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REVIEW

Macrophage activation syndrome as part of systemic juvenile idiopathic arthritis: diagnosis, genetics, pathophysiology and treatment

A Ravelli[1], AA Grom[2], EM Behrens[3] and RQ Cron[4]

Macrophage activation syndrome (MAS) is a severe, frequently fatal complication of systemic juvenile idiopathic arthritis (sJIA) with features of hemophagocytosis leading to coagulopathy, pancytopenia, and liver and central nervous system dysfunction. MAS is overt in 10% of children with sJIA but occurs subclinically in another 30–40%. It is difficult to distinguish sJIA disease flare from MAS. Development of criteria for establishing MAS as part of sJIA are under way and will hopefully prove sensitive and specific. Mutations in cytolytic pathway genes are increasingly being recognized in children who develop MAS as part of sJIA. Identification of these mutations may someday assist in MAS diagnosis. Defects in cytolytic genes have provided murine models of MAS to study pathophysiology and treatment. Recently, the first mouse model of MAS not requiring infection but rather dependent on repeated stimulation through Toll-like receptors was reported. This provides a model of MAS that may more accurately reflect MAS pathology in the setting of autoinflammation or autoimmunity. This model confirms the importance of a balance between pro- and anti-inflammatory cytokines. There has been remarkable progress in the use of anti-pro-inflammatory cytokine therapy, particularly against interleukin-1, in the treatment of secondary forms of MAS, such as in sJIA.

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INTRODUCTION

Macrophage activation syndrome (MAS) is a serious, potentially fatal complication of rheumatic diseases, which is seen most frequently in systemic juvenile idiopathic arthritis (sJIA) and in its adult equivalent, adult-onset Still disease,[1–3] although it is increasingly reported in other pediatric inflammatory disorders, namely juvenile systemic lupus erythematosus[4] and Kawasaki disease.[5–7] In recent years, this condition has also been observed in periodic fever syndromes.[8,9] MAS may occur spontaneously, as a complication of active underlying disease, or may be triggered by an infection, a change in drug therapy or a toxic effect of a medication, including biologics.[10] Clinically, patients with MAS present with non-remitting high fever, pancytopenia, hepatosplenomegaly, hepatic dysfunction, encephalopathy, coagulation abnormalities and sharply increased levels of ferritin (Table 1). The pathognomonic feature of the syndrome is seen on bone marrow examination, which reveals numerous morphologically benign macrophages exhibiting hemophagocytic activity (Figure 1). Such cells may also be found in lymph nodes and spleen, but they may infiltrate almost any organ in the body and may account for many of the systemic features of the syndrome. As MAS bears a close resemblance to a group of histiocytic disorders collectively known as hemophagocytic lymphohistiocytosis (HLH), it is currently classified among the secondary, or acquired, forms of HLH.[11,12]

DIAGNOSTIC CRITERIA FOR MAS IN sJIA

MAS is a severe condition that can pursue a rapidly fatal course. Prompt recognition of its clinical and laboratory features and immediate therapeutic intervention are, therefore, imperative. However, diagnosis of MAS can be difficult and hard to distinguish from sepsis-like syndromes, although it may also be associated with sepsis, especially in cases of intraphagocytic pathogen infections. In addition, the recognition of subclinical forms of MAS in sJIA[13,14] underscores the importance of establishing criteria sensitive enough to distinguish MAS from routine disease flare. Differentiation of MAS from these conditions is critical to select the appropriate therapeutic approach. The difficulties in making the diagnosis and recent therapeutic advances (see below) emphasize the need of diagnostic tools and well-established diagnostic guidelines. Diagnostic criteria will also be important for research purposes and use in literature reports.

The recognition that MAS is clinically similar to HLH has led some to propose the use of the HLH diagnostic guidelines for establishing a diagnosis of MAS. However, HLH criteria developed primarily for homozygous genetic disorders leading to hemophagocytosis[15] are not necessarily of timely use in defining/identifying MAS in the setting of sJIA. The main shortcoming of HLH criteria in MAS is due to the fact that certain criteria may not apply to patients with sJIA. Owing to the prominent inflammatory expression of the latter disease, the occurrence of a relative

[1]Department of Pediatrics, Università degli Studi di Genova, Genova, Italy; [2]Department of Pediatrics, University of Cincinnati, Cincinnati, OH, USA; [3]Department of Pediatrics, University of Pennsylvania, Philadelphia, PA, USA and [4]Department of Pediatrics, Children's Hospital of Alabama, University of Alabama, Birmingham, AL, USA. Correspondence: Professor RQ Cron, Children's Hospital of Alabama, University of Alabama, 1600 7th Avenue South, CPP suite #210, Birmingham, AL 35233-1711, USA.
E-mail: rcron@peds.uab.edu
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Table 1. Main clinical, laboratory and pathological features of macrophage activation syndrome

Clinical features
Non-remitting high fever
Hepatomegaly
Splenomegaly
Lymphadenopathy
Hemorrhages
Central nervous system dysfunction
Laboratory features
Cytopenia
Abnormal liver function tests
Coagulopathy
Decreased erythrocyte sedimentation rate
Hypertriglyceridemia
Hyponatremia
Hypoalbuminemia
Hyperferritinemia
Elevated sCD25 and sCD163
Histopathological features
Macrophage hemophagocytosis in the bone marrow
Increased CD163 staining of the bone marrow

