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MACROPHAGE ACTIVATION SYNDROME AND CYTOKINE DIRECTED THERAPIES

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Abstract

Macrophage activation syndrome (MAS) is an episode of overwhelming inflammation that occurs most commonly in children with systemic juvenile idiopathic arthritis. It is characterized by expansion and activation of T lymphocytes and hemophagocytic macrophages, and bears great similarity to hemophagocytic lymphohistiocytosis (HLH). This disorder has substantial morbidity and mortality, and there is frequently a delay in recognition and initiation of treatment. Here, we will review what is known about the pathogenesis of MAS and in particular its similarities to HLH. The development of MAS is characterized by a cytokine storm, with the elaboration of numerous proinflammatory cytokines. We will examine the evidence for various cytokines in the initiation and pathogenesis of MAS, and discuss how new biologic therapies may alter the risk of MAS. Finally we will review current treatment options for MAS, and examine how cytokine-directed therapy could serve as novel treatment modalities.

Keywords

macrophage activation syndrome; hemophagocytic lymphohistiocytosis; systemic juvenile idiopathic arthritis; Still's disease; IL-1; IL-6; IL-18 and IFN γ

DEFINITIONS

Macrophage activation syndrome (MAS) is a serious, potentially fatal complication of rheumatic diseases caused by excessive activation and expansion of T lymphocytes and macrophages that exhibit hemophagocytic activity [1–4]. In MAS, this excessive cellular

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activation and expansion leads to a hyperinflammatory state associated with three cardinal features: cytopenias, liver dysfunction, and coagulopathy resembling disseminated intravascular coagulation. MAS is also associated with extreme hyperferritinemia. It is a life-threatening condition, and the reported mortality rates are approximately 20–30% [5, 6].

Since MAS is characterized by expansion of tissue macrophages, or *histiocytes*, exhibiting hemophagocytic activity, and is often triggered by infections or modifications in the drug therapy, the term "*reactive hemophagocytic lymphohistiocytosis*" has been preferred by some authors to classify this condition [7, 8]. Indeed, MAS may belong to a group of histiocytic disorders collectively known as *hemophagocytic lymphohistiocytosis or HLH*. This term describes a spectrum of disease processes characterized by accumulations of histologically benign well-differentiated mononuclear cells with a macrophage phenotype, as well as T cell proliferation [9, 10]. In the contemporary classification of histiocytic disorders, HLH is subdivided into *primary*, or *familial HLH*, and *secondary*, or *reactive HLH*, though clinically they may be difficult to distinguish from each other. *Familial HLH (FHLH)* is a constellation of rare autosomal recessive immune disorders linked to genetic defects in various genes affecting the cytolytic pathway (see below). The clinical symptoms of FHLH usually become evident within the first months of life. Secondary HLH tends to occur in older children and adults and more often is associated with an identifiable infectious episode, most notably Epstein-Barr virus (EBV) or cytomegalovirus (CMV) infection. The group of secondary hemophagocytic disorders also includes malignancy associated HLH. However, the distinction between primary and secondary HLH is becoming increasingly blurred as the genetic basis for these conditions is further delineated [11, 12].

EPIDEMIOLOGY

MAS is seen most frequently in children with systemic juvenile idiopathic arthritis (SJIA) and in its adult equivalent, adult-onset Still's disease (AOSD) [3, 5]. However, it is increasingly reported in other rheumatic disease of childhood, including pediatric systemic lupus erythematosus [13, 14], Kawasaki disease [14–16], juvenile dermatomyositis, antiphospholipid syndrome and mixed connective tissue disease [17]. Episodes of MAS appear to be most commonly triggered by infections, particularly viral infections, or during periods of high disease activity including disease onset [17, 18]. Notably MAS can also occur in adults with rheumatic disease. One recent systematic review found that while more than half of MAS episodes reported in the literature were in children with SJIA, there were significant numbers of adults with SLE, AOSD and rheumatoid arthritis who experienced MAS [19].

The epidemiologic studies of MAS have been complicated by the lack of defined diagnostic criteria (see below). In most cases reported in the literature, the diagnosis of MAS was initially suspected based on the development of cytopenias, coagulopathy, and extreme hyperferritinemia. The pathognomonic feature of this syndrome is usually found upon bone marrow examination: numerous, morphologically benign macrophages exhibiting hemophagocytic activity. Most pediatric rheumatologist now agree that approximately 7–17% of patients with SJIA develop overt MAS [6, 20] while mild "subclinical" MAS may be seen in as many as one third of patients with active systemic disease [21, 22]. Bone

marrow examination in these patients typically reveals extensive expansion of highly activated macrophages with only few of these cells exhibiting overt hemophagocytic activity.

DIAGNOSTIC APPROACH

There are no validated diagnostic criteria for MAS, and early diagnosis is often difficult due to similarities with a SJIA flare and/or sepsis-like syndromes. This is further complicated by the fact that MAS may in fact be triggered by a SJIA flare or sepsis, especially in case of intraphagocytic pathogen infections. As a general rule, impending MAS should be strongly suspected in a patient with SJIA who develops persistent fevers, a fall in the ESR and drop in platelet count, particularly in a combination with increase in serum D-dimer and ferritin levels. Ideally, the diagnosis of MAS should be confirmed by the demonstration of hemophagocytosis in the bone marrow. However, the demonstration of hemophagocytosis may be difficult due to sampling error, particularly at the early stages of the syndrome. In such cases, additional staining of the bone marrow with anti-CD163 antibodies may be helpful. In the setting of MAS, this usually reveals massive expansion of highly activated histiocytes [21, 22].

