Macrophage activation syndrome in systemic juvenile idiopathic arthritis

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ABSTRACT

Macrophage activation syndrome (MAS) is a severe, potentially life-threatening complication of systemic juvenile idiopathic arthritis (s-JIA). An immunological feature is the excessive activation and proliferation of T lymphocytes and macrophages. Massive hypercytokinemia is strongly associated with its pathogenesis, particularly the overproduction of interleukin (IL)-1, IL-6 and IL-18; interferon (IFN)-γ; and tumor necrosis factor (TNF)-α. Furthermore, heterozygous mutations in causative genes for primary hemophagocytic lymphohistiocytosis and in vivo exposure to highly elevated levels of IL-6 and IL-18 might induce natural killer cell dysfunction and decrease their numbers, respectively. A proper diagnosis is important to begin appropriate therapeutic interventions and change an unfavorable prognosis. The 2016 ACR/EULAR classification criteria for MAS have a high diagnostic performance; however, the diagnostic sensitivity for onset is relatively low. Therefore, careful monitoring of laboratory values during the course of MAS is necessary to diagnose it early in s-JIA. Further studies on the diagnosis and monitoring of disease activity using serum cytokine profile and a targeted cytokine strategy are required.

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1. Introduction

Macrophage activation syndrome (MAS) is a severe, potentially life-threatening complication of rheumatic diseases, which is classified as a secondary form of hemophagocytic lymphohistiocytosis (HLH). MAS most often occurs in patients with systemic juvenile idiopathic arthritis (s-JIA) [1]. s-JIA is characterized by arthritis and other systemic features such as spiking fever, salmon-colored skin rash, hepatosplenomegaly, generalized lymphadenopathy, and serositis [2]. MAS is complicated in 7–14% of children with s-JIA [3,4]. Furthermore, subclinical or occult MAS may occur in another 30–40% of patients. MAS [5], which develops at the onset of s-JIA in >20% of patients, may occur spontaneously or may be triggered by an infection or change in medication [6–9].

MAS is characterized clinically by a non-remitting high fever, hepatosplenomegaly, cytopenia in all three blood cell lines, hepatic dysfunction, coagulopathy, central nervous system dysfunction, hyperferritinemia, and natural killer (NK) cell dysfunction [1]. A pathognomonic feature is the presence of hemophagocytic macrophages found in the bone marrow [1].

2. Pathogenesis of MAS

An immunological feature of MAS is the excessive activation and proliferation of T lymphocytes and macrophages. Massive hypercytokinemia is strongly associated with its pathogenesis. In particular, the overproduction of interleukin (IL)-1, IL-6, IL-12 and IL-18; interferon (IFN)-γ; and tumor necrosis factor (TNF)-α is closely associated with the pathogenesis of MAS complicating s-JIA (Figure 1) [10]. Inadequate production of IL-10, a regulatory cytokine that counter-regulates IFN-γ, also might be related to the development of MAS in s-JIA [11,12].

Furthermore, decreased NK-cell function, which is a characteristic feature of primary HLH (pHLH), has been observed in s-JIA and MAS [13–17]. Reduced NK cytotoxic activity is associated with heterozygous mutations in causative genes for pHLH in some patients [13,18]. Moreover, in vivo exposure to highly elevated IL-6 and IL-18 might induce NK-cell dysfunction and their decrease, respectively [19–23].

2.1. Genetic causes of impaired cytolytic function in s-JIA and MAS

pHLH is caused by mutations that affect the granule-mediated cytolytic pathway, including the PRF1,
UNC13D, STX11, and STXBP2 genes in cytotoxic T lymphocytes (CTLs) and NK cells [24–30]. The reduced cytolytic function of these cells leads to a persistent ongoing immune response and overproduction of inflammatory cytokines [25,26]. CTLs and NK cells perform their cytotoxic functions by releasing toxic granules containing perforin and granzymes. Once these cells are activated, cytotoxic granules are released into the immunological synapse between the antigen-presenting cells and the CTL and NK cells. Perforin forms a pore in the antigen-presenting cell, allowing granzyme B to enter the cytoplasm and trigger apoptosis.

