

# Macrophage activation syndrome and reactive hemophagocytic lymphohistiocytosis: the same entities?

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## Purpose of the review

One of the most perplexing features of systemic-onset juvenile rheumatoid arthritis is the association with macrophage activation syndrome, a life-threatening complication caused by excessive activation and proliferation of T cells and macrophages. The main purpose of the review is to summarize current understanding of the relation between macrophage activation syndrome and other clinically similar hemophagocytic disorders.

## Recent findings

Clinically, macrophage activation syndrome has strong similarities with familial and virus-associated reactive hemophagocytic lymphohistiocytosis. The better understood familial hemophagocytic lymphohistiocytosis is a constellation of rare, autosomal recessive immune disorders. The most consistent immunologic abnormalities in patients with familial hemophagocytic lymphohistiocytosis are decreased natural killer and cytotoxic cell functions. In approximately one third of familial hemophagocytic lymphohistiocytosis patients, these immunologic abnormalities are secondary to mutations in the gene encoding perforin, a protein that mediates cytotoxic activity of natural killer and cytotoxic CD8<sup>+</sup> T cells. Several recent studies have suggested that profoundly depressed natural killer cell activity and abnormal levels of perforin expression may be a feature of macrophage activation syndrome in systemic-onset juvenile rheumatoid arthritis as well. Although it has been proposed that in both hemophagocytic lymphohistiocytosis and macrophage activation syndrome, natural killer and cytotoxic cell dysfunction may lead to inadequate control of cellular immune responses, the exact nature of such dysregulation and the relation between macrophage activation syndrome and hemophagocytic lymphohistiocytosis still remain to be determined.

## Keywords

Systemic onset juvenile rheumatoid arthritis, macrophage activation syndrome, hemophagocytic lymphohistiocytosis, perforin, natural killer cells.

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## Abbreviations

<b>HLH</b>	hemophagocytic lymphohistiocytosis
<b>MAS</b>	macrophage activation syndrome
<b>NK</b>	natural killer
<b>soJRA</b>	systemic-onset juvenile rheumatoid arthritis

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The term “macrophage activation syndrome” (MAS) is familiar to most pediatric rheumatologists. In the field of hematology–oncology, however, a clinically similar condition is defined as “reactive hemophagocytic lymphohistiocytosis.” Based on the data reported over the past year, the similarities between the two syndromes are not limited to only clinical features. This review summarizes the current knowledge of the common pathogenic mechanisms suggesting that the terminology, indeed, may need to be revised.

## Macrophage activation syndrome and reactive hemophagocytic lymphohistiocytosis

In rheumatology, the term “macrophage activation syndrome” refers to a set of clinical symptoms caused by the excessive activation and proliferation of well differentiated macrophages. It was first described as a complication of systemic-onset juvenile rheumatoid arthritis (soJRA). In 1985, Hadchouel *et al.* described seven JRA patients who acutely presented with a hemorrhagic syndrome associated with mental status changes, hepatosplenomegaly, increased serum levels of liver enzymes, and a sharp fall in blood cell counts and erythrocyte sedimentation rate [1]. Six of these patients had the systemic form of JRA. Since in most of these patients, bone marrow aspiration revealed the presence of numerous non-neoplastic histiocytes with prominent hemophagocytic activity, the authors suggested that this severe complication might be induced by excessive activation of macrophages. The term “macrophage activation syndrome” was eventually introduced by Stephan *et al.* in 1993 in a follow-up article originating from the same center [2].

Over the following years, few more reports from various countries described a number of patients with very similar symptoms [3–8]. Although more recently, MAS has been also observed in a small number of patients with many other rheumatic disorders (polyarticular juvenile rheumatoid arthritis [1,8], systemic lupus erythematosus, rheumatoid arthritis, sarcoidosis [9], dermatomyositis, and chronic infantile neurologic cutaneous and articular syndrome [8]), it is by far most common in soJRA. The reason for increased incidence of MAS in soJRA remains unclear (See Ravelli [10] for detailed discussion of the clinical presentation).