In contrast to MAS, the diagnosis of HLH is typically established based on the diagnostic criteria developed by the International Histiocyte Society [23]. This diagnosis can be made if patients have a known molecular diagnosis consistent with HLH, or meet at least 5 of 8 clinical or laboratory criteria (Table 1). Unfortunately, the HLH diagnostic criteria when applied to SJIA patients with suspected MAS are not sufficiently sensitive to diagnose the condition early in its development when treatment is most successful. Some of the HLH markers, such as lymphadenopathy, splenomegaly and hyperferritinemia, are common features of active SJIA itself and, therefore, do not distinguish MAS from a conventional SJIA flare. Other criteria, such as cytopenias and hypofibrinogenemia, become evident only in the later stages of MAS, as SJIA patients often have increased white blood cell and platelet counts as well as serum levels of fibrinogen as a part of the inflammatory response. Finally, other criteria such as NK cell function and bone marrow examination are time consuming and may unnecessarily delay administration of specific therapy.

Attempts to modify the HLH criteria to increase the sensitivity and specificity for the diagnosis of MAS in rheumatic conditions including SJIA have been initiated [24, 25]. One consideration is that the relative decrease in white blood cell count, platelets, or fibrinogen rather than the low absolute numbers required by the HLH criteria may be more useful in establishing an early diagnosis. Another consideration is that the minimum threshold level for hyperferritinemia required for the diagnosis of HLH (500 µg/L) may be too low, as many patients with active SJIA (in the absence of MAS) have ferritin levels above that threshold. In contrast, in the acute phase of MAS, ferritin levels generally peak above 5,000 ug/L. The first proposed criteria for diagnosis of MAS in patients with SJIA were by Ravelli and colleagues [24]. For these criteria, patients with SJIA diagnosed with MAS by clinical and laboratory features were compared to SJIA patients with high disease activity but no evidence of MAS. Clinical and laboratory features that were the strongest discriminators for MAS were identified, and when used in combination were 100% sensitive and 95–97%

specific for MAS (Table 1). However, these criteria have not been further validated in a large cohort of patients, or examined in patients with MAS without SJIA.

Recently, an international collaborative project was started to develop a new and robust set of diagnostic criteria for MAS in patients with SJIA [25]. As the first step in this project, the Delphi survey technique was used to identify the clinical features most pertinent to the diagnosis of MAS. The 9 features ranked the highest by the 232 respondent physicians are shown in Table 1. These candidate diagnostic criteria are currently being evaluated for their performance in the early recognition of MAS.

PATHOPHYSIOLOGY

The main pathophysiologic feature of MAS is excessive activation and expansion of cytotoxic CD8+T cells and macrophages. These activated immune cells produce large amounts of proinflammatory cytokines creating “a cytokine storm”. In clinically similar primary HLH, the uncontrolled expansion of T cells and macrophages has been linked to decreased NK cell and cytotoxic T cell function. Indeed, various defects in the granule-dependant cytotoxic activity of lymphocytes seen in several hereditary hemophagocytic syndromes, highlight the importance of the cytolytic function in downregulation of certain immune responses [26–31]. Furthermore, depressed cytolytic activity has been seen in MAS as well [32]. Normally, cytotoxic cells induce apoptosis of cells infected with intracellular microbes or cells undergoing malignant transformation. In some circumstances, cytotoxic cells may also be directly involved in induction of apoptosis of activated macrophages and T cells during the contraction stage of the immune response. It has been proposed that in both HLH and MAS, failure to induce apoptosis due to cytotoxic dysfunction leads to prolonged expansion of T cells and macrophages and escalating production of proinflammatory cytokines. The exact pathway linking cytolytic abnormalities in MAS patients with excessive expansion of macrophages is not clear. However, the hemophagocytic activity of macrophages, the pathognomonic feature of MAS, appears to be induced by chronic stimulation of macrophages with cytokines. The identification of cytokines that play the pivotal role in this process is an area of active research, since selectively targeting these cytokines may be a very effective therapeutic strategy.

Genetic defects in Primary HLH

In primary HLH, the uncontrolled proliferation of T cells and macrophages has been linked to various genetic defects leading to decreased NK cell and cytotoxic T cell function. In about 30% of FHLH patients this is due to mutations in the gene encoding perforin [26]. Perforin is a protein which cytolytic cells utilize to induce apoptosis of target cells such as tumor cells or cells infected by viruses. In about 10% of patients with primary HLH, the disease is caused by mutations in another gene, MUNC13-4 [27]. The protein encoded by this gene is involved in the release of perforin into the immune synapse with a target cell. Although the cytolytic cells of patients with MUNC13-4 mutations produce sufficient amounts of perforin, their ability to kill target cells is greatly diminished. More recently, mutations in two other genes encoding proteins that facilitate granule fusion in intracellular trafficking events leading to the release of perforin have been linked to the development of primary HLH: Syntaxin 11, a member of the SNARE protein family [28], and syntaxin

binding protein 2 (STXBP2, known as MUNC18–2) [29, 30]. Finally, there are several syndromic immunodeficiency syndromes that predispose to HLH which are caused by mutations in gene products involved in function of lytic granules. These including Griscelli syndrome type II (Rab27a), Chediak Higashi syndrome (Lyst) and Hermansky-Pudlak syndrome type II (AP3 β 1) [31].