Four steps are required for the release of cytotoxic granules: (1) sorting these proteins to the correct vesicle, (2) polarizing the vesicle to the immune synapse, (3) docking the vesicle with the plasma membrane, and (4) priming and fusing the vesicle (Figure 2) [31]. Munc 13-4 encoded by the UNC13D gene is necessary for priming [27]. Syntaxin 11, which is encoded by the STX11 gene, and syntaxin-binding protein 2, which is encoded by the STXBP2 gene, are essential for fusion [29,30].

pHLH is classified into five subtypes. pHHLH1-linked locus on chromosome 9q21.3 was reported in 1999 in four consanguineous families in Pakistan [32]; however, to date, no causative genes have been identified. pHHLH2 is caused by the mutations of the PRF1 gene-encoding perforin protein [28]. pHHLH3-5 is caused by mutations of the UNC13D, STX11, and STXBP2 genes [27,29,30].

Mutations of the RAB27A gene-encoding Rab27a protein, a MUNC13-4 effector molecule, have been linked to the development of Griscelli syndrome type 2 [33]. Mutations of the Lyst gene-encoding LYST protein, which is essential for lysosomal trafficking and protein sorting, have been identified to cause Chediak–Higashi syndrome [34]. Mutations of the AP3B1 gene-encoding AP3 beta chain protein, which is essential for the trafficking from Golgi to granules, can cause Hermansky–Pudlak syndrome [35]. These three disorders can be complicated by pHHLH with partial albinism.

Previous studies investigating mutations in the pHHLH genes, including PRF1, UNC13D, STX11, STXBP2, and RAB27A, in patients with s-JIA have revealed that more than one-third of patients had monoallelic mutations [13]. Furthermore, the frequency of rare protein-altering variants in UNC13D, STXBP2, and LYST were higher in patients with MAS (36%) as compared to those without (14%) [18]. These results indicate that genetic mutations affecting granule-mediated cytolytic function might increase susceptibility to MAS in some patients with s-JIA, although further studies, including those with Japanese patients, are necessary to define the link between these mutations and MAS predisposition.

2.2. Acquired NK-cell dysfunction and IL-6 and IL-18 overproduction in s-JIA

Recent investigations have revealed that the innate proinflammatory cytokines IL-1, IL-6, and IL-18

Figure 1. MAS pathophysiology. The immunological feature of macrophage activation syndrome (MAS) is the excessive activation and proliferation of T lymphocytes and macrophages. Overproduction of interleukin (IL)-6 and IL-18, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, namely, cytokine storm, is closely associated with pathogenesis. In contrast, inadequate production of IL-10, a regulatory cytokine to counter-regulate IFN-γ, might be related to the development of MAS. Heterozygous mutations in causative genes for primary hemophagocytic lymphohistiocytosis related to granule-mediated cytolytic pathway and in vivo exposure highly elevated IL-6 and IL-18 levels and induced natural killer (NK) cell dysfunction and decreased the number of cells.
play important roles in the pathogenesis of s-JIA [10]. NK-cell development, number, and function are modulated by IL-6 and IL-18 [19–23]. In an IL-6 transgenic mouse that developed MAS, elevated levels of IL-6 induced reductions in perforin and granzyme B expression in the NK cells without effects on degranulation that improved after treatment with IL-6 inhibition [20]. Furthermore, similar changes have been observed in NK cells from patients with s-JIA [20]. These findings indicate that IL-6 is involved in the amplification of inflammatory responses and suggest the role of chronic IL-6 overproduction in NK-cell dysfunction and MAS development.

The introduction of tocilizumab (TCZ), a humanized monoclonal antibody against the human IL-6 receptor, has brought about a paradigm shift in the treatment of s-JIA [36,37]. However, MAS still can develop in some patients with s-JIA during treatment with TCZ [38–41]. This finding indicates that blocking IL-6 alone cannot completely prevent the onset of MAS.

