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Not all Hemophagocytes are Created Equally: Appreciating the Heterogeneity of the Hemophagocytic Syndromes

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Abstract

Purpose of Review—The deadly Macrophage Activation Syndrome (MAS) constitutes one of the few rheumatologic emergencies. MAS is part of a larger group of diseases referred to as hemophagocytic syndromes that are seen in infections, malignancies, or genetic immunodeficiencies. Because of the clinical similarity of these diseases, many clinicians are tempted to approach them all similarly, both in diagnostic criteria and treatment paradigms. New work in the field suggests that not all hemophagocytic syndromes are equal. We will review the latest literature from both human and murine models related to the diagnosis, etiology, and treatment of hemophagocytic syndromes including MAS.

Recent Findings—More specific diagnostic criteria for the different hemophagocytic syndromes are being developed. Animal models suggest at least two different mechanisms by which hemophagocytic syndromes arise: enhanced antigen presentation and excessive Toll-like receptor signaling. Work in humans suggests different cytokine profiles, and different treatment strategies for the variety of hemophagocytic syndromes.

Summary—The recent studies reviewed in this article suggest that despite clinical similarities the different hemophagocytic syndromes are indeed likely heterogeneous. Diagnostic criteria and treatment strategies tailored to the underlying disease or genetic context are needed and will hopefully be addressed by future work in this field.

Keywords

Hemophagocytosis; Macrophage Activation Syndrome; Cytokine

Introduction

Macrophage Activation Syndrome (MAS) is a potentially life-threatening complication of a variety of inflammatory diseases. It is characterized by prolonged fever, peripheral cytopenias, splenomegaly, hepatitis, and often multiple organ dysfunction in the context of overwhelming inflammation. The term MAS is often reserved for this syndrome when it complicates rheumatic illnesses, particularly systemic Juvenile Idiopathic Arthritis (sJIA), systemic lupus erythematosus (SLE), or Kawasaki Disease (KD). Other autoimmune/ autoinflammatory disease that have reported associations with MAS include ankylosing spondylitis, polyarteritis nodosa, dermatomyositis, and adult rheumatoid arthritis. It bears many clinical similarities to a disorder known as familial Hemophagocytic Lymphohistiocytosis (fHLH) that is caused by any of a number of genetic defects resulting in impaired cellular cytotoxicity. Reactive HLH is a term applied to this constellation of

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symptoms complicating infections (particularly with Epstein-Barr Virus, EBV) or malignancy (particularly Cutaneous T-cell lymphomas), although some authors have extended the moniker MAS to these populations as well. The pathologic hallmark of all these diseases is the appearance of hemophagocytes (HPCs): activated macrophages that have engulfed other hematopoietic elements. HPCs can be found in the bone marrow, lymph nodes, spleen, or a variety of other organs. Being characterized by the presence of HPCs, MAS and HLH are grouped under the term hemophagocytic syndromes.

Drs. Grom and Mellins provided an excellent overview of this topic in 2010 (1), and we would direct the unacquainted reader to this and other reviews of the field. The current review will touch on recent advances in diagnosis and treatment, but will particularly focus on novel insights into the pathogenesis of hemophagocytic syndromes.

Diagnosis

The diagnoses of hemophagocytic syndromes must be accompanied by a high level of clinical suspicion and occur in the context of an evaluation for a variety of cytokine storm syndromes. There is an increasing appreciation for the similarities between MAS, HLH, and severe infection/sepsis that includes the frequent identification of HPCs in septic patients (2, 3). Recent work in animal models also supports a likely overlap between sepsis, severe allergic reaction, and MAS/HLH (4, 5). This overlap makes it tempting to consider all of these syndromes as roughly equivalent. However, the diagnostic distinctions between these diseases, while currently lacking a complete mechanistic understanding, are of critical importance in selecting the appropriate therapeutic strategy.

When followed by specific genetic testing, the criteria proposed by the Histiocyte Society in 2004 remain the gold standard for identification of patients with fHLH (6). These criteria contain an evaluation of a patient's Natural Killer (NK) cell cytotoxic function and level of soluble IL-2 receptor α (sIL2R α /sCD25). Since *Stepp et al* first described perforin gene mutations associated with HLH in 1999 (7), several other gene products critical for proper packaging, exocytosis, or function of cytotoxic granules have been described, including Munc13-4, Munc18-2, Syntaxin 11, Rab27a, and Lyst (reviewed in (8)). This list will continue to grow as whole exome/genome sequencing becomes more widely utilized. The HLH-04 criteria are, however, suboptimal for general diagnosis of hemophagocytic syndromes. First, NK cytotoxic function and sIL2R α testing are not rapidly or widely available. Second, many investigators have noted the absence of HPCs early in the course of disease, both in MAS and in fHLH (9, 10). Third, these criteria do not perform well in distinguishing MAS from a flare of the primary disease in which it occurs (especially rheumatic diseases such as sJIA and SLE) or from sepsis.