Although familial cases of MAS in SJIA have not been reported, SJIA/MAS patients have functional defects in the exosome degranulation pathway [32] and these abnormalities are associated with specific MUNC13-4 [33, 34] and perforin [35] gene polymorphisms. This suggests that there may be genetic predisposition to MAS, and could allow for the identification of a subset of patients at particular risk for this syndrome.

Clues from animal models

Some clues to the pathophysiology of MAS are provided by animal models of HLH. First, Jordan et al demonstrated that perforin-deficient mice infected with lymphochoriomeningitic Virus (LCMV) developed fevers, splenomegaly, pancytopenia, extreme hyperferritinemia, and hypercytokinemia, as well as histological features including hemophagocytosis characteristic of HLH [36]. More importantly, these clinical features were prevented by the administration of anti-CD8 antibodies or neutralization of IFN γ , while antibodies against CD4 and the neutralization of other inflammatory cytokines, including TNF- α had no effect. Of note, the effects of neutralization of IL-1 β have not been studied in this model. These results suggest that IFN γ producing CD8+ T cells were central in the pathogenesis of the hemphagocytic syndrome in this model [36]. Similar results have been obtained in mice deficient in other HLH-associated genes including Munc13-4 and Rab27a [37, 38]. These animals also developed an HLH-like picture upon infection with LCMV in an IFN γ dependent manner.

In all these models, however, the HLH-like clinical features emerge only in response to viral infections. Although a viral illness is a very common trigger of hemophagocytic syndromes, many FHLH patients develop the first symptom of the disease spontaneously without an identifiable infection [11]. Similarly, MAS is often associated with a flare of underlying SJIA rather than infection. These considerations prompted a search for other animal models that would not be dependent on an infectious trigger. Recent reports showing the critical need for the TLR signaling adaptor MyD88 in the development of HLH-like disease in LCMV infected *MUNC13-4* deficient mice [39], combined with the evidence of persistently activated TLR/IL1R signaling pathways in SJIA [40, 41] provided a rationale for repeated activation of TLR to replicate the environment that would allow MAS to develop in a genetically predisposed host. Indeed, wild-type mice given repeated TLR9 stimulation develop some MAS features including hepatic dysfunction and cytopenias [42]. Interestingly, this model appears to be only partially IFN γ dependent, and in contrast to the models of primary HLH, IFN γ in these animals appears to be produced mainly by dendritic cells and NK cells, but not by CD8 T lymphocytes. Furthermore, in this model, many clinical features including hemophagocytosis do not appear to depend on IFN γ . Although the findings in this model are intriguing, their relevance to the disease in humans still needs to be elucidated.

“Cytokine storm” in MAS

In both MAS and HLH, strikingly high levels of circulating cytokines and natural i-cytokine-inhibitors such as soluble TNF receptors and IL1R antagonists_[RL2] have been reported in many studies [43–45]. These include pro-inflammatory cytokines derived from lymphocytes such as IFN- γ and IL-2 as well as cytokines that are of monocyte and macrophage origin including IL-1 β , TNF α , IL-6 and IL-18. Based on these observations, the term “cytokine storm” has been used by many authors to characterize the immune response seen in MAS. Notably, patients with FHLH and MAS also show elevated levels of regulatory cytokines such as IL-10 [45–47]. This cytokine has several antiinflammatory properties including reducing cytokine production by macrophages [48, 49], and may contribute to hemophagocytosis [42]. Patients who exhibit fulminant MAS may represent those where regulatory pathways such as IL-10 are overwhelmed, leading to uncontrolled inflammation. This is supported by animal studies described above, where TLR9 stimulation concordant with blockade of the IL-10 receptor led to more severe disease [42].

However, despite growing evidence for a “cytokine storm” in MAS, the data must be interpreted with caution. Although in general circulating cytokine determinations are useful in disease, an elevated cytokine level in a particular pathologic condition does not necessarily establish causality. This is true even for those cytokines that have a high degree of correlation with a severity of disease. In contrast, changes in the clinical presentation in response to blocking a specific cytokine provides the best evidence for a role of the cytokine in disease pathogenesis. Below we further examine the several cytokines that are increased in MAS and examine their putative role in the pathogenesis of this disease (FIGURE 1).