We previously have reported that serum IL-18 levels were extremely elevated in patients with active s-JIA, and these further increased in patients with MAS [42]. Increased serum IL-18 levels were specific to MAS with s-JIA because other forms of HLH are associated with lower serum IL-18 levels. Furthermore, we have identified two distinct groups of patients with s-JIA having specific clinical features on the basis of their IL-6 and IL-18 levels [43]. Patients in the IL-6–dominant group (IL-18/IL-6 > 1000) were more likely to develop MAS [44].

Moreover, recent studies have revealed a close association between IL-18 and the development of MAS [45]. Canna et al. have identified a new monogenic autoinflammatory disease associated with gain-of-function mutations of the NLRC4 gene, an inflammasome sensor that causes a disease characterized by early-onset, recurrent MAS [45]. Mutations of NLRC4 cause and trigger constitutive caspase-1 cleavage and increased production of IL-18 [45]. The most characteristic immunological finding was extremely high IL-18 levels equal to those in patients with MAS and s-JIA, providing evidence that NLRC4 is particularly important in regulating IL-18 production and in further supporting the role of IL-18 as a predisposing factor for MAS. These findings indicate that IL-18 is another mediator in s-JIA and that its overproduction may be closely related to the development of MAS.

IL-18 is an interferon-γ-inducing factor mainly produced by activated macrophages that enhances the proliferation and activity of T cells and NK cells. IL-18 is the most effective at regulating NK-cell activity. Previous studies have reported that NK-cell dysfunction and a decreased number of cells were found in s-JIA [16,21–23]. A previous report has shown that exposure to high concentrations of IL-18 can induce NK-cell death [21–23]. Furthermore, de Jager et al. have reported that impaired NK-cell function in s-JIA involves a defect in the IL-18 receptor β phosphorylation [22]. These findings indicate that IL-18 exposure may decrease the number of NK cells and also impair their functions.
Table 1. Relationship between cytokines and clinical manifestations of MAS.

| Cytokines | Clinical symptoms and laboratory findings |
|-----------|------------------------------------------|
| IFN-γ     | Fever, Cytopenia, Hemophagocytosis, Disseminated intravascular coagulation, Hypoalbuminemia |
| TNF-α     | Fever, Cachexia, Neurological symptoms, Cytopenia, Hypertriglyceridemia, Liver injury, Disseminated intravascular coagulation, Hypoalbuminemia, Hyperferritinemia |
| IL-1β      | Fever, Acute phase proteins production, Cytopenia, Hyperferritinemia |
| IL-6       | Fever, Acute phase proteins production, Anemia, Acute kidney Injury, NK cell dysfunction |
| IL-18      | Liver injury, NK cell dysfunction |

We previously have reported the case of an infant born to a mother with adult-onset Still’s disease (AOSD), presenting with transient impairment of NK-cell function associated with increased serum IL-18 levels, transmitting from the mother to the infant [46]. Furthermore, infants born to mothers with AOSD can develop MAS and show extremely elevated levels of IL-18 [47]. These findings indicate that the nonfunctional IL-18/NK-cell axis induced by high serum IL-18 levels may be closely associated with MAS development in s-JIA.

We have demonstrated that NK-cell activation by IL-18 is impaired in patients with active s-JIA [23]. Furthermore, NK-cell activation is recovered by exogenous IL-18 stimulation in patients with s-JIA in whom serum IL-18 levels decreased after treatment began [23]. Furthermore, NK-cell activation by IL-18 stimulation is negatively correlated with serum IL-18 levels [23]. On the basis of these findings, exposure to high IL-18 levels may induce NK-cell exhaustion and secondary transient NK-cell dysfunction.

2.3. IFN-γ as the key effector cytokine in MAS

Elevated serum IFN-γ and IFN-γ-induced chemokine levels are significantly correlated with disease activity in pHLH, infection-associated secondary HLH, and MAS [48–52]. Furthermore, the clinical symptoms of pHLH and MAS have been shown to be inhibited by anti-IFN-γ antibody treatments [53,54]. These findings indicate that IFN-γ plays a pivotal role in MAS as well as in HLH.

