9 in this model results in cytopenia, hepatosplenomegaly, hepatitis, hyperferritinemia, and hemophagocytosis (85). The production of IFN-γ is required for the development of anemia, thrombocytopenia and hepatitis in this model. Interestingly, neither the absence of B and T lymphocytes ($Rag2^{-/-}$ mice) nor depletion of NK cells affects disease development, although some clinical features are attenuated in $Rag2^{-/-}$ $Il2rg^{-/-}$ mice with complete absence of B lymphocytes, T lymphocytes, and NK cells.

Instead, CpG DNA-induced MAS appears to be more dependent on myeloid cells. The excess production of IFN-γ is dependent on IL-12 primarily produced by inflammatory monocytes (86, 87). Prominent extramedullary myelopoiesis and accumulation of inflammatory monocytes are evident in CpG-treated mice and features of MAS are reversed with monocyte/macrophage depletion using clodronate-containing liposomes or antibodies to macrophage colony-stimulating factor (87, 88). CpG-induced MAS is also associated with increased production of IL-10, which is observed in patients with MAS (89). This cytokine plays a protective role in the hyperinflammatory response, as IL-10 blockade leads to fulminant MAS and lethal disease in CpG-treated mice (85). Curiously, host microbiota also contribute to the development of cytokine storm in this model, as the manifestations of MAS are significantly attenuated in germ-free mice and in wild-type (WT) mice treated with broad spectrum antibiotics (90).

LPS-induced MAS in IL-6 transgenic mice

IL-6 possesses multiple proinflammatory roles, and elevated IL-6 levels in the peripheral blood is associated with different types of CSS. While transgenic mice engineered to overproduce human IL-6 do not show evidence of MAS at baseline, Strippoli et al. demonstrated that a single dose of the TLR4 ligand lipopolysaccharide (LPS) or TLR3 ligand Poly I:C is sufficient to induce hyperinflammation and early mortality in these mice (91). IL-6 transgenic mice treated with LPS exhibit anemia, neutropenia, hyperferritinemia, and a cytokine storm that includes high levels of TNF, IL-1β, and IL-18. Hemophagocytosis has not been described in this model. Development of the MAS-like syndrome in IL-6 transgenic mice also requires IFN-γ; neutralizing antibodies to IFN-γ reduced ferritin levels and improved survival and body weight recovery (92).

Other models of MAS induced by TLR agonists

Another TLR-driven model of MAS was established by Wang et al. to mimic infection-associated MAS. Sequential treatment with a viral TLR agonist (Poly I:C or R837, a TLR7 agonist) followed by a non-lethal dose of LPS, but not in the reversed order, elicits a lethal MAS-like disease characterized by pancytopenia, hyperferritinemia, and hemophagocytosis (93). Curiously, IFN-γ is dispensable in this model (93).

Chronic activation of Tlr7 alone is also sufficient to drive the development of anemia, thrombocytopenia, and hemophagocytosis in mice (94). Studies using a transgenic strain that overexpresses Tlr7 identified a population of monocyte-derived inflammatory macrophages

that are responsible for the manifestations of cytopenia and hemophagocytosis. Signaling through interferon regulatory factor-5 (Irf5) downstream of Tlr7 is essential for the development of these macrophages. Interestingly, polymorphisms in *IRF5* are associated with MAS susceptibility in patients with pediatric Still disease (95).

The connection between TLR signaling, LCMV infection, and the development of MAS is reinforced by a study by Ohyagi and colleagues (96). However, they found that the uptake of erythrocytes by monocyte-derived dendritic cells is essential for the production of IL-10, which inhibits the hyperinflammatory response. This notion supports the finding that human hemophagocytes exhibit a transcriptomic signature of alternative activation (97). Further studies are needed to determine the role of hemophagocytes in CSS (98).