IL-1—IL-1 β is a proinflammatory cytokine produced primarily by monocytes and macrophages. It is present as an inactive form pro-IL-1 β ; however, upon activation of cells it is cleaved by caspase-1 to the biologically active form. IL-1 β signals through its receptor and causes lymphocyte and endothelial activation as well as production of other inflammatory cytokines including IL-6 [50]. IL-1 β is believed to be central to the pathogenesis of SJIA. Newly diagnosed SJIA patients show an IL-1-drive gene expression profile [41, 51], and serum from patients with active SJIA triggers the induction of IL-1 related genes in monocytes from healthy donors [40]. Indeed, large series [52–54] as well as randomized trials [55, 56] have shown that IL-1 blockade could induce long-lasting clinical remission in >50% of SJIA patients. However, the precise role of IL-1 β in MAS is not known. *On the one hand*, the hematological changes in MAS are not characteristic of an IL-1-mediated disease. In IL-1-mediated diseases such as cyropyrin-associated periodic fever syndrome, familial Mediterranean fever, AOSD and SJIA, neutrophilia is present and falls rapidly with IL-1 blocking therapy [57]. Similarly, platelet counts are elevated in these syndromes and also fall rapidly with IL-1 blockade. In contrast, neutropenia rather than neutrophilia is a feature of MAS, and the most salient hematological change is thrombocytopenia, not high platelet counts. Elevated levels of hepatic enzymes in the circulation are often observed in MAS but rarely in IL-1-mediated syndromes. Finally, patients with FHLH do not exhibit increased serum concentrations of IL-1 α or IL-1 β [44].

On the other hand, in some SJIA patients with MAS IL-1 blockade resulted in complete resolution of the MAS symptoms (see below). These clinical observations suggest that IL-1 still may have a role in MAS pathogenesis. Another possibility is that the effect of IL-1 blocking therapies in these patients may not be direct, but rather mediated through a reduction in production of other cytokines such as IL-18.

IL-6—IL-6 is a pleiotropic cytokine produced in the early stages of inflammation and is central in driving the acute-phase response. Patients with SJIA also demonstrate increased levels of IL-6, which correlate with disease activity [58, 59]. However, like IL-1 β , the role of IL-6 in the pathogenesis of MAS remains unknown and not well studied. One study of patients with MAS demonstrated the presence of IL-6 producing activated macrophages obtained from liver biopsies [60]. In addition, IL-6 is elevated in patients with HLH, although not as markedly as in syndromes such as sepsis [43, 61]. Interestingly, one study of IL-6 overexpressing transgenic mice found that prolonged exposure to IL-6 *in vivo* led to an exaggerated inflammatory response to TLR ligands with some clinical features reminiscent of MAS including cytopenias and hyperferritinemia [62]. These findings suggest that IL-6 may amplify the inflammatory response to infections and contribute to a “cytokine storm”. Indeed, recent studies of SJIA patients receiving the IL-6 monoclonal antibody tocilizumab have reported a very low rate of MAS episodes [63], and other reported cases appear to have a significantly milder clinical course [64].

TNF α —TNF α is a pleomorphic cytokine implicated in the pathogenesis of several inflammatory diseases. It is produced largely by monocytes and macrophages that are activated by toll-like receptor ligands such as endotoxin as well as cytokines such as IL-18, and stimulates local endothelial cells as well as lymphocytes [65]. Elevated levels of TNF α have been found in the synovium of children with oligoarticular and polyarticular JIA, and TNF α -blocking agents have dramatically improved the clinical outcomes and quality of life of these patients [65, 66]. However, the role of TNF α in SJIA appears to be more limited. While serum levels of TNF α are increased in patients with SJIA, levels are comparable to those seen in patients with other systemic inflammatory disease such as Kawasaki disease [67]. Similarly, while there are reports of good clinical response in SJIA patients with TNF-blocking agents, the results have been inconsistent [68], and these agents are not included in the consensus treatment plans for new-onset SJIA [69].

The role of TNF α in MAS and other hemophagocytic syndromes is unclear. Several animal models of HLH have shown increased levels of TNF α [36, 38, 70], likely driven by IL-18 [70]. However there are conflicting results as to the pathologic role of increased TNF α , as one report showed this cytokine may mediate tissue damage [71] while others have found no effect of neutralizing this cytokine [36]. TNF α similarly does not appear to be central to the pathogenesis of MAS in patients with SJIA. Serum levels of TNF α are only modestly elevated, and are not significantly higher than those seen in active SJIA [67, 72]. There are some reports of MAS that was refractory to standard therapies that showed rapid improvement with TNF blockade [73–75]. However, there are numerous cases described in the literature of MAS in the setting of TNF α therapy [8, 19] as well as reports of patients whose MAS worsened upon initiation of therapy [76]. In addition, neutralization of TNF α in

some animal models of arthritis did not have a major impact on the clinical presentation of the hemophagocytic syndrome [36]. Taken together these findings may suggest that elevated TNF α levels reflect the degree of underlying cellular activation rather than playing a causative role in MAS.

IL-18—IL-18 is a unique cytokine in the IL-1 family, and is constitutively present in keratinocytes, epithelial cells and blood monocytes [77]. Because the IL-18 precursor is inactive, caspase-1 is required for processing and secretion of the active cytokine. IL-18 induces production of IFN γ by NK cells and T cells as well as TNF α and chemokine secretion by macrophages [78]. Another unique feature of IL-18 is the existence of a naturally occurring, high affinity binding protein termed the IL-18 binding protein (IL-18BP). IL-18BP is endogenously produced in healthy subjects and is elevated in many pathologic conditions including chronic liver disease [79] where evidence suggests it may play a role in the perpetuation of the inflammatory response.