IL-10 is an immunosuppressive cytokine that counter-regulates IFN-γ. In patients with s-JIA, plasma IL-10 levels are lower as compared with proinflammatory mediators [12]. Furthermore, B lymphocytes show decreased IL-10 production ex vivo and after in vitro stimulation [12]. Furthermore, IL-10 polymorphisms are associated with decreased IL-10 activity [11]. In a mouse model of MAS with repeated TLR9 stimulation, IL-10 receptor blocking increased disease severity [55]. These findings indicate that defective IL-10 production and overproduction of IFN-γ contribute to the pathogenesis of MAS.

3. Clinical features of MAS

MAS is clinically characterized by sustained fever, hepatomegaly with liver dysfunction, splenomegaly, lymphadenopathy, and variable neurologic symptoms. Once MAS develops, fever patterns change from spiking to continuous, and the fever is usually high grade (>38.5°C), prolonged, and unresponsive to anti-infection treatment. Hemorrhagic symptoms and respiratory distress are sometimes observed. The characteristic laboratory findings of MAS are leukocytopenia, thrombocytopenia, coagulation disorders including a decrease in plasma fibrinogen levels and increase in FDP and D-dimer levels, increased liver enzymes, elevated triglycerides, abnormally high ferritin levels, and NK-cell dysfunction. Hemophagocytosis found in bone marrow puncture is a hallmark of MAS, although hemophagocytes are not essential for the diagnosis of HLH/MAS and are found in patients with juvenile arthritis without overt MAS.

These abnormal findings are closely associated with the overproduction of inflammatory cytokines and can be explained by the known effects of key cytokines such as IFN-γ, TNF; and IL-1β, IL-6, and IL-18. Table 1 shows the relationship between these and the characteristic clinical features of MAS.

Cytopenia results from both hemophagocytosis in the bone marrow and depression of hematopoiesis by IFN-γ, IL-1β, and TNF-α. Coagulopathy is associated with fibrin deficiency caused by liver dysfunction, and DIC develops as a result of IFN-γ and TNF overproduction. Liver dysfunction is associated with IL-10, which contributes to the development of cholestasis and causes liver damage. Hypertriglyceridemia is the consequence of the inhibition of lipoprotein lipase by TNF-α. Massive elevation of serum ferritin levels may be caused by IL-1β and TNF elevation and also reflects the activation of M2c-type macrophages. Elevation of urinary β2 microglobulin is induced by IFN-γ.

Monitoring the serum cytokine profile is useful for assessing the disease activity of s-JIA and MAS. However, it can be difficult to monitor cytokine
levels in general hospitals. Thus, monitoring serum cytokine-inducible protein levels is useful to assess the disease activity of MAS (Table 2).

4. Diagnosis of MAS

4.1. Classification criteria

MAS is diagnosed using the 2016 ACR/EULAR classification criteria for MAS complicating s-JIA (Table 3) [56]. In this criteria, a febrile patient with known or suspected s-JIA is classified as having MAS if the patient has ferritin > 684 ng/L and at least two of the following laboratory abnormalities: platelets > 181 x 10^9/mL, AST > 48 U/L, triglycerides > 1.76 mmol/L (156 mg/dL), and fibrinogen > 3.6 g/L (360 mg/mL).

We previously have validated these classification criteria in Japanese children with MAS [57]. These criteria had a high diagnostic performance; however, the diagnostic sensitivity for MAS onset was relatively low. Therefore, careful monitoring of laboratory values during the course of MAS, including platelet counts, plasma fibrinogen, FDP and FDP D-dimer levels, serum AST, LDH, and triglycerides, is necessary for the early diagnosis of MAS in s-JIA.

4.2. Differentiation from pHLH

It is important to differentiate MAS from pHLH, because treatment strategies and outcomes are significantly different. The MAS/HLH (MH) score uses the following items to make the differential diagnosis: age at onset, neutrophil count, fibrinogen, platelet count, and hemoglobin (Table 4) [58]. According to statistical weight, each item is assigned a score. The cut-off value of the MH score best in discriminating between MAS and pHLH is 60 (score range, 0–123 points), with a sensitivity of 91% and a specificity of 93% [58].