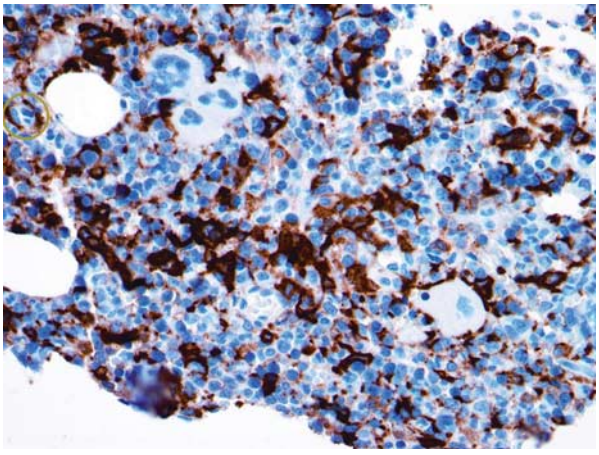

Figure 1. CD163 staining of histiocytes/macrophages in the bone marrow of a patient with MAS. A bone marrow biopsy from a child with MAS was stained with anti-CD163 (haptoglobin receptor) antibody and analyzed under high power magnification. Numerous activated histiocytes/macrophages are present revealing hemophagocytic activity. A particularly phagoctyic histiocyte is circled in green in the upper left hand corner of the figure.

decrease in white blood cell count, platelets or fibrinogen, rather than the absolute decrease required by the HLH criteria, may be more relevant in making an early diagnosis. Indeed, sJIA patients often have increased white blood cell and platelet counts as well as increased serum levels of fibrinogen as part of the inflammatory response seen in this disease. Therefore, when these patients develop MAS, they may only reach the degree of cytopenia and hypofibrinogenemia seen in HLH at the late stages of the syndrome, when their management becomes challenging. Another problem is that the minimum threshold level for hyperferritinemia required for the diagnosis of HLH ($500\,\mu g\,l^{-1}$) is not suitable to detect MAS in children with sJIA. It is well known that many patients with active sJIA, in the absence of MAS, have ferritin levels above that threshold.[16] In the acute phase of MAS, ferritin levels generally peak to more than $5000\,\mu g\,l^{-1}$. Thus, use of a $500\text{-}\mu g\,l^{-1}$ threshold may not help to discriminate MAS from a flare of sJIA. Other HLH criteria that are not readily applicable to MAS are the demonstration of low or absent natural killer cell activity or soluble interleukin-2 (IL-2) receptor alpha chain (CD25) above normal limits for age, as these tests are not routinely performed in pediatric rheumatology settings. We recently found that the diagnostic guidelines for HLH were highly specific in patients with sJIA-associated MAS, but lacked sensitivity (manuscript in preparation).

Previously, preliminary diagnostic guidelines for MAS complicating sJIA were published.[17] Laboratory criteria include decreased platelet count ($\leqslant 262 \times 10^9\,l^{-1}$), elevated levels of aspartate aminotransferase ($>59\,U\,l^{-1}$), decreased white blood cell count ($\leqslant 4.0 \times 10^9\,l^{-1}$) and hypofibrinogenemia ($\leqslant 2.5\,g\,l^{-1}$). Clinical criteria include hepatomegaly, hemorrhagic manifestations and central nervous system dysfunction. The diagnosis of MAS requires the presence of any two or more laboratory criteria, or any two or three or more clinical and laboratory criteria. The demonstration of macrophage hemophagocytosis in the bone marrow aspirate is required only in doubtful cases. As noted in patients with HLH[18] and in several instances of MAS,[17] the bone marrow aspirate does not always show hemophagocytosis, and, furthermore, hemophagocytosis is not always demonstrable in the initial stages of the disease. Repeat bone marrow aspirate over time may eventually demonstrate hemophagocytosis. These criteria have the advantage of being data-driven and not merely based on expert consensus. However, the study underlying their development has several limitations, including the lack of several laboratory measurements in a number of patients and insufficient data for some of the laboratory parameters evaluated. Moreover, the criteria have yet to be validated.

In recent years, an international collaborative effort was started, which is aimed at developing a new and robust set of diagnostic criteria for MAS complicating sJIA, based on the combination of expert consensus and the analysis of real patient data. The first step of the project, with a specific aim to identify candidate items using international consensus formation through the Delphi survey technique, has just been accomplished.[19] A total of 505 pediatric rheumatologists belonging to three large networks were sent a questionnaire that listed 28 clinical, laboratory and histopathological features that were thought most likely to be helpful and relevant in the diagnosis of MAS complicating sJIA indentified through literature review. Respondents were asked to select the 10 features that they felt were most important and to rank-order the selected features by assigning 10 to the most important one, and end with 1 as the least important. The following nine features were selected by more than 50% of the 232 respondents and were given most frequently the highest ranks: falling platelet count, hyperferritinemia, evidence of macrophage hemophagocytosis in the bone marrow, increased liver enzymes, falling leukocyte count, persistent continuous fever $\geqslant 38\,^{\circ}C$, falling ESR, hypofibrinogenemia and hypertriglyceridemia. These features may be the best candidates to be part of the final set of diagnostic criteria for the syndrome.