The diagnostic hallmarks of this syndrome are found in bone marrow aspiration: numerous, well differentiated macrophages (or histiocytes) actively phagocytosing hematopoietic elements. Therefore, the term “reactive hemophagocytic lymphohistiocytosis” has been used by some authors to classify this condition. Furthermore, over the past year, several pediatric rheumatologists have suggested that the term “macrophage activation syndrome” is misleading and should be replaced with the term “reactive hemophagocytic lymphohistiocytosis” [11,12]. This would provide for better understanding between pediatric rheumatologists and hematologists and would make pediatric rheumatologists more familiar with treatment options used to control hemophagocytic lymphohistiocytosis.

Indeed, “hemophagocytic lymphohistiocytosis” is a more general term that applies to a wide spectrum of disease processes characterized by accumulations of histologically benign, well differentiated mononuclear cells with a macrophage phenotype [13,14]. Since such mononuclear phagocytes represent a subset of histiocytes that are distinct from Langerhans cells, this entity should be distinguished from Langerhans cell histiocytosis as well as other dendritic cell disorders. Hemophagocytic lymphohistiocytosis (HLH) can be further divided into at least two major groups of conditions that are often difficult to distinguish from each other: primary, or familial hemophagocytic lymphohistiocytosis, and secondary, or acquired HLH. Familial HLH is a constellation of rare autosomal recessive immune disorders. Symptoms of familial HLH are usually evident within the first 2 months of life, although initial presentation as late as 22 years of age has been reported as well [15]. If left untreated, Familial HLH can be rapidly fatal. Most patients require allogeneic hematopoietic stem cell transplantation for full control of the disease.

The group of secondary hemophagocytic disorders includes infection-associated (or reactive) HLH and malignancy-associated HLH [13]. Secondary HLH tends to occur in older children or even in adolescents or adults. Among the infectious triggers, two members of the herpes family, Epstein-Barr virus and cytomegalovirus, are

most common. If treated aggressively, these patients often recover with no need for a long-term immunosuppressive therapy. In some cases, secondary HLH may be a self-limiting complication, and patients are able to recover after receiving only supportive treatment.

### Immunologic abnormalities in hemophagocytic lymphohistiocytosis

While the etiology of HLH is unknown, it appears that there is an underlying abnormality in immunoregulation that contributes to the lack of control of an exaggerated immune response [14]. The clinical findings during the acute phase of HLH can largely be explained as a consequence of the prolonged production of cytokines and chemokines originating presumably from activated macrophages and T cells. Excess of circulating interleukin 1 beta, tumor necrosis factor alpha, interleukin 6, and interferon gamma is likely to contribute to the early and persistent findings of fevers, hyperlipidemia, and endothelial activation responsible in part for coagulopathy, as well as later sequelae including tissue infiltration by lymphocytes and histiocytes, hepatic triaditis, central nervous system vasculitis and demyelination, and bone marrow hyperplasia. Hemophagocytosis, the pathognomonic feature of the syndrome, is a hallmark of cytokine driven over activation of macrophages.

The most consistent immunologic abnormality described in these patients has been global impairment of cytotoxic function. Thus, Egeler *et al.* showed that most patients with familial hemophagocytic lymphohistiocytosis had normal quantitative serum immunoglobulin levels [16]. Phenotypic analysis of lymphocyte subsets showed that most patients had surprisingly normal absolute lymphocyte counts and normal distribution of mature T-cell subsets. CD4/CD8 ratios were normal in most patients. In contrast, the NK function was markedly decreased or absent in most patients. Cytotoxic activity of CD8<sup>+</sup> cells was also defective [16,17]. One subtype of familial HLH, accounting for approximately 30% of cases in the United States, has been recently associated with mutations in the gene encoding perforin (PRF1), a protein that mediates cytotoxic activity of NK and T cells [18]. These patients usually have normal numbers of NK cells, but either very low or absent perforin expression in all cytotoxic cell types.