The disease most associated with MAS is sJIA, and while features of MAS are seen in up to 50% of sJIA patients (9), fulminant MAS occurs in roughly 10%. The difficulty in distinguishing MAS from an sJIA flare has led *Ravelli et al* to investigate diagnostic criteria for this purpose. Using retrospective chart reviews and surveying experts, they identified certain clinical features suggestive of MAS versus sJIA flare, such as CNS dysfunction, hepatitis, and disseminated intravascular coagulopathy (DIC). Laboratory features suggestive of MAS versus sJIA flare included dropping platelet or leukocyte count, low erythrocyte sedimentation rate, low fibrinogen, high ferritin, and presence of bone marrow hemophagocytes (11, 12). The criteria proposed by Ravelli are listed in Table 1 (11). It should be noted that these criteria are only to be used in distinguishing MAS from a flare of sJIA, and not as general diagnostic criteria. While useful for inclusion of MAS patients for retrospective studies, the performance of these criteria has not yet been tested prospectively.

Additionally, a few recent studies have highlighted other tests of potential diagnostic utility. In both MAS and HLH, very high ferritin levels have been used as markers of macrophage and dendritic cell activation. Recent data suggest that very high ferritin levels, and failure of the ferritin to fall dramatically with treatment, are poor prognostic signs across all HLH subtypes (13). Neopterin, an acute phase reactant and byproduct of nitric oxide synthesis, is produced by activated macrophages and was evaluated in patients with suspected HLH (familial and reactive). Neopterin was found to be elevated in HLH patients versus a comparator population with juvenile dermatomyositis and correlated strongly with ferritin (14). CD107a, also known as lysosomal-associated membrane protein 1 (LAMP1), is a membrane protein expressed on the surface of cytotoxic cells following degranulation. Patients with genetic defects in degranulation associated with fHLH were shown to have defective CD107a mobilization to the cell surface upon *ex vivo* stimulation, making this a rapid functional assay defects in degranulation (15).

Pathogenesis

Recent work has provided new details into the mechanisms that underlie disease in both primary HLH and in secondary forms of HLH/MAS.

Novel insights into cytotoxic defects

It is well established that defects in the production, transport, exocytosis, or function of CD8 T-cell or NK cell cytotoxic granules can lead to HLH. The variety of such defects includes the pore-forming protein perforin itself, but also proteins critical for sorting of lysosomal protein (AP3 β 1, as seen in Hermansky-Pudlak Syndrome), lysosomal fission (Lyst, as seen in Chediak-Higashi Syndrome), tethering (Rab27a), lysosomal packaging & priming of membrane fusion (Munc13-4), and membrane fusion (Munc18-2 & Syntaxin 11). The diseases related to these defects and the murine models associated with each have been recently and thoroughly reviewed (8). In each model, uncontrolled CD8 T-cell production of interferon γ (IFN γ) appears to be the primary driver of disease.

Adding to the paradigm that IFN γ is a critical proinflammatory mediator is a recent study by Zoller *et al* showing that IFN γ alone delivered via osmotic pump is sufficient to cause hemophagocytosis and anemia (16). They further show that the anemia seen in various IFN γ -mediated disease models is dependent on monocytes responding to IFN γ , and that mice with monocytes that lack the ability to respond to IFN γ are protected against both anemia and hemophagocytosis. They call this process the “Consumptive Anemia of Inflammation,” and show evidence to suggest that hemophagocytosis is a macropinocytic process (as opposed to receptor-mediated) similar to phagocytosis of apoptotic cells. These data support IFN γ as a critical cytokine for driving hemophagocytic diseases. This study also supports a pathogenic role for HPCs by correlating the absence of anemia and hemophagocytosis to monocytes incapable of responding to IFN γ . This is in contrast to data suggesting HPCs may be bystander or regulatory cells based on expression of scavenger receptors or markers of alternative activation (4, 17).

The same group also recently described a mechanism by which the combination of viral infection and cytotoxic dysfunction might result in HLH. Lykens *et al* show that, rather than excessive viremia, enhanced antigen presentation is responsible for T cell hyperactivation (in both CD8 and CD4 compartments) in perforin deficient/LCMV-infected mice, and this activation does not correlate with viral load (18). Rather than seeing enlarged antigen presenting cell (APC) populations, they observed an enhanced ability of APCs that matured in a perforin-deficient environment to activate T-cells regardless of the T-cell perforin status. These data suggest that a major function of cellular cytotoxicity is the regulation of antigen presentation, and not merely the killing of infected cells. They also suggest that

HLH is caused by problems with the regulation of inflammation, rather than a primary problem with excessive proinflammatory stimuli (like uncontrolled viremia). Further, these data support targeting APC populations as a viable strategy for the treatment of hemophagocytic syndromes, particularly in patients with a defect in cytotoxicity.