Questionnaire respondents were intentionally not asked to indicate the threshold level for each laboratory test that they felt was optimal for early identification of MAS. This objective is intended to be pursued in the subsequent phase of the project, which is under way and is aimed at collecting real data from patients with sJIA-associated MAS and patients with conditions that may be confused with MAS, including sJIA flare without MAS and febrile systemic infection controls. This process is also meant to enable a data-driven assessment of the relative sensitivity and specificity of clinical, laboratory and histopathological features in discriminating MAS from the confusable conditions. Notably, the data collection is structured is such a way that it may establish whether laboratory criteria for the syndrome are better assessed in terms of absolute threshold values, or percentage change over the

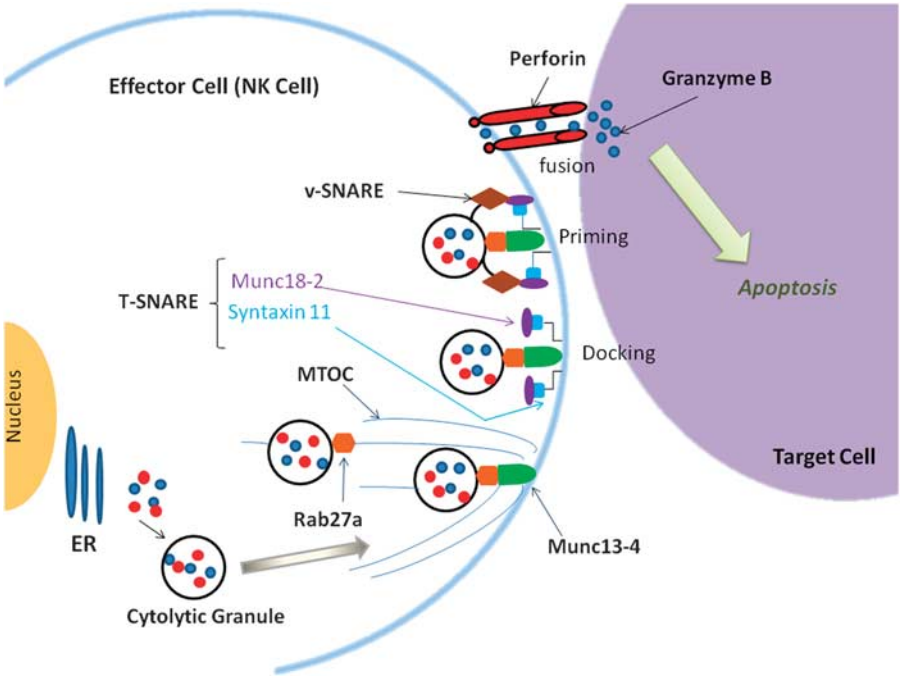

Figure 2. Cytolytic pathway proteins mutated in MAS. A diagram of the immunologic synapse between a cytolytic (to the left) lymphocyte and an APC (to the right) is portrayed. Proteins involved in the cytolytic pathway that can be found mutated in MAS and HLH include Rab27a, Munc13-4, Syntaxin 11, Munc18-2 and perforin.

preceding days or both. The ultimate goal of the project is to develop a core set of criteria for the diagnosis MAS in sJIA that is timely, feasible and both highly sensitive and specific.

GENETIC FACTORS FOR MAS IN sJIA

The etiology of MAS remains elusive. However, MAS bears strong resemblance to a group of histiocytic disorders collectively known as HLH.[11,20] HLH is a term that describes a spectrum of disease processes characterized by the accumulations of well-differentiated mononuclear cells with a macrophage phenotype exhibiting hemophagocytic activity. HLH is further subdivided into primary or familial (FHLH) and secondary or reactive (ReHLH).[21,22] FHLH is a constellation of rare autosomal recessive immune disorders resulting from homozygous deficiency in cytolytic pathway proteins.[15] Its clinical symptoms usually become evident within the first 2 months of life. ReHLH tends to occur in older children and more often is associated with an identifiable infectious episode or autoimmune condition.[21] However, distinctions between primary and secondary HLH are becoming increasingly blurred as new genetic causes are identified, some of which are associated with less severe and somewhat distinct clinical presentations occurring later in life.[23] Some of these may present later in life due to heterozygous or compound heterozygous mutations in cytolytic pathway genes that confer a partial dominant negative effect on cytolysis.

The pathological mechanisms of HLH are not fully understood. In primary HLH, the uncontrolled expansion of T cells and macrophages has been linked to decreased NK cell and cytotoxic T-cell function[21,24] due to mutations in various genes whose products are involved in the cytolytic pathway.[21,25–27] The cytotoxic activity of these cells is mediated by the release of specialized cytotoxic granules that contain several classes of proteins expressed only in cytotoxic cells, including perforin and granzymes. Once cytotoxic cells are activated, these granules are delivered to the surface of the cells and the contents are released at the immunologic synapse with the target cell (Figure 2). Perforin aids in delivering of the contents of the granules into the cytoplasm of the target cell, while granzymes trigger apoptosis once in the cytoplasm of the target cell. In 15–40% of patients with FHLH, cytolytic dysfunction is due to mutations in the gene encoding perforin.[26] Mutations in another gene, *MUNC13-4*, have been implicated in the development of hemophagocytosis in about 10–30% of patients with inherited HLH.[25] The protein encoded by the *MUNC13-4* gene is important for docking and fusion of the cytotoxic granules with the cytoplasmic membrane (Figure 2). Although the cytolytic cells of the patients with FHLH caused by *MUNC13-4* mutations produce sufficient amounts of perforin, the poor ability to deliver the content of the cytolytic granules to the immunologic synapse with the target cell leads to profoundly decreased cytolytic activity. More recently, mutations in two other genes encoding proteins that facilitate granule fusion in intracellular trafficking events 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*, also known as *MUNC18-2*)[27] (Figure 2).