MAS driven by hyperactive metabolic pathways

In the model of MAS driven by sequential treatment Poly I:C followed by LPS, Wang et al. demonstrated that the inflammatory response was dependent on cellular immunometabolism. Inhibition of glycolysis by 2-deoxyglucose, but not inhibition of individual cytokines, is sufficient to block the development of the lethal MAS-like disease (93). Supporting this view, constitutive activation of the metabolic regulator mechanist target of rapamycin complex 1 (mTORC1) via conditional deletion of *Tsc2* can drive the development of an MAS-like disease with cytopenia, hepatosplenomegaly, increase ferritin levels and fulminant hemophagocytosis (99). Because mTORC1 signals downstream of multiple cytokines, this pathway may serve as a nexus for input from multiple proinflammatory mediators. Interestingly, MAS is also associated with inborn errors of cellular metabolism in humans (41, 100).

Heterogeneity in the murine models of CSS and relevance to human disease

The murine models of HLH and MAS share the pathognomonic features of cytokine storm, hepatosplenomegaly, cytopenia and hemophagocytosis. There are also clear differences among these models, which is, perhaps, expected given the heterogenous causes of CSS in humans, despite the overlapping clinical manifestations. Therefore, lessons from these models are helpful in determining the mechanism and appropriate intervention for the different types of CSS.

A key discrepancy between the mouse models is the role of IFN-γ. Excess production of IFN-γ is a hallmark of familial HLH and MAS in patients, and blockade of IFN-γ is effective for the treatment of familial HLH refractory to other interventions (101, 102). IFN-γ is critically important for disease development in the perforin-deficiency model of HLH, CpG-induced MAS, and LPS-induced MAS in IL-6 transgenic mice (47, 85, 92). Overproduction of IFN-γ alone is sufficient to drive hemophagocytosis and anemia (103). However, repeated TLR9 activation in combination with IL-10 receptor blockade can trigger fulminant MAS in the absence of IFN-γ signaling (86). MAS secondary to sequential administration of poly I:C and LPS is also unresponsive to IFN-γ blockade (93). The development of CSS in the absence of IFN-γ is particularly relevant in rare cases of HLH that develop in patients lacking the IFN-γ receptor (104).

Another difference among the murine models is the role of activated T lymphocytes. Ineffective killing of activated lymphocytes by $CD8^+$ T cells and NK cells is central to the biology of HLH as illustrated by the perforin-deficiency model (47, 80). In contrast, neither T lymphocytes nor NK cells are required in the development of MAS triggered by CpG or Poly I:C / LPS (85, 93). Mononuclear phagocytes including monocytes, macrophages, and dendritic cells appear to play a more prominent in the models of CSS induced by TLR ligands. These observations further suggest the existence of multiple pathways of hyperinflammation that can lead to similar clinical manifestations.

Given the involvement of multiple cytokines in CSS, therapeutic approaches that modulate common mediators of these cytokines may be more effective than focusing on a single target with variable involvement. Indeed, inhibitors of Janus kinases (JAK) are increasing used in the treatment of HLH as they can inhibit the signaling pathways of IFN-γ among many other cytokines and growth factors (105). In the perforin-deficiency model of HLH and fulminant MAS induced by CpG and IL-10 blockade, treatment with ruxolitinib showed additional protective effects beyond IFN-γ inhibition, including reductions in splenomegaly, $CD8^+$ T cell activation, and neutrophil activation (106). A recent study further illustrate that combined treatment with ruxolitinib and anti-IFN-γ antibodies may be even more effective for the treatment of familial HLH in mice (107).

Taken together, murine models of HLH and MAS have significantly improved our mechanistic understanding of CSS. They help to demonstrate the multifaceted immunology of CSS, ranging from defective perforin mediated cytolysis to dominantly active inflammasomopathies to non-genetic infection triggers. These models not only provide a platform to study the different pathways that contribute to hyperinflammation, but they allow for evaluation of novel therapeutics. However, researchers should always be mindful of the potential translational gap between animal models and human disease. The phase 1 clinical trial on the CD28 superantagonist TGN1412, which appeared safe in murine and non-human primate models but resulted in rapid development of a severe CSS in healthy volunteers, is an important reminder that differences in host biology can be associated with very different outcomes (108).

Treatment of CSS

Treatment of CSS is focused on removing the inciting trigger(s) and calming the hyperinflammatory response. With the many different paths that lead to CSS, the choice treatment should be based on available evidence and tailored to each patient. Evaluation of novel therapeutic options should consider the lessons learned from decades of failure in the search of more effective treatment for sepsis (109). The cause of CSS, contribution of host genetics, severity and heterogeneity of clinical manifestations as well as utility of animal models for the particular form of CSS should be taken into consideration for treatment selection and clinical trial design.