Novel Pathogenic Pathways in HLH/MAS

The current literature is beginning to clarify the extent to which MAS and HLH are overlapping diseases. While inclusion in HLH studies generally follows the HLH-04 criteria (6), determination of which patients have MAS is often made at the investigators' discretion and varies from study to study. Nonetheless, studies showing impaired NK cell function, perforin gene defects, or Munc13-4 gene defects in some patients with sJIA complicated by MAS support impaired cytotoxic function as an important causative factor in at least a subset of MAS patients (19–21). Other data, however, suggest heterogeneity within MAS/reactive HLH depending on the underlying disease. *Maruyama and Inokuma* evaluated selected cytokines in predominantly adult females with various rheumatic illnesses. They found that macrophage colony stimulating factor (M-CSF) and IL-18 were elevated in all MAS patients, but SLE patients had more M-CSF while Adult-onset Still's Disease patients had substantially more IL-18 (22). Further supporting heterogeneity in MAS, *Shimizu et al* measured selected cytokines in MAS associated with sJIA, KD, and EBV (23). They found IL-18 highly elevated in the sJIA-MAS patients regardless of disease activity, neopterin elevations restricted to EBV-HLH patients, and IL-6 elevations most impressive in KD-MAS. While these data do little to clarify pathogenic mechanisms, they underscore the necessity of studying MAS in the context of its underlying disease.

In an attempt to examine human cells in an animal model, *Sato et al* infected immunodeficient mice reconstituted with normal human stem cells with EBV (24). These mice develop HLH with cytopenias, hemophagocytosis, expansion of activated CD8+ T-cells, and elevated IFN γ . In contrast to the previous study, cytokinemia and T-cell expansion in this model correlated with viral load, and most T-cells were not EBV-specific, suggesting that the antigen-presenting ability of host APCs may not be the sole determinant of disease. This study did not examine cytotoxic defects directly, and thus it is unclear why genetically "normal" human hematopoietic cells are prone to the development of HLH in this context, but may relate to the antigen-presenting ability of infected B-cells.

Further illustrating the difficulty of extending conclusions from murine models to fHLH patients, *Sumegi and colleagues* performed peripheral blood mononuclear cell (PBMC) microarrays on patients felt to have fHLH in comparison to healthy controls (25). Curiously, IFN γ and IFN γ -inducible genes were not significantly upregulated. Various other proinflammatory genes were highly upregulated, including those encoding IL-1 α & β , IL-6 and IL-8, as well as anti-inflammatory genes such as those encoding the IL-1 receptor antagonist, IL-10, and SOCS3. Interestingly, they observed downregulation of a number of genes associated with NK and CD8 T-cell activation, Toll-like Receptor (TLR) signaling, and genes regulating apoptosis. The active downregulation of such genes in sick patients suggests these may be pathways important for disease initiation.

TLR signaling has recently emerged as a potentially important mechanism for disease causation in HLH and MAS. A recent study by *Krebs et al* showed that signaling through molecules critical for TLR function may be critical for development of HLH in mice with defective cytotoxicity (26). This group has previously shown that mice lacking functional Munc13-4 develop an HLH-like syndrome after exposure to LCMV (27). The recent study demonstrates that mice defective in Munc13-4 that are also defective in MyD88 (a critical signaling protein downstream of most TLRs and the IL-1 receptor) are almost entirely protected from developing HLH after LCMV infection. Such mice are able to clear the virus

but do not have the same signs of APC and T-cell activation as mice with only the cytotoxic defect.

Additionally, stimulation through TLR9 alone was recently shown to result in an MAS-like syndrome in wild-type mice, but with the absence of HPCs (28). Many, if not most, MAS patients have no identified defects in cytotoxicity. Thus, this model is important in that it illustrates a distinct pathogenic mechanism for an MAS/HLH phenotype without dependence on a genetically mediated cytotoxic defect or viral infection. Like other models utilizing mice defective in cytotoxicity infected with LCMV, mice repeatedly stimulated through TLR9 did not develop disease in the absence of IFN γ . TLR9-stimulation may be particularly suited to inducing MAS, as TLR9 is implicated in SLE pathogenesis (29), and EBV is a DNA virus known to signal through TLR9 (30). Further, mice repeatedly stimulated through TLR9 who cannot respond to IL-10 develop far more severe disease including the production of HPCs (28). This, in concert with the human PBMC data showing upregulation of IL-10-related pathways (25), suggests IL-10 may be a critical regulator of MAS and hemophagocytosis. A recent human study evaluating Interferon Regulatory Factor 5 (IRF5) polymorphisms in sJIA corroborates a role for TLR signaling in MAS/HLH pathogenesis. IRF5 is another crucial signaling molecule downstream of a number of TLRs, including TLR9. The odds of developing MAS as a complication of sJIA were 4-fold higher in patients harboring homozygous activating IRF5 polymorphisms than in sJIA patients without the polymorphism (31).