Defects in the granule-dependent cytotoxic functions of lymphocytes have also been implicated in two other genetic diseases associated with the hemophagocytic syndrome. Thus, mutations in the gene encoding Rab27a, one of the MUNC13-4 effector molecules (Figure 2), have been linked to the development of Griscelli syndrome type 2.[29] Mutations in the *Lyst* gene have been identified as a cause of Chediak–Higashi syndrome. Both disorders can be complicated by the development of HLH.[30]

HLH following exposure to Epstein-Barr virus (EBV) and occasionally other viruses, termed fulminant infectious mononucleosis, is the most frequent life-threatening complication of X-linked lymphoproliferative syndrome (XLP). XLP1 is caused by hemizygous mutations in the *SH2D1A* gene encoding SAP (SLAM-associated protein), which leads to abnormal NK cell responses and invariant NKT cell deficiency.[31] XLP2 is caused by mutations in *BIRC4*, which encodes XIAP, and has been described as an X-linked

form of FHLH.[32] Recent observations suggest that lymphocytes from patients with both types of XLP demonstrate decreased activation-induced apoptosis that contributes to the uncontrolled lymphoproliferation. Taken all together, these genetic disorders still account for less than half of the diagnosed cases of HLH in children, including many familial cases still awaiting molecular definition.[21]

The presence of the defects in the granule-dependent cytotoxic activity of lymphocytes in several diseases associated with hemophagocytic syndromes highlights the importance of this function in restraining some inflammatory responses.[33-36] The exact mechanisms that link deficient NK cell and cytotoxic T-cell functions with expansion of activated macrophages are not clear. One possible explanation is related to the fact that poor cytolytic activity seen in HLH patients may lead to diminished ability to control some infections. More specifically, NK cells and cytotoxic T lymphocytes fail to kill infected cells and, thus, to remove the antigen-presenting cell source of antigenic stimulation. Such persistent antigen stimulation leads, in turn, to persistent antigen-driven activation and proliferation of T cells associated with escalating production of cytokines that stimulate macrophages. It has also been shown that abnormal cytotoxic cells may fail to provide appropriate apoptotic signals for removal of activated macrophages and T cells during the contraction stage of some immune responses leading to persistent expansion of T cells and macrophages secreting proinflammatory cytokines.[34,35,37] As a result of continuous stimulation with proinflammatory cytokines (most notably, interferon gamma (IFNγ)), macrophages will become hemophagocytic.[33,38]

Although familial cases of MAS in sJIA have not been reported, as in FHLH, sJIA/MAS patients may also have functional defects in the exosome degranulation pathway.[39-41] Furthermore, these functional abnormalities are associated with SNPs in the FHLH-associated genes, including *PRF1*[40] and *MUNC13-4*.[42,43] In addition, sJIA/MAS patients with bi-allelic mutations in the *MUNC13-4* gene reported in FHLH have been described,[43] suggesting that there is likely a genetic component that overlaps between MAS and FHLH. Interestingly, mutations *in MUNC13-4* also affect the degranulation of neutrophils[44] and platelets.[45] Not surprisingly, FHLH patients with bi-allelic mutations in *MUNC13-4* often have atypical clinical features with some overlap with sJIA. Preliminary sequencing of the FHLH-associated genes in a small cohort of sJIA/MAS patients identified new sequence variants, as well as known heterozygous mutations, in several genes, most notably *MUNC13-4*, *STXBP2* and *BIRC4* (Grom, Cron unpublished observations). The exact prevalence of such sequence variants and their impact on the cytolytic function of CD8 T or NK cells and ultimately on the clinical phenotype still need to be clarified. One possibility is that these heterozygous mutations still affect the cytolytic function through a gene dosage effect. Alternatively, the defects may be in multiple genes. Consistent with this idea, FHLH in patients who are heterozygous for the A91 V perforin variation is often associated with other heterozygous genetic defects.[46]

Another recent study from Japan identified *IRF5* (interferon regulatory factor 5) gene polymorphisms as risk factors for MAS development in patients with sJIA.[47] Given the presumed role that IFNγ has in the pathophysiology of MAS (see further), this observation is very intriguing and needs to be confirmed in other ethnic groups. Thus, the genetics of MAS in children with sJIA likely shares many of the same defects as in infants who present with FHLH.

PROPOSED PATHOPHYSIOLOGY OF MAS IN CHILDREN WITH sJIA

Because of the rarity of MAS and the difficulty in performing mechanistic human immunology studies, much of our knowledge regarding the immunologic pathoetiology of MAS has relied on animal studies. Initially, animal models of primary HLH were used to dissect the mechanisms behind this related disease. Jordan *et al.*[33] described the first such model, a mouse deficient in the gene for perforin that developed a primary HLH syndrome upon infection with lymphochoriomeningitic virus. Depletion or neutralization of various cytokines and cellular populations revealed a critical role for both IFNγ and CD8 T cells in the development of disease. These results lead to the proposition of a model whereby defects in cytotoxic granule function prevent the ability of immune cells to clear infected, activated antigen-presenting cells. The continued presence of these antigen-presenting cells results in the repeated, uninterrupted stimulation of CD8 T cells and in turn, the production of IFNγ. The accumulation of large amounts of IFNγ in this process leads to cytokine toxicity, and the various elements of the disease (Figure 3).