In MAS, serum IL-18 levels are elevated out of proportion compared with other cytokines [80, 81]. This is in distinct contrast to IL-18 levels in other diseases such as rheumatoid arthritis [82], sepsis [83], lupus erythematosus [84, 85], granulomatosis with polyangiitis [86], and chronic liver disease [79]. The most commonly used ELISA for circulating IL-18 detects about 100 pg/mL in the serum of healthy humans, whereas in diseases characterized by systemic inflammation, the levels rarely exceed 300 pg/mL. In sharp contrast, in MAS, levels of circulating IL-18 can be in the nanogram per milliliter range [57, 80]. However, levels of free IL-18 also correlate very strongly with underlying disease activity in SJIA [80] and AOSD [82] and decrease with clinical remission. Notably serum IL-18 levels in patients during MAS were indistinguishable from those of patients with active SJIA [67]. This may suggest that elevated IL-18 levels are not specific to MAS but rather distinguish patients with uncontrolled inflammation who are susceptible to MAS. On the other hand, this could also be consistent with the hypothesis that SJIA with MAS is not a distinct disease variant, but rather the severe end of a spectrum that includes all SJIA patients and incorporates subclinical MAS.

In patients with MAS secondary to infections, autoimmune disease, lymphoma, or cancer, the concentrations of serum IL-18 were highly increased, while in contrast the concentrations of IL-18BP were only moderately elevated, resulting in a high level of biologically active free IL-18 [80]. Free IL-18, but not other cytokines, significantly correlated with overall clinical status as well as many specific features of MAS including anemia, hypertriglyceridemia, and hyperferritinemia, along with markers of Th1 lymphocyte activation including elevated concentrations of IFN γ and soluble IL-2. Interestingly, IL-18 is a strong stimulator of NK cell activity, but despite high IL-18 levels, in vitro NK-cell cytolytic activity is severely impaired in MAS patients, due to both NK-cell lymphopenia but also intrinsic NK-cell functional deficiency [87]. Thus, a severe IL-18/IL-18BP imbalance may result in T lymphocyte and macrophage activation, which escapes immunoregulatory control by NK-cell cytotoxicity. As a result, in patients with underlying inflammatory diseases this creates conditions favoring the development of secondary MAS. The regulation of IL-18BP production in MAS may also affect disease outcome. Both IL-18 and IL-18BP are markedly upregulated by IFN γ , thus providing a negative feedback mechanism

modulating IL-18 activity [88]. While activation of mononuclear cells from patients with FHLH leads to highly increased production of IFN γ , the resultant induction of IL-18 BP is markedly reduced [89]. If IL-18 indeed plays a major role in perpetuating MAS, the failure of IFN γ to induce IL-18BP negative feedback may constitute a fundamental pathogenetic mechanism.

However, one recent study suggests that IL-18 may have a more limited role in MAS. Chiossone and colleagues examined perforin-deficient mice infected with murine cytomegalovirus, which leads to uncontrolled viral replication along with pancytopenia, hepatic dysfunction, hemophagocytosis and death [70]. Administration of synthetic IL-18BP ameliorated liver damage in the perforin-deficient mice. However, the animals still produced a substantial amount of proinflammatory cytokines, and there was no change in overall survival. Further work is needed to better characterize the role of IL-18 in other model systems.

IFN γ —IFN γ is a proinflammatory cytokine produced by T lymphocytes and NK cells when activated by antigen-presenting cells via IL-12 and IL-18. Its primary function is to profoundly activate monocytes and macrophages [90]. Activated macrophages are divided into several general classes based upon stimuli and their resulting polarization, with M1 macrophages driven by IFN γ into a classical proinflammatory phenotype characterized by increased microbicidal capacity, heightened responses to TLR ligands and upregulated antigen processing and presentation. These cells are potent producers of proinflammatory cytokines including IL-6, IL-12 and IL-23, as well as the chemokines IP-10, MIG and I-TAC which recruit polarized Th1 cells as well as NK cells [91, 92]. There is also evidence that IFN γ may be a critical driver of hemophagocytosis by these activated macrophages [93]. In contrast, macrophages can also be “alternatively activated” by other stimuli including IL-4 to exhibit an anti-inflammatory, wound-healing phenotype [94]. These macrophages are likely a heterogeneous population that encompasses distinct cellular phenotypes involved in activating polarized Th2 cells, immunoregulation and wound healing [92].

The role of IFN γ in SJIA has not been fully characterized. However, IFN γ does not appear to play a central role in the pathogenesis of SJIA without MAS. Patients with both active and inactive SJIA do not exhibit increased serum IFN γ [52, 95]. In addition, 3 independent gene expression studies have failed to find a prominent IFN γ induced signature in the peripheral blood monocytes of children who do not exhibit MAS clinical features [40, 41, 96].