Immune cells as therapeutic targets

For familial HLH, cytolytic lymphocytes (CTL and NK cells) are primary drivers of disease secondary to their defective lysis of APC/target cells resulting in prolonged engagement and

excess proinflammatory cytokine production (e.g. IFN-γ). Recently, increased percentages of activated $CD8^+$ T cells ($CD4^{dim}$, $CD38^{high}$, HLA-DR^+) have been noted in both HLH (110) and MAS (111) patient populations. This provides additional rationale for the use of etoposide, an inhibitor of topoisomerase II, to target proliferating lymphocytes in HLH (Table II) (11). The glucocorticoid, dexamethasone (good central nervous system penetration), is given with etoposide as a broadly immunosuppressant targeting most all immune cell types (11). For refractory HLH requiring salvage therapy, almetuzumab (anti-CD52; targets lymphocytes and myeloid cells for depletion) (112) and anti-thymocyte globlulin (ATG; targets T lymphocytes for depletion) (113) have been employed to control familial HLH prior to stem cell transplantation (Table II).

Another cellular target for treating HLH includes rituximab (anti-CD20; targets B lymphocytes for depletion) specifically for HLH in the setting of EBV infection (Table II). The rationale derives in part from the fact that EBV generally targets B cells (T cells can also be infected), but most of the benefit of rituximab in treating EBV triggered HLH has been noted in the primary immunodeficiency syndromes, such as XLP (114). Rituximab has the most survival benefit in treating EBV associated HLH when the serum ferritin is ≤1,000 ng/mL and the EBV viral load is ≤1,500 copies/mL (114). Nevertheless, survival in the setting of EBV HLH is not ideal. Rituximab therapy has also been used to treat thrombotic microangiopathy (TMA), a potential coincident condition associated with MAS/HLH with poor survival (115). In addition, eculizumab (anti-C5, complement) may improve TMA survival by preventing terminal complement activation on endothelial cells (116). The use of biologics targeting various cell types is often carried out in conjunction with etoposide-based protocols, which in the best of centers still yields mortality rates near 40% (117). Targeting cytokines broadly via the nuclear factor of activated T cells (NFAT) inhibitor, cyclosporin A has been dropped from the etoposide-based protocols for increased side effects without additional benefit (117). However, glucocorticoids remain a frequent mainstay of CSS therapy and work in part by inhibiting cytokine production (Table II).

Cytokines as therapeutic targets

Another approach to treating HLH/CSS, which may in part target cytokines via anti-cytokine antibodies, is intravenous immunoglobulin (IVIg) (Table II). This has primarily found success in non-EBV infection triggered CSS (118). Targeting cytokines broadly with plasmapheresis in the setting of CSS has also been reported as beneficial (119). More recently, the targeting of individual pro-inflammatory cytokines has gained acceptance in treating CSS. Initially, targeting tumor necrosis factor (TNF) was anecdotally reported to sometimes help or to hinder treatment of CSS in various case reports/series (17). While targeting TNF has not gained traction, targeting IL-1 in the setting of CSS has gained wider acceptance (Table II).

The first reported beneficial use of IL-1 blockade with the recombinant human IL-1 receptor antagonist (rhIL-1Ra) anakinra for severe CSS was in a child with a histiocytic disorder (120). Anakinra was seen as an attractive therapeutic in the setting of CSS because of its recombinant nature, short half-life (4-6 hours), quick action, large therapeutic window, and prior established safety profile (121, 122). Anakinra has been particularly useful in

treating rheumatic triggers of MAS/CSS, particularly in those with Still disease (123, 124). Anakinra may also play a role in CSS associated with sepsis (122). And while malignancy associated HLH/CSS is associated with high mortality rates (125), anakinra has anecdotally been reported to benefit some in this setting (126). Most recently, anakinra has also notably improved survival in the setting of severe COVID-19 CSS (127).