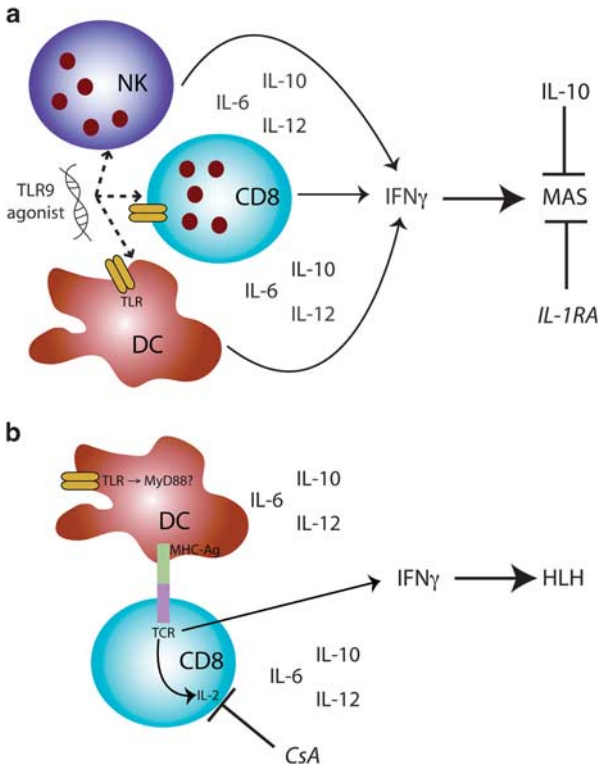

Figure 3. Proposed pathophysiology contributing to MAS/HLH and associated cytokine storm. (**a**) MAS and (**b**) HLH result from the inability of a CD8 T cell to lyse an APC due to cytolytic pathway defects resulting in sustained immune cell activation and a cytokine storm of pro-inflammatory cytokines, including IFNγ. Blockade of IL-1 (with IL-1RA, anakinra) can help control the cytokine storm, and IL-10 expression can help prevent hemophagocytosis in a model of MAS. CsA can also help prevent the cytokine storm in models of MAS/HLH. CD8, CD8 T cells; DC, dendritic cell; MHC-Ag, major histocompatibility complex coupled to antigen; NK, natural killer cell; TCR, T-cell receptor; TLR, Toll-like receptor.

These results, and the predictions made by this model, have subsequently been confirmed in other mouse models that are defective in many of the other human genes associated with primary HLH. Rab27a is important in docking the perforin laden vesicle at the cell membrane for release.[48] Munc13-4 is likely important in activating Syntaxin 11 that in turn is important for vesicle fusion.[25,28] Absence of any these molecules prevents the release of perforin and therefore destruction of target antigen-presenting cells (Figure 2). Accordingly, mice deficient in the genes encoding Munc13-4[49] and Rab27a[50,51] also develop HLH upon infection with lymphochoriomeningitic virus in an IFNγ-dependent manner.

In many instances of sJIA/MAS, no such defects in cytotoxic cell function have been identified, or have only variable penetrance. This has lead to a search for alternate mechanisms to explain the origin of the syndrome in this context as the above described model does not account for these situations. Recently, a new murine model of MAS induced by repeated stimulation of Toll-like receptor (TLR) 9 may provide some insight.[52] The rationale for this model involves multiple observations. Gene signatures consistent with chronic TLR/IL-1β signaling are present in sJIA patients.[53] Lupus, another rheumatic condition associated with MAS,[4] has also long been associated with hyperactive TLR9 function.[54] EBV, perhaps the most common infectious trigger of secondary HLH, is a DNA virus that triggers TLR9.[55] Furthermore, as opposed to patients with EBV-mononucleosis, patients with EBV-HLH have very high titers of viral particles in the blood,[56] meaning that there is an excess of TLR9 ligand present in these patients. As described above, polymorphisms of the TLR9 signaling molecule IRF5 that result in the hyperactivation have been strongly associated with sJIA-related MAS.[47] Taken together, these observations suggest that situations of repeated activation of TLR9 will replicate the environment that allows MAS to develop in the susceptible genetically predisposed host.

Indeed, mice given repeated TLR9 stimulation develop many of the features of MAS. Furthermore, the disease in this model is also IFNγ dependent, drawing a connection back to primary HLH[52] (Figure 3). Unlike primary HLH, this IFNγ is arising from different sources than CD8 T cells, including dendritic cells and NK cells. These results suggest that pathogenic IFNγ can arise from multiple initial immunologic insults, and, that while the end stage clinical syndrome may be similar, the origin of the hypercytokinemia may be different in different disease settings. Another observation made from these studies was the important regulatory role of IL-10 in controlling disease. Mice given repeated TLR9 stimulation concordant with blockade of the IL-10 receptor developed much more fulminant disease. This is consistent with human data where gene signatures of IL-10 responses are prominent in patients with HLH and sJIA/MAS, suggesting the importance of IL-10 in the immune response.[57] Intriguingly, polymorphisms of IL-10 associated with decreased function of IL-10 are associated with sJIA.[58,59] It is possible that the combined immunologic insult of hyperactive TLR/IL-1β signaling in combination with decreased IL-10 function may result in a predisposition to MAS. It will be of interest to explore the combined haplotype of *IRF5* and *IL-10* gene polymorphisms to see if this provides increased power to identify at risk patients. It is also possible that IL-10 function serves to explain the phenomenon of occult or subclinical MAS, in that these patients may have higher IL-10 function than those developing fulminant MAS. This increase in IL-10 function would be able to maintain their state of relative compensation (Figure 3). Interestingly, some of these ideas may apply to primary HLH as well, as a recent report shows the critical need for the TLR signaling adaptor MyD88 in the development of disease in lymphochoriomeningitic virus-infected *MUNC13-4*-deficient mice.[60]