However, multiple lines of evidence suggest an essential role for IFN γ in the pathogenesis of MAS. IFN γ appears central to the pathogenesis of primary, familial forms of HLH. First, IFN γ expression is highly elevated in these patients, out of proportion to other proinflammatory cytokines such as TNF α and IL-6, and rapidly returns to normal upon effective treatment [43, 47, 97]. These children also exhibit increased levels of the IFN γ -induced chemokines IP-10 and MIG [98] and cytokine soluble TWEAK [99]. Secondly, multiple independent animal models of familial HLH have also demonstrated increased levels of IFN γ , and neutralization of this cytokine dramatically improves survival [36, 38,

42]. Taken together, these findings support IFN γ blockade as a novel therapy for HLH, and a clinical trial is currently in progress [100]. There is also increasing evidence for the role of IFN γ in SJIA patients who develop MAS. MAS episodes are frequently triggered by viral infections, which are known activators of IFN γ -induced pathways [101]. Indeed, both children [67, 102] and adults [72] who experience MAS exhibit increased levels of serum IFN γ , and higher levels of IFN γ were observed in patients who succumbed to MAS [72]. Patients with MAS also show significant proliferation of IFN γ producing T-cells in tissue [60]. The precise molecular phenotype of macrophages in patients with MAS has not been defined. However, children with MAS do exhibit increased levels of neopterin, an IFN-driven product of activated macrophages [103]. In addition, levels of neopterin could distinguish patients with MAS from those with active SJIA without MAS clinical features [67]. Taken together, there is strong evidence that IFN γ -induced pathways are central to the pathogenesis of MAS in patients with SJIA.

TREATMENT OF MAS

Standard non-biologic treatments

MAS is a life-threatening condition associated with high mortality rates. Therefore, early recognition and immediate therapeutic intervention to produce a rapid response is critical. Most clinicians start with intravenous methylprednisolone pulse therapy (e.g. 30 mg/kg for three consecutive days) followed by 2–3 mg/kg/day in 2–4 divided doses. If response to steroids is not immediately evident, parenteral administration of cyclosporine A (CsA) (2–7 mg/kg/day) is usually initiated [2, 4, 104]. Although high IV doses of CsA have been associated with the increased risk for the posterior reversible encephalopathy syndrome, in most MAS patients, addition of CsA not only provides rapid control of the symptoms, but also avoids excessive use of steroids [5]. Patients in whom MAS remains active, despite the use of corticosteroids and CsA, present a serious challenge. In these patients, one might consider using the HLH-2004 treatment protocol developed by the International Histiocyte Society [23]. This protocol, in addition to steroids and CsA, includes etoposide (or VP16), a podophyllatoxin derivative that inhibits DNA synthesis by forming a complex with topoisomerase II and DNA. Although successful use of etoposide in MAS has been reported, potential toxicity of the drug is a major concern, particularly in patients with hepatic impairment. Etoposide is metabolized by the liver and then both unchanged drug and its metabolites are excreted through kidneys. Since patients who may require the use of etoposide are very likely to have hepatic and renal involvement, caution should be exercised to properly adjust the dosage and thus limit the potential side effects such as severe bone marrow suppression. Reports describing deaths with the use of etoposide caused by severe bone marrow suppression and overwhelming infection have been published [105, 106]. Recently, it has been suggested that in patients unresponsive to the combination of steroids and cyclosporine A, particularly in those with renal and hepatic impairment, anti-thymocyte globulin (ATG) might be a safer alternative to etoposide [107, 108]. ATG depletes both CD4 $^{+}$ and CD8 $^{+}$ T cells through complement-dependant cell lysis. Mild depletion of monocytes is noted in some patients as well. Although in the reported cases this treatment was tolerated well, one must remember infusion reactions are frequently reported with the

use of ATG and adequate laboratory and supportive medical resources must be readily available if this treatment is used.

Biologic agents

The utility of biologic drugs in MAS treatment remains unclear. Although TNF α inhibiting agents have been reported to be effective in occasional MAS patients, other reports describe patients in whom MAS developed while they were on TNF α -inhibiting agents (see above). Since, at least in SJIA, MAS episodes are often triggered by the disease flare, biologics that neutralize IL-1, a cytokine that plays a pivotal role in SJIA pathogenesis, have been tried by some authors. As with TNF α -inhibiting agents the results have been conflicting (see below). Intravenous immune globulin treatment has been a successful treatment in virus-associated Reactive HLH [109–111]. Rituximab, an anti-CD20 antibody that depletes B lymphocytes, has been successfully used in EBV-induced lymphoproliferative disease [112, 113] and could be considered in EBV-driven MAS.

Interleukin-1 blockade and MAS—The exact role of IL-1 in the development of MAS remains unclear. Since MAS episodes are often triggered by SJIA flare, it is reasonable to expect at least some response to IL-1 inhibition due to a better control of the underlying disease. On the other hand, although linked, MAS and SJIA differ in terms of the underlying pathophysiology and, as a result, respond differently to therapies. There are several reports of SJIA-associated MAS dramatically benefiting from anakinra, a recombinant IL-1 receptor antagonist, after inadequate response to corticosteroids and CsA [114–116]. For those severely ill children, IL-1 blockade has been remarkably effective in a relative brief time frame. Successful treatment of refractory AOSD with IL-1 blockade complicated by MAS has been reported as well [117]. However, in a recent report summarizing the experience with the use of anakinra in SJIA in several pediatric rheumatology centers, one of 23 patients developed MAS while being treated with anakinra [54]. Moreover, in a more recent report describing 46 SJIA patients treated with anakinra at disease onset, anakinra was a suspected MAS trigger in 5 children at doses of 1–2 mg/kg/day [53]. However, the exact cause and effect was difficult to establish in these patients and permanent discontinuation of anakinra was unnecessary for any of those children. Interestingly, there is some data that IL-1 blockade may alter the responsiveness of monocytes to other cytokines including IFN γ . Blood monocytes from patients treated with anakinra showed an upregulation of the IFN γ -induced signature [55], and have enhanced STAT1 phosphorylation in response to IFN γ treatment compared to control cells [95]. This could suggest a mechanism to potentiate MAS by leading to exaggerated immune response to a viral illness.