While early during the pandemic blockade of IL-6 appeared to improve survival of severe COVID-19 CSS, meta-analyses of multiple clinical trials have been less enthusiastic about the benefit of anti-IL-6 or anti-IL-6R mAb approaches for hospitalized COVID-19 patients (128). However, IL-6 blockade with tocilizumab (anti-IL-6R mAb) has received United States Food and Drug Administration (FDA) approval in treating iatrogenic CRS associated with chimeric T cell treatment for refractory hematopoietic malignancies (129) (Table II). The FDA has also recently approved of emapalumab (anti-IFN-γ mAb) for primary/familial HLH (102) (Table II), bringing the murine model full circle (47). IFN-γ over-expression occurs in the setting of familial HLH as the result of decreased perforin-mediated cytolysis, but IFN-γ is not the only increased cytokine noted (49). This perhaps explains the benefit of adding JAK inhibition (Table II) to anti-IFN-γ mAb therapy in treating CSS/HLH (107). JAK inhibition with baricitinib has also been touted to treat severe COVID-19 CSS (130). While the coronavirus is the trigger for the deadly COVID-19 CSS (131), the appropriate cytokine(s) to target for this pandemic remains unclear (132).

Conclusions

CSS are gaining wider recognition as deadly complications from a wide variety of genetic, infectious, rheumatic, oncologic, and other conditions, ranging from familial HLH to EBV to Still disease to T cell lymphoma to Castleman disease (7, 8, 17). While there are multiple potential CSS triggers with genetic contributions, including worldwide pandemics (19, 69), the defective host immune response is largely responsible for the inflammatory mortality, best characterized in the setting of familial HLH (47). Following identification of the genes responsible for familial HLH in humans, genetic murine models have been established to explore the pathophysiology of CSS/HLH (21). Defects in perforin-mediated cytolysis, though, are not just restricted to familial HLH, but also contribute to the much more common secondary forms of HLH/MAS/CSS (19, 55, 64, 70). Ineffective NK cell and CTL lysis of targets cell via perforin result in prolonged engagement of the lytic lymphocyte with its target cell producing excess pro-inflammatory cytokines believed responsible for the multi-organ dysfunction of CSS (48, 49). Other immunologic pathways can also be disturbed in CSS (5).

More recently, gene defects resulting in constitutive inflammasome activation have been identified (4, 5). Mutations in *CDC42* (75, 76) and *NLRC4* are associated with CSS characterized by high levels of the inflammasome product, IL-18 (133). IL-18 as a target to treat autoinflammatory CSS is currently being explored (134). Another, inflammasome product, IL-1, is already a well-established target in treating various forms of CSS, including Still disease (2). Targeting of other individual cytokines have received FDA approval for various CSS (IL-6 blockade for CRS, IFN-γ blockade for familial HLH) (102, 129). Broader cytokine inhibition with JAK inhibitors is also being explored for treatment of CSS/HLH

(135). Again, murine models have proven valuable to study this therapeutic approach (136), as well as murine models exploring non-hereditary forms of CSS (85). Beyond broad immunosuppression with glucocorticoids and chemotherapy (etoposide), targeted cytokine approaches are proving efficacious and less toxic in saving lives of those afflicted with CSS (7, 8). As we continue to expand our knowledge of the multifaceted immunology of various CSS scenarios via murine and human studies (3), we will be able to provide a precision medicine approach to treating the frequently fatal entity of CSS (137).

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Abbreviations used in this article:

APC	antigen presenting cell
CAR-T	chimeric antigen receptor T cell
CMV	cytomegalovirus
CRS	cytokine release syndrome
COVID-19	coronavirus disease 2019
CSS	cytokine storm syndrome
CTL	cytolytic T lymphocyte
EBV	Epstein-Barr virus
FDA	Food and Drug Administration
HLH	hemophagocytic lymphohistiocytosis
IFN-γ	interferon-gamma
IVIg,	intravenous immunoglobulin
JAK	Janus kinase
LCMV	lymphocytic choriomeningitis virus
mAb	monoclonal antibody
MAS	macrophage activation syndrome
MIS-C	multisystem inflammatory syndrome in children