The immunological mechanisms behind other animal models of HLH/MAS are less well defined. Infection of rabbits with Herpesvirus papio results in a clinical syndrome similar to EBV-HLH.[61,62] These animals develop anti-red blood cell antibodies, which were suggested to contribute to hemophagocytosis. This is unlike other models and human disease, where anti-red blood cell antibodies are usually not found, making this model difficult to interpret. Mice deficient in asparaginyl endopeptidase (AEP), an enzyme important for lysosomal degradation, were also reported to develop an HLH-like syndrome including fever, cytopenia, splenomegaly and erythrophagocytosis.[63] Different from human disease, the phagocytozing histiocytes in this model were only consuming red blood cells and not other hematopoetic cells. This was likely due to the fact that these red blood cells had deformed membranes as a result of the *AEP* deficiency. Additionally different from human disease, these mice do not display a hypercytokinemia. Thus, the *AEP*-deficient mice may represent a distinct pathologic process from MAS, perhaps more similar to a lysosomal storage disease. As in humans, mice deficient in the gene encoding *SAP* develop hypogammaglobulinemia and are susceptible to HLH secondary to herpesvirus infections.[64] The precise immune mechanisms that lead to HLH in *SAP* deficiency are not clear. The most obvious link is that *SAP*-deficient NK cells also have impaired cytotoxicity.[65] Infection of Sv129S6 mice with *Salmonella enterica* serotype Typhimurium led to a syndrome of secondary HLH consistent with human disease.[66] This model has not yet been carefully immunologically dissected to provide insight into the mechanisms of disease.

There is much for room for continued exploration of the immunology underlying MAS. What is the source and nature of the IL-10 response? Almost every immune cell in the mouse has been described to produce IL-10 under the correct stimulus. T cells themselves might be a source of the IL-10, but this might be surprising under the very strong Th1 milieu of HLH/MAS. Recently, B cells have been noted to be an important source of regulatory IL-10.[67] A role for these cells in HLH/MAS might provide novel targets for therapeutic options. Myeloid derived IL-10, from dendritic cells and macrophages may also have a role in regulating the potentially hyperactive antigen presentation process that drives HLH.[35]

Although CD8 T cells are clearly the source of the pathogenic IFNγ in perforin-deficient HLH, the IFNγ producing cell is less clear in other forms. Which cells are producing IFNγ in EBV-HLH or sJIA-associated MAS remain untested. In the TLR9-induced mouse model of MAS, a mixed population of dendritic cells, T cells and NK cells produced IFNγ. Ultimately, as the identity of the IFNγ producing cell is likely to be different depending on the initiating stimulus, the producer may be less important than the cell upon which the pathogenic IFNγ is acting. It has been recently reported that IFNγ is sufficient to induce hemophagocytes, and that the IFNγ receptor is required on macrophages for this effect in a STAT1-dependent manner.[68] Whether the IFNγ responding macrophage becomes a hemophagocyte, or whether it induces another cell type to become a hemophagocyte is unclear. Furthermore, although the presence of hemophagocytosis is correlated with development of anemia[68] and worsened disease activity,[52] there is of yet no direct demonstration that hemophagocytes are directly pathogenic. In fact, some authors have found potentially anti-inflammatory/regulatory properties for hemophagocytes.[69] Certainly CD163, highly expressed on human hemophagocytes, is a marker strongly associated with regulatory M2 macrophage differentiation. Better characterization of the function of the hemophagocyte is clearly needed to understand these syndromes.

What other cytokines are having a role? What other immunologic insults can lead to a similar hyper-IFNγ state? How can we modulate the IFNγ and IL-10 axes to treat disease? Answers to these questions have relevance not only for MAS but also for 'cytokine storm' syndromes in general, such as sepsis or systemic inflammatory response syndrome. Continued work in animal models, as well as in translating these findings back to the human syndromes both remain important goals.

TRADITIONAL THERAPY FOR MAS IN THE SETTING OF sJIA

Although animal models of MAS may lead to novel and improved therapies, treatment of MAS as part of sJIA has traditionally developed from anecdotal experience. The first reported therapy, and still used today, for MAS as part of sJIA is high dose corticosteroids. Hadchouel *et al*.[70] in France described seven children with JIA (six with sJIA) complicated by acute hemorrhagic, hepatic and neurologic manifestations (later recognized as MAS).

The seven JIA patients were treated with high dose steroids and five survived.[70] A similar benefit was reported for high dose steroids in nine children with MAS (seven with sJIA) in England.[3] More recently, glucocorticoids, along with other immunosuppressive therapy, were reported to be effective in 13 children with sJIA in China[71] and in 6 children with sJIA in India.[72] Hence, high dose steroids are routinely used to treat MAS as part of sJIA.