4.3. Treatment with biologics

The clinical use of IL-1- and IL-6-blocking biologic agents have striking and long-lasting effects on s-JIA, even in patients receiving other therapies [36,37,59–61]. However, despite the efficacy of these biologics, patients with s-JIA are still at risk for developing MAS [38–41]. The rate of MAS complications in patients being treated with canakinumab and TCZ were 2.8 and 1.8 per 100 patients, respectively, which is similar to the rates reported for patients not being treated with biologics [40]. Furthermore, these biologics can modify the clinical and laboratory features of MAS associated with S-JIA [40,41]. MAS classification criteria were less likely to classify patients diagnosed with MAS while being treated with canakinumab (77.1%) and TCZ (54.3%) because of an absence of fever or insufficient ferritin elevation, compared with the patients not receiving these biologics [40]. These findings indicate that using the 2016 ACR/EULAR criteria for these patients may not be appropriate. Care must be taken to not underdiagnose MAS using these criteria.

4.4. Biomarkers for diagnosis

A proper diagnosis of MAS is important for beginning an appropriate therapeutic intervention and for changing an unfavorable prognosis. However, it often is difficult to distinguish MAS from s-JIA flares, especially in its early stages. Therefore, identifying a promising indicator of disease activity as well as a useful marker to predict the transition to MAS from acute-phase s-JIA is desired.

Table 2. Cytokine inducible proteins.

| Cytokine inducible proteins | Related cytokines | Pathological conditions indicated by the findings |
|-----------------------------|-------------------|--------------------------------------------------|
| Urinary Jx2 microglobulin   | IFN-γ             | Overexpression of HLA class I molecule           |
| Soluble IL-2 receptor       | IFN-γ             | T cell activation                                |
| AST and LDH                 | TNF-α             | Mitochondria injury: apoptosis and necrosis      |
| Triglyceride                | TNF-α             | Suppression of lipoprotein lipase                |
| FDP D-dimer                 | IFN-γ and TNF-α   | Coagulopathy, Endothelial cell injury            |
| Ferritin                    | IFN-γ and TNF-α   | Iron metabolism abnormality                      |
|                             |                   | Reticuloendothelial abnormality                  |
|                             |                   | M2c macrophage activation                       |

IL-18 levels in the serum are significantly increased in active s-JIA [42,62,63]. Therefore, IL-18 increasingly has been recognized as a biomarker for s-JIA diagnosis and its disease activity [63]. The levels of IL-18 increase more in patients with MAS [42,62,63]. We have found that patients with s-JIA who have extremely high serum IL-18 levels (>47,750 pg/mL) were more likely to develop MAS [44]. However, no significant differences existed in serum IL-18 levels in s-JIA patients with MAS whether measured before or during MAS. These findings indicate that IL-18 is causatively involved in the development of MAS, and serum IL-18 levels are useful for predicting MAS in s-JIA.

IL-18 is a well-known IFN-γ-inducing cytokine. Recently, high levels of free IL-18 (i.e., IL-18 not bound to IL-18-binding protein) have been found to indicate the risk for developing MAS [62]. Increased free IL-18 and induction of IFN-γ might be closely
related to the development of MAS in s-JIA. IFN-γ plays a central role in the pathogenesis of HLH and MAS. Serum levels of IFN-γ and IFN-γ-induced chemokines are markedly elevated in pHLH, in infection-related secondary HLH, and in MAS [48–52]. These levels are significantly correlated with disease activity [51,52]. TNF-α is another key cytokine in the pathogenesis of HLH and MAS [10]. Serum levels of sTNFR-II and sTNFR-II/I ratio reflecting TNF-α production are significantly elevated in patients with MAS [64]. These levels also are significantly correlated with disease activity.