NFAT	nuclear factor of activated T cells
NK	natural killer
Ra	receptor antagonist
rh	recombinant human
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
TLR	toll-like receptor
TMA	thrombotic microangiopathy
TNF	tumor necrosis factor
XLP	X-linked lymphoproliferative
WT	wild-type

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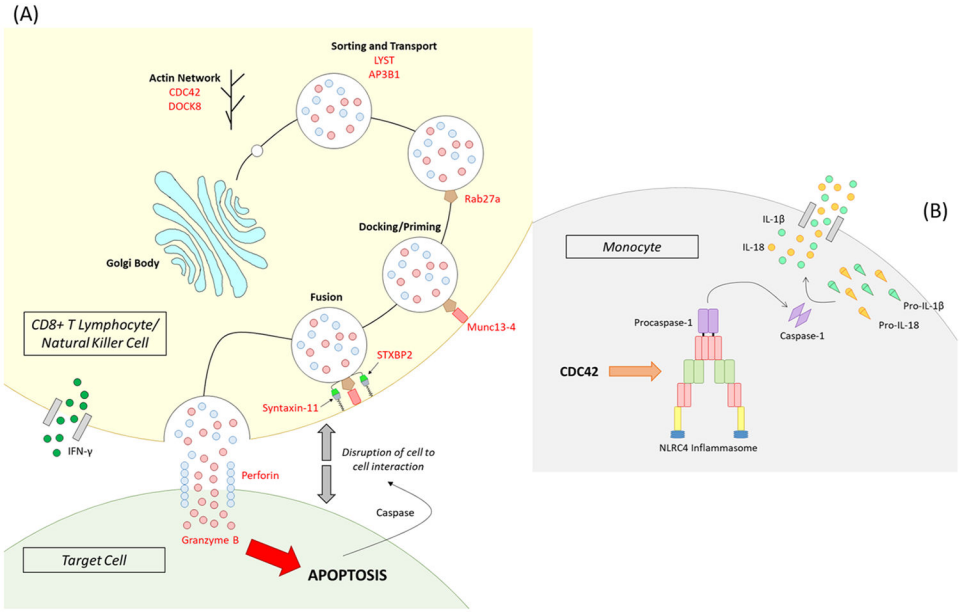

FIGURE 1.

Cytolytic and autoinflammatory genetic pathways to cytokine storm syndrome. A) A cytolytic lymphocyte (top) lyses an antigen presenting cell (target cell below) via the perforin pathway by delivery of granzyme B to the target cell. Intact proteins of perforin, syntaxin 11, STXB2, Munc13-4, Rab27a, LYST, and AP3B1 are required for cytolytic granule sorting, transport, docking, priming, and fusion to the cell membrane so perforin can be released into the immunologic synapse to form a pore to deliver granzyme B resulting in caspase dependent apoptosis of the target cell. CDC42 and DOCK8 are important for trafficking of cytolytic granules along the actin cytoskeleton. B) Monocyte derived cell populations can also produce high levels of IL-1β and IL-18 when inflammasome gene products like NLRC4 are mutated or stimulated (e.g. CDC42) to activate caspase-1 from procaspase-1 resulting in conversion of pro-IL-1β and pro-IL-18 into active IL-1β and IL-18, respectively. This figure was generated by Dr. Daniel D. Reiff (University of Alabama at Birmingham).