Treatment

A number of case reports and small case series have recently emerged that suggest new treatment strategies for MAS and HLH. Unsurprisingly, many of these modalities focus on the context in which the hemophagocytic syndrome occurs to guide treatment.

The protocol set forth in HLH-04 remains the primary treatment for patients diagnosed with fHLH (6). The risks of infection, severe side effects, treatment-related malignancies, and death related to this protocol are quite high (2, 32), and much empiric work has been done in both fHLH and MAS patients to reduce or avoid these risks. In fHLH, allogeneic bone marrow transplant (BMT) remains the only potentially-curative option, but reduced-intensity induction or use of specific T-cell depleting agents have shown promise (32, 33). Based on successes with the use of IL-1 blocking agents, primarily anakinra, in patients with sJIA, some investigators have reported success with higher doses in the treatment of MAS complicating sJIA as well as other rheumatic illnesses (34, 35). Fewer reports exist of successes with inhibitors of Tumor Necrosis Factor α (TNF α) in MAS (36), concordant with animal models suggesting this cytokine is not a critical driver of HLH/MAS (26, 28, 37). The use of IL-6 antagonism has not yet been reported as a treatment for MAS, perhaps out of concern for worsening of leukopenia. Additionally, the use of rituximab, ostensibly to deplete infected B-cells, has been used with success in conjunction with immunosuppressive chemotherapy in EBV-HLH (38).

Conclusion

We have presented several novel lines of evidence to suggest that, despite clinical similarities, it may be both incorrect and dangerous to consider the hemophagocytic syndromes as a single entity. Recent human and murine investigations into these diseases demonstrate that different fundamental insults (cytotoxicity defects versus heightened TLR activity) can give rise to the syndrome. Furthermore, heterogeneity is highlighted clinically by the different mixes of cytokines found in the serum depending on the underlying disease. Therefore, it is likely wise to continue considering fHLH, the various rheumatic-associated

MAS syndromes, and reactive HLH as separate entities. Clinically, their different underlying pathoetiologies will require different treatment protocols. From a research perspective, considering what are likely different diseases as a single group may confound our ability to make important discoveries. In practice, this is evident from the fact that sJIA-MAS can be successfully treated with IL-1 β blockade, EBV-HLH with rituximab, and yet tHLH requires allogeneic BMT, further illustrating the need to tailor therapy to the clinical scenario rather using the same strict protocol for all. A major goal of future research should be to more completely define the initiating and propagating mechanisms of disease in these various scenarios in order that we may identify at risk patients, and rationally select the therapies to treat them.

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Key Points

- Although they share a similar clinical phenotype, it is likely that the different hemophagocytic syndromes arise from different pathogenic mechanisms
- Treatment of the different hemophagocytic syndromes is likely to require individualization to the particular clinical scenario rather than a common protocol for all.
- Because of the likely different in pathogenic mechanisms, it is important to separate the distinct hemophagocytic syndromes in research design.

Table 1

Ravelli preliminary diagnostic criteria for MAS complicating SJIA, Adapted from (11)

The diagnosis of MAS requires the presence of any 2 or more laboratory criteria or of any 2 or 3 or more clinical and/or laboratory criteria. A bone marrow aspirate for the demonstration of haemophagocytosis may be required only in doubtful cases.	
Laboratory criteria	
1. Decreased platelet count ($\leq 262 \times 10^9/L$)	
2. Elevated levels of aspartate aminotransferase ($> 59 U/L$)	
3. Decreased white blood cell count ($\leq 4.0 \times 10^9/L$)	
4. Hypofibrinogenemia ($\leq 2.5 g/L$)	
Clinical criteria	
1. Central nervous system dysfunction (irritability, disorientation, lethargy, headache, seizures, coma)	
2. Hemorrhages (purpura, easy bruising, mucosal bleeding)	
3. Hepatomegaly (≥ 3 cm below the costal arch)	
Histopathological criterion	
Evidence of macrophage hemophagocytosis in the bone marrow aspirate	