Serum cytokine levels with a radar chart in patients with MAS in different background rheumatic diseases and Epstein–Barr virus-associated HLH are shown in Figure 3. The pattern of the cytokine profile is characteristic in each background. The massive increase of serum IL-18 levels is characteristic of s-JIA. Furthermore, we have compared the accuracy of serum biomarkers, including IFN-γ-related molecules and TNF-α-related molecules, for the diagnosis of MAS [65]. Receiver operating characteristic (ROC) curve analysis has revealed that serum neopterin levels had the highest area under the ROC curve value, and neopterin was the most accurate biomarker for the diagnosis of MAS [65]. From these findings, a combination of serum IL-18 and neopterin levels might be useful to diagnose MAS and to differentiate MAS from s-JIA flares or from other secondary HLH.

5. Treatment of MAS

The intravenous infusion of a high-dose glucocorticoid is the basic treatment for MAS. Methylprednisolone pulse therapy (30 mg/kg/time, 3 days as one course, one to two courses) is widely used as a first-line treatment. The intravenous infusion of dexamethasone palmitate (2.5–5.0 mg, every 12 h; half a dose for infants) also is effective. In addition, cyclosporine to suppress T cell activation and restrain mitochondrial permeability conversion by TNF-α is used widely (continuous intravenous infusion, 1–1.5 mg/kg/day) [66,67]. However, patients treated with corticosteroids and cyclosporine are at high risk for developing posterior reversible encephalopathy syndrome [68,69]; therefore, tightly

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Table 3. 2016 ACR/EULAR classification criteria for MAS complicating s-JIA.

| Parameter                                      | Criteria                      |
|-----------------------------------------------|-------------------------------|
| Ferritin (ng/L)                               | > 684                          |
| Platelets (×10^9/L)                           | > 181                          |
| AST (U/L)                                     | > 48                           |
| Triglycerides (mmol/L)                        | > 1.76                         |
| Fibrinogen (g/L)                              | > 3.6                          |

Table 4. The MAS/HLH score.

| Item       | Points for scoring |
|------------|--------------------|
| Age at onset (year) | 0 (>1.6), 37 (≤1.6) |
| Neutrophil count (×10^9/L) | 1 (>1.6), 37 (≤1.6) |
| Fibrinogen (mg/dL) | 2 (>1.6), 37 (≤1.6) |
| Splenomegaly | 3 (>1.6), 37 (≤1.6) |
| Platelet count (×10^9/L) | 4 (>1.6), 37 (≤1.6) |
| Hemoglobin (g/dL) | 5 (>1.6), 37 (≤1.6) |

MH score ≥ 60 is best in discriminating MAS and pHLH.

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Figure 3. (A–J) Cytokine profiles with radar charts in patients with MAS in different background rheumatic diseases and Epstein–Barr virus associated HLH. Representative profiles of serum cytokines including, neopterin, IL-6, IL-18, and sTNF-RII are shown for in patients with MAS in different background rheumatic diseases and Epstein–Barr virus associated HLH. s-JIA: systemic juvenile idiopathic arthritis, KD: Kawasaki disease, SLE: systemic lupus erythematosus, JDM: juvenile dermatomyositis, HC: healthy controls, MAS: macrophage activation syndrome, EBV-HLH: Epstein–Barr virus associated hemophagocytic lymphohistiocytosis.
controlling patient blood pressure is essential. In addition, anticoagulant therapy is needed. Apheresis therapy, including plasma exchange and leukocytapheresis, has been performed in refractory cases to corticosteroids and cyclosporine [70–72].

Recently, rapid and dramatic benefits have been seen with the use of emaparumab, an anti-IFNγ monoclonal antibody, in patients with pHLH and MAS [53,54]. High-dose anakinra, an IL-1 receptor antagonist, has also been reported to be effective [73]. Additional therapeutic protocols are needed.

6. Conclusions

MAS is a fatal complication of s-JIA. Its proper and timely diagnosis is essential to initiate appropriate therapeutic interventions and to improve unfavorable outcomes. The 2016 ACR/EULAR classification criteria for MAS complications in s-JIA have a high diagnostic performance; however, their diagnostic sensitivity for MAS onset is relatively low. Therefore, careful monitoring of laboratory values during the course of MAS is necessary for early diagnosis. Remarkable progress in the use of biologics, particularly against IL-1 and IFN-γ, has been seen in treatment. Additional effective and safe therapeutic strategies for MAS are desired.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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