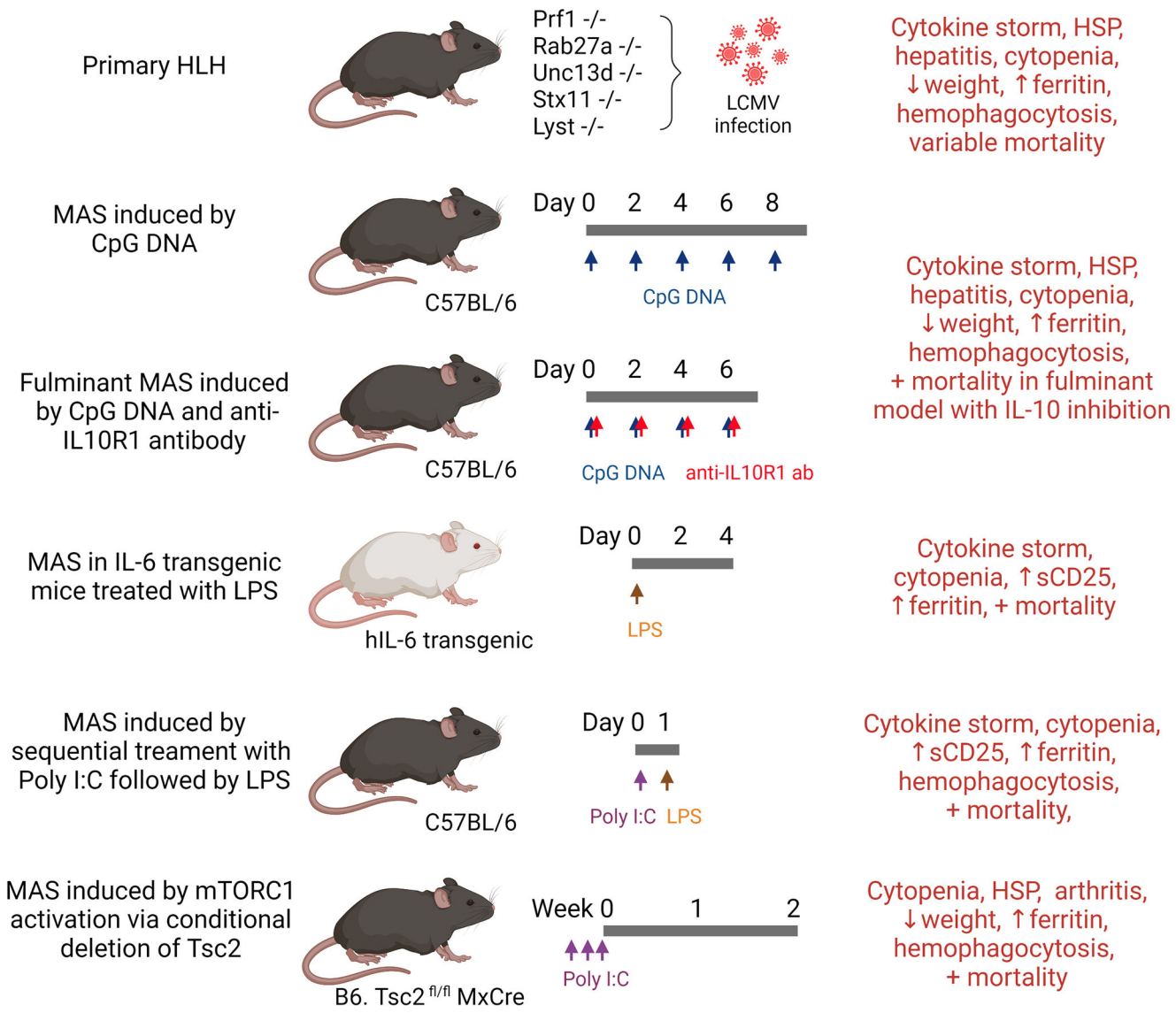

FIGURE 2.

Murine models of cytokine storm syndrome. Primary HLH can be modeled by infecting mice with deficiency of *PRF1*, *RAB27A*, *UNC13D*, and *STX11* with LCMV. Manifestations of MAS can be induced in WT mice or IL-6 transgenic mice using TLR ligands. Constitutive activation of the metabolic regulator mTORC1 by conditional deletion of Tsc2 is also shown to trigger an MAS-like disease. The typical timeline of disease development and key manifestations of MAS are displayed for each model. Abbreviations: CpG, 5'—Cytosine—phosphate—Guanine—3'; HLH, hemophagocytic lymphohistiocytosis; HSP, hepatosplenomegaly; LPS, lipopolysaccharide; MAS, macrophage activation syndrome; poly I:C, polyinosinic-polycytidylic acid; sCD25, soluble IL-2 receptor-α.

Table 1.