In addition to corticosteroids, cyclosporine A (CsA) has become a staple in the therapy of MAS as a complication of sJIA. After some initial cases reported in the mid-1990's[73-75] in 2001, a report from France highlighted the utility of treating 24 patients (18 with sJIA) with MAS using CsA in pulse steroid failures or in 5 patients as a first line therapy.[76] Similarly, the protocol for treating primary or FHLH employs both corticosteroids (although at much lower doses) and CsA as part of the treatment protocol.[15] CsA is thought to preferentially target lymphocytes by inhibiting the NFAT family of transcription factors that are critical for the activation of a wide array of cytokine genes.[77] One of the likely benefits of CsA is dampening of the cytokine storm that occurs during MAS,[37] and, thus, lymphocyte-targeted immunosuppression is a key component of treating MAS as part of sJIA.

Another relatively lymphocyte specific therapy that has been explored for treating severe sJIA and MAS is cyclophosphamide. Anecdotal success has been reported with this chemotherapeutic agent[3,78] but it has not received a lot of attention of late for treating MAS as part of sJIA. Another chemotherapeutic agent used to treat MAS is etoposide, which is part of the protocol developed for treating FHLH.[15] This protocol carries a not insignificant risk of mortality both pre- and post-bone marrow transplantation, and may not be appropriate as first-line therapy for MAS as part of sJIA.[79] Autologous hematopoetic stem cell transplantation has been used specifically for treating cases of severe sJIA,[80] but the occurrence of several fatal complications due to MAS has suggested better control of systemic disease before transplantation is necessary.[81] Whether or not a less aggressive use of etoposide for treating secondary forms of MAS, such as those complicating sJIA, will be beneficial, remains unclear. Meanwhile, the advent and use of a variety of biologic agents is beginning to replace cytotoxic agents as first-line therapy for secondary forms of HLH/MAS.

One of the first biologic agents to be used for MAS is intravenous immunoglobulin. Success of intravenous immunoglobulin requires treatment early in the course of MAS.[82] Although often useful for the treatment of infection-triggered MAS[83,84] and a few children with sJIA,[72,85] children with MAS as part of sJIA are often refractory to intravenous immunoglobulin.[86] A more aggressive approach using anti-thymocyte globulin has been proposed for FHLH[87] and has been used successfully in two patients with probable MAS.[88] Nevertheless, there is a significant risk of serious infection and mortality with anti-thymocyte globulin use,[87] and less aggressive but effective biologic therapies have found recent success in treating MAS as part of sJIA.

A relative explosion of novel biologic therapies is now available for the treatment of rheumatic disorders. Rituximab, a B-cell depleting anti-CD20 antibody developed for the treatment of B-cell lymphoma, has found a variety of indications to treat rheumatic disorders ranging from arthritis to lupus to vasculitis and beyond.[89] Rituximab has also recently been reported to achieve remission in a high percentage of children with refractory sJIA.[90] In addition, rituximab has anecdotally been used to effectively treat EBV-associated HLH/MAS in the setting of EBV infection.[91,92] To date, no cases of rituximab therapy for MAS associated with sJIA have been reported. However, anti-cytokine therapies are finding a niche in the treatment of MAS as part of sJIA.

The first cytokine inhibitors used to treat MAS as part of sJIA were TNF blockers. Initially, there was some excitement as TNF inhibition was reported to effectively treat many cases of MAS including several children with sJIA.[93-101] This early enthusiasm has been tempered by the notion that TNF inhibitors may trigger MAS in some instances.[102-109] Although cause and effect is certainly difficult to formally prove in these circumstances, the fact that MAS can develop in the setting of TNF inhibition is concerning.[110,111] While TNF inhibition may not be the ideal therapy for MAS, particularly in the setting of a child with sJIA, therapy directed at two other pro-inflammatory cytokines, IL-1 and IL-6, seems promising.

IL-1 BLOCKADE FOR THE TREATMENT OF MAS IN CHILDREN WITH sJIA

The first reported use of the IL-1 receptor antagonist, anakinra, to purposely target MAS was in a critically ill child with a severe inflammatory condition and resulted in a remarkable improvement in a relatively brief time frame.[112] Although not formally defined as having MAS, two severely ill children with sJIA also received substantial benefit from anakinra treatment.[113] Soon after, anakinra was shown to markedly improve both systemic and arthritic features in a cohort of conventional treatment refractory sJIA patients.[114] Two independent groups concluded that, in addition to the $\sim$10% risk of developing overt MAS as part of sJIA, another 30–40% of sJIA patients may have occult or subclinical MAS during sJIA disease flare that can eventually lead to overt MAS.[13,14] This, along with the similarity of many clinical and laboratory features shared by MAS and sJIA flare, led to the concept that MAS may be an inherent aspect of sJIA pathophysiology in a large percentage of patients. The fact that anakinra was shown to be highly effective for sJIA[114,115] suggested anakinra would also be a valuable treatment for MAS as part of sJIA.