Canakinumab, a monoclonal antibody directed against IL-1 β , is another IL-1 blocking biologic agent [56]. Although canakinumab is an effective treatment in SJIA, it does not appear to have a significant effect on reducing the risk of MAS, even in patients whose underlying SJIA is well controlled [56 and Grom et al, submitted]. Infections were the most prevalent trigger for MAS in this group. Furthermore, the overall clinical features of MAS in patients treated with canakinumab do not appear to be modified by treatment.

Interleukin-6 blockade and MAS—IL-6 blockade, via the anti-IL-6 receptor monoclonal antibody tocilizumab, has proven highly efficacious in treating SJIA [63, 118]. IL-6 is produced by activated macrophages in MAS, and one animal model suggests IL-6 may amplify the response of macrophages to proinflammatory stimuli [62]. There are no reports of MAS treated specifically with tocilizumab. In contrast, in a Phase III clinical trial in SJIA, MAS was observed in 3 patients receiving IL-6 blockade with tocilizumab. This corresponded to 1.5 MAS cases per 100 patient years (all patients followed up to Week 104 post-infusion as of May 31 2011), in patients whose underlying SJIA responded well to the treatment [63]. Another recent report from Japan described a patient with severe AOSD who showed a very good initial response to tocilizumab, but then rapidly progressed to develop MAS [119]. Also concerning are reports of patients with SJIA and a history of MAS who rapidly developed cytopenias upon initiation of tocilizumab therapy which led to discontinuation of the therapy [120]. Furthermore, it has been suggested that the treatment with tocilizumab may mask some of MAS features. Shimizu and colleagues described several SJIA patients who developed MAS while on tocilizumab and showed remarkably that the CRP levels remained normal, while the increase in the levels of ferritin was relatively modest [64]. This may be related to the fact that IL-6 is the strongest inducer of CRP synthesis by the liver cells. It also has a strong effect on the production of ferritin.

In the US, as of March 31, 2012, 41 cases of MAS in patients treated with tocilizumab had been registered in the FDA Adverse Events Reporting System (AERS) database. For 23 of these 41 patients, tocilizumab had been prescribed to treat JIA (presumed to be SJIA), 2 to treat AOSD, 8 to treat rheumatoid arthritis, 1 to treat Castleman's disease, and 7 patients had no indication reported. For 12 patients, 4 of whom had JIA, MAS was fatal. Since this database does not provide information about the total number of patients receiving tocilizumab nor the time of exposure, neither crude incidence nor incidence rate of MAS can be determined using this dataset. However, in Japan, where tocilizumab is approved to treat rheumatoid arthritis and SJIA, the Chugai pharmaceuticals website provides information for Japanese patients participating in a registry. Based on this dataset, between 16 April 2008 and 28 August 2012 there were 33 cases of MAS reported in 517 patients with SJIA treated with tocilizumab. This equates to a crude incidence of 6.38% and a fatality rate of 10%. Since the duration of exposure is not reported, it is not possible to calculate a time adjusted rate of MAS. This crude incidence is slightly lower than the range of 7–17% reported in the literature.

FUTURE THERAPIES FOR MAS

Despite significant advances in our understanding of MAS, there is great need for new and specific therapies for this condition. Cytokine-directed therapies have the potential to target the effector cells of MAS without the myelo suppressive side-effects of current therapies such as etoposide. In this regard, an intriguing target is IFN γ . There is significant clinical and animal data implicating IFN γ in the pathogenesis of both primary familial and reactive forms of HLH, and clinical trials are currently underway to test IFN γ blockade in these patients. However, there are significant knowledge gaps regarding the role of IFN γ in MAS. The phenotype of activated mononuclear phagocytes in MAS is largely unexplored. Most studies have examined peripheral blood mononuclear cells, which may not provide a full

picture as activated phagocytes traffic to tissues such as the liver and bone marrow. More information is needed on the phenotype of these tissue effector cells, and their secreted products. It is also unknown whether monocytes from patients with SJIA exhibit altered responses to IFN γ that would contribute to development of MAS clinical features. However, we recently demonstrated that monocytes from patients with SJIA had enhanced responses to IFN γ , with significantly greater expression of STAT1 and a trend towards increased expression of IFN γ -induced chemokines IP-10 and MIG, compared with healthy controls [95]. It is not known why monocytes from patients with SJIA would have altered responsiveness to IFN γ . However, this may reflect the elevated levels of pro inflammatory cytokines including IL-1 and IL-6 associated with the underlying disease. This is supported by recent findings in genetically normal animals where chronic stimulation through TLR activation or IL-6 predisposed to development of MAS clinical features [42, 62]. Further work is needed to dissect the signaling pathways that lead to “cytokine storm” in MAS, and to determine how these effector molecules can be most effectively targeted to help resolve this frequently fatal episode of inflammation.