Diseases under the cytokine storm syndrome umbrella

Cause of CSS	Example	Notable features
Genetic		
Familial HLH	Homozygous *PRF1* deficiency	Approximately half of familial HLH in North America
Secondary HLH	Dominant-negative *STXBP2* mutation	Early-onset; often triggered by viral infection
Autoinflammatory	*NLRC4* activating mutation	Associated with colitis
Primary immunodeficiency	X-linked lymphoproliferative disease (XLP1/2)	EBV-induced
Metabolic	Lysinic protein intolerance (*SLC7A7* mutation)	Splenomegaly
Systemic illness		
Chronic inflammation	MAS in systemic juvenile idiopathic arthritis	Rash, arthritis
Hematologic malignancy	T cell leukemia	Poor outcome
Lymphoproliferative disorder	Multicentric Castleman disease	HHV8 association
Infectious disease		
Sepsis and septic shock	bacterial, viral and fungal pathogens	Poor NK cell function
Herpes virus family	Epstein-Barr virus	High mortality
Influenza	H1N1	HLH gene mutations
Hemorrhagic fever virus	Dengue	Extreme hyperferritinemia
SARS-CoV-2	COVID-19-associated ARDS and MIS-C	Severe pneumonia in ARDS; myocarditis in MIS-C
Other associations		
CAR-T cell therapy	Cytokine release syndrome	Frequent central nervous system involvement
Immune dysregulation	Pregnancy	Infectious triggers common
Medications	Anti-CD28 monoclonal antibody	Multiple cytokine release

Abbreviations: ARDS, acute respiratory distress syndrome; CAR-T; chimeric antigen receptor T-cell; COVID-19, coronavirus disease of 2019; EBV, Epstein-Barr virus; HHV8, human herpesvirus-8; H1N1, hemagglutinin-1 neuraminidase-1; HLH, hemophagocytic lymphohistiocytosis; MAS, macrophage activation syndrome; MIS-C, multisystem inflammatory syndrome in children; NK, natural killer; NLRC4, NOD-like receptor family CARD domain containing 4; PRF1, perforin-1; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SLC7A7, solute carrier family 7 member 7 ; STXBP2, syntaxin binding protein-2.

Table 2.

Immunologic therapeutics used in treatment of cytokine storm syndrome

Therapeutic agent	Target	Mechanism of action	Application
Etoposide (topoisomerase II inhibitor)	T lymphocytes	Inhibits cell proliferation	HLH
Almetuzumab (anti-CD52)	Lymphocytes, monocytes	Depletes leukocytes	HLH
Rituximab (anti-CD20 mAb)	B lymphocytes	Depletes B lymphocytes	EBV-MAS
Anti-thymocyte globulin (ATG)	T lymphocytes	Depletes T lymphocytes	HLH
Cyclosporin A (calcineurin inhibitor)	IL-2, IFN-γ, others	Inhibits cell proliferation and effector functions	HLH, MAS
Anakinra (rhIL-1Ra)	IL-1	Blocks IL-1 from receptor binding	MAS, CRS
Tocilizumab (anti-IL-6R mAb)	IL-6	Blocks IL-6 from receptor binding	MAS, CRS
Emapalumab (anti-IFN-γ mAb)	IFN-γ	Neutralizes IFN-γ	HLH, MAS
Tadekinig alfa (rhIL-18BP)	IL-18	Blocks IL-18 from receptor binding	NLRC4-MAS, XIAP
Ruxolitinib (JAK1/2 inhibitor)	IFN-γ, IL-6, IL-12, others	Inhibits cytokine signaling	HLH, MAS, severe COVID-19
Plasmapheresis	Multiple cytokines	Remove proinflammatory mediators	severe COVID-19
Dexamethasone (glucocorticoids)	Multiple cytokines	Broad immunosuppression	Most CSS
Intravenous immunoglobulin (IVIG)	Multiple cytokines	unknown	MAS, MIS-C

Abbreviations: BP, binding protein; COVID-19, coronavirus disease of 2019; CRS, cytokine release syndrome; EBV, Epstein-Barr virus; HLH, hemophagocytic lymphohistiocytosis; IFN, interferon; JAK, Janus kinase; mAb, monoclonal antibody; MAS, macrophage activation syndrome; MIS-C, multisystem inflammatory syndrome in children; NLRC4, NOD-like receptor family CARD domain containing 4; Ra, receptor antagonist; rh, recombinant human; XIAP, X-linked inhibitor of apoptosis protein.