Patients with virus-associated HLH also have very low or absent cytolytic NK cell activity. However, in contrast to familial hemophagocytic lymphohistiocytosis, this phenomenon appears to be related to profoundly decreased numbers of NK cells rather than impaired perforin expression. In fact, perforin expression in both CD8<sup>+</sup> and CD56<sup>+</sup> cytotoxic cells is often mildly increased. It appears that NK function may completely recover in some of these patients after the resolution of the acute phase of the syndrome [19].

Interestingly, NK cells are also affected in the Chediak-Higashi syndrome, another disease associated with the development of lymphohistiocytic expansion and hemophagocytosis. In this disease, numbers of NK cells are normal, but the cells are characterized by the presence of abnormal granules containing perforin in the cytoplasm. The genetic defect in this condition appears to be a mutation in the gene encoding one of the cytoskeletal proteins, leading to impaired ability to mobilize perforin [20]. As a result the cytolytic activity of such NK cells is greatly diminished.

Two other genetic conditions associated with hemophagocytic lymphohistiocytosis are Griscelli syndrome and X-linked lymphoproliferative disease. In Griscelli syndrome, hemophagocytic complications are related to the deficiency of RAB27a, a cytoplasmic protein involved in cytotoxic granule release [21], while X-linked lymphoproliferative disease is caused by mutation in an adaptor protein SH2DIA that has been implicated in the regulation of T-cell activation and NK effector function [22,23]. In both syndromes, cytotoxic functions are greatly impaired.

### **Perforin expression and natural killer function in macrophage activation syndrome**

Two recent studies suggested that abnormal perforin expression and NK dysfunction may be relevant to the pathogenesis of MAS in soJRA as well. Thus, Wulfraat *et al.* demonstrated reduced perforin expression in two subsets of CD8<sup>+</sup> lymphocytes (CD45RA<sup>-</sup>, CD28<sup>-</sup> and CD45RA<sup>+</sup>, CD28<sup>-</sup>) in patients with active systemic JRA compared with other forms of JRA and healthy controls [24•]. They suggested that this feature may be responsible for the increased incidence of MAS in soJRA. Interestingly, perforin expression returned to the normal levels after autologous hematopoietic stem cell transplantation performed in four patients. This observation suggests that depressed perforin expression is not likely to be caused by a genetic abnormality but rather induced by the underlying disease (*eg*, soJRA).

Another recent study focused on the assessment of NK cell function and perforin expression in seven patients with macrophage activation syndrome presenting as a complication of soJRA [25•]. NK activity in peripheral blood collected during the acute stage of MAS was profoundly depressed in all seven patients. Furthermore, two major patterns of immunologic abnormalities were delineated. In four of seven patients studied, the authors observed low NK activity associated with very low numbers of NK cells, but mildly increased levels of perforin expression in NK T cells and cytotoxic CD8<sup>+</sup> lymphocytes, a pattern somewhat similar to that in virus-associated HLH. In contrast, in three remaining patients, very low NK activity was associated with only mildly

decreased numbers of NK cells, but very low levels of perforin expression in all cytotoxic cell types. This pattern is very similar to that seen in carriers of familial hemophagocytic lymphohistiocytosis (FHLH). Remarkably, two of these patients had a history of multiple previous episodes of MAS. Previous reports suggested that depressed NK function and decreased absolute numbers of NK cells may also be a feature that distinguishes the patients with systemic juvenile rheumatoid arthritis from those with other forms of juvenile rheumatoid arthritis [26]. These observations may provide another clue to the understanding of the reasons for increased incidence of MAS in soJRA.

### **Natural killer cell function and cellular immune responses**

The exact mechanisms that would link deficient NK cell function with expansion of activated macrophages are not clear. One possible explanation is that decreased NK function may be responsible for diminished ability to clear the infecting pathogen and remove the source of antigenic stimulation at early stages of infection [27]. This would lead to persistent, antigen-driven T-cell activation associated with increased production of cytokines that might stimulate macrophages.

Another possible explanation is related to the recently suggested “immunoregulatory” role of NK cells. Indeed, increasing evidence suggests that the role of NK cells is not limited to defense against intracellular microbes and tumor cells. NK cells have also been implicated in regulation of homeostasis and certain adaptive immune responses. Thus several recent studies utilizing perforin-deficient and NK