Several cases of sJIA-associated MAS dramatically benefiting from anakinra after inadequate response to corticosteroids and CsA have now been reported[86,115-118] (Table 2). For those severely ill children, IL-1 blockade has been remarkably effective in a relative brief time frame. Similarly, treatment refractory adult Still disease (likely related or identical to sJIA but in adults) with MAS has been described as having miraculously responded to anakinra therapy by several groups.[119-128] Although anakinra appears to be extremely safe, as a recombinant human protein with a short half-life ($\sim$3 hours),[129] and a large therapeutic window (1–48 mg kg^{-1} per day),[118,130] there has been a report of hepatitis attributed to anakinra in children with sJIA.[131] Moreover, there has also been the suggestion that anakinra triggered MAS in two children with sJIA[132,133] but once again, cause and effect is difficult to establish. In a large case series of 46 sJIA patients treated with anakinra at disease onset, anakinra was a potential MAS trigger in five children at doses of 1–2 mg kg^{-1} per day.[115] However, dose escalation of anakinra often seemed to help control MAS, and permanent discontinuation of anakinra was unnecessary for any of the children.[115] The overall published experience for the use of IL-1 blockade therapy for the treatment of refractory MAS as part of sJIA has been highly favorable (Table 2).

Like IL-1 inhibition, IL-6 blockade, via an anti-IL-6 receptor monoclonal antibody (tocilizumab), has proven highly efficacious in treating sJIA.[134] Whether tocilizumab will be similarly helpful in treating MAS in sJIA remains unclear at present, as there has been a case of MAS attributed to IL-6 blockade.[135] However, because many children with sJIA flare of disease are in a state of MAS[13,14] and respond favorably to IL-6 blockade, it is likely that blocking the actions of this cytokine will also benefit MAS in children with sJIA. Co-stimulatory blockade with CTLA-4-Ig has been anecdotally beneficial in children with severe sJIA[118] but its place in treating MAS is unknown. Nevertheless, there is building evidence that biologic therapies, particularly IL-1 inhibitors, are a welcome addition to corticosteroids and CsA in treating MAS associated with sJIA.[86] Time will tell if novel biologic therapies in the pipeline also hold promise for the treatment of MAS in children with sJIA.

Table 2. Benefit of IL-1 blockade in treating MAS as part of sJIA

Patient no.	*Age (yrs)*[a]	*Sex*	*Dx*	*Immuno-suppression*	*Anakinra dose (mg kg^{-1} per d)*	*Ferritin (ng ml^{-1})*[b]	*Outcome of MAS*	*First Author*	*Reference no.*
1	13	F	sJIA	St, CsA, IVIg	1	132 206	Resolved	Kelly	117
2	17	M	sJIA	St, CsA, IVIg	2	4787	Resolved	Miettunen	86
3	9	F	sJIA	St, CsA, IVIg	2	2279	Resolved	Miettunen	86
4	12	M	sJIA	St, CsA, IVIg	2	3141	Resolved	Miettunen	86
5	8	F	sJIA	St, CsA, IVIg	2	423	Resolved	Miettunen	86
6	1	F	sJIA	St, CsA, IVIg	2	438	Resolved	Miettunen	86
7	15	M	sJIA	St, CsA, IVIg	2	1055	Resolved	Miettunen	86
8	13	M	sJIA	St, CsA, IVIg	2	1285	Resolved	Miettunen	86
9	8	F	sJIA	St, CsA, Et	2	$>10\,000$	Resolved	Miettunen	86
10	4	F	sJIA	St, CsA, MTX, Ab	11	869	Resolved	Record	118
11	10	F	sJIA	St, CsA, MTX, Ab, Cyc, IVIg	9	15 693	Resolved	Record	118
12	8	M	sJIA	St	2	19 674	Resolved	Bruck	116
13	12	F	sJIA	St	2	28 000	Resolved	Bruck	116

Abbreviations: Ab, abatacept; CsA, cyclosporine A; Cyc, cyclosphophamide; Dx, diagnosis; Et, etoposide; F, female; IVIg, intravenous immunoglobulin; M, male; MAS, macrophage activation syndrome; MTX, methotrexate; sJIA, systemic juvenile idiopathic arthritis; St, steroids. [a]Age when MAS treated with anakinra. [b]Level before anakinra treatment.

SUMMARY

MAS is becoming an increasingly recognized, sometimes fatal, complication of sJIA disease flare. Because of the urgency in making the diagnosis of MAS and instituting appropriate therapy in a timely fashion, efforts are ongoing to establish timely, practical, and highly sensitive and specific criteria for recognizing MAS in children with sJIA. Progress is also being made in understanding the genetics and pathophysiology of MAS in children with sJIA. Preliminary studies suggest that children with sJIA and MAS frequently possess heterozygous mutations/polymorphisms in cytolytic pathway genes, which are disrupted in a homozygous fashion in infants with the related condition, FHLH. Knocking out these genes in mice has provided a tool for studying infection triggered MAS/HLH, and recently a novel murine model of non-infectious triggered MAS, which is dependent on repeated TLR stimulation, is imparting added insights into the pro- and anti-inflammatory milieu of the MAS cytokine storm. Understanding the balance of cytokines in the cytokine storm of MAS is crucial to the efforts in developing and utilizing the available cytokine-targeted biologic therapies. The rapid and dramatic benefits seen with the use of the recombinant human protein, anakinra, is generating substantial enthusiasm for treating MAS in the setting of sJIA with IL-1 blockade. Future therapeutic protocols for the treatment of MAS as part of sJIA will likely include a combination of high dose corticosteroids, CsA and anti-pro-inflammatory cytokine treatment, such as blockade of IL-1.

CONFLICT OF INTEREST

Drs Cron and Behrens who serve as consultants to Genentech for the use of Actemra (tocilizumab) to treat sJIA. Dr Cron also serves as a consultant to Novartis on the use of canakinumab to treat sJIA. Other authors declare no conflicts of interest.

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