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Practice points

- MAS should be suspected in patients with SJIA who develop *persistent* fevers, a fall in the ESR and platelet count, particularly in a combination with coagulopathy and increasing ferritin levels
- Initial treatment is intravenous methylprednisolone pulse therapy (e.g. 30 mg/kg for three consecutive days) followed by 2–3 mg/kg/day in 2–4 divided doses. For patients who fail to respond to steroids we recommend cyclosporine.
- The impact of biologic therapy with IL-1 and IL-6 blocking agents on the course MAS is not fully known, but patients clearly remain at risk for MAS while on these therapies even if the underlying SJIA is well controlled. Furthermore, CRP levels may remain normal in SJIA patients developing MAS while treated with tocilizumab.

Research agenda

- The emerging evidence supporting a “cytokine storm” as central to the pathogenesis of MAS supports clinical studies of cytokine-directed therapy for these patients
- Despite more than 20 years of knowledge of MAS as a complication of SJIA, it remains unclear why these patients are particularly susceptible to this syndrome
- The introduction of biologic therapy has revolutionized the management of patients with SJIA; however, the impact of these therapies on the risk of MAS is largely unknown

Summary

MAS is an episode of overwhelming inflammation characterized by expansion and activation of T lymphocytes and hemophagocytic macrophages. It occurs most frequently in patients with SJIA and can occur at diagnosis and during periods of high disease activity. Clinically and pathologically it bears great similarity to HLH, and is often characterized as a form of secondary or reactive HLH. Central to its pathogenesis is the elaboration of a cytokine storm, with markedly increased levels of numerous proinflammatory cytokines including IL-1, IL-6, IL-18, TNF α and IFN γ . However, there is significant overlap between this cytokine profile and that seen in active SJIA without MAS, and there is limited data linking particular cytokines to the pathogenesis of MAS. There is however increasing evidence that IFN γ may play a central role in the pathogenesis of this condition. The mainstay of treatment of MAS remains corticosteroids, with other medications including cyclosporine used in those who fail to respond. The emerging pathologic role of the cytokine storm in MAS suggests that cytokine-directed therapy could be an attractive target for novel therapeutics in this condition.

Figure 1.

"Cytokine storm" and the development of MAS. MAS can develop in the setting of high SJIA disease activity, which is associated with increased cytokine levels including IL-1, IL-6, IL-18 and TNF α . MAS can also be triggered by viral infections, wherein pathogen-associated molecular patterns (PAMPs) are recognized by toll-like receptors (TLR) and trigger further secretion of inflammatory cytokines. Notably the proinflammatory environment including elevated IL-6 can enhance signaling through TLR. Infection also leads to activation and proliferation of CD8+ T cells and NK cells, including secretion of IFN γ . Increased IL-18 levels further drive IFN γ production by these activated lymphocytes. This surge in IFN γ leads to activation of macrophages that acquire a proinflammatory phenotype and generate high levels of chemokines and cytokines. These activated macrophages, along with CD8+ T cells, traffic to tissue including the bone marrow and liver and lead to the cytopenia, liver dysfunction and coagulopathy associated with MAS.

Table 1

Proposed criteria or features useful in the diagnosis of MAS.

HLH-2004 Criteria [23]		Ravelli Criteria [24]	MAS Study Group [25]	
OR	1	A molecular diagnosis consistent with HLH (i.e. reported mutations found in either PRF1 or MUNC13-4, STX11, STXBP2, Rab27a, SH2D1A or BIRC4)	Laboratory criteria: <ul style="list-style-type: none">Decreased platelet count ($262 \times 10^9/L$)Elevated aspartate aminotransferase (>59 U/L)Decreased white blood count ($4.0 \times 10^9/L$)Hypofibrinogenemia (2.5 g/L)	<ul style="list-style-type: none">Falling platelet count;HyperferritinemiaEvidence of macrophage hemophagocytosis in the bone marrowIncreased liver enzymesFalling leukocyte countPersistent continuous fever $38^{\circ}C$Falling ESRHypofibrinogenemiaHypertriglyceridemia
	2	At least 5 of the 8 diagnostic criteria for HLH listed below <ul style="list-style-type: none">Persistent feverSplenomegalyCytopenias (affecting 2 of 3 lineages in the peripheral blood):Hemoglobin <90 g/L, Platelets <100×10⁹/L, Neutrophils < 1.0×10⁹/LHypertriglyceremia (fasting triglycerides 3.0 mmol/L) and/or hypofibrinogenemia (1.5 g/L)Hemophagocytosis in bone marrow or spleen or lymph nodes, no evidence of malignancySerum ferritin 500 µg/LLow or absent NK cell activity (according to local laboratory reference)Increased serum sIL2Rα (according to local laboratory reference)	Clinical criteria: <ul style="list-style-type: none">Central nervous systemic dysfunction (irritability, disorientation, lethargy, headache, seizures, coma)Hemorrhages (purpura, easy bruising, mucosal bleeding)Hepatomegaly (3 cm below the costal margin)	
Criteria 1 OR 2 fulfilled		2 or more laboratory criteria OR any 2 or more clinical and/or laboratory criteria	Most frequent features identified by responding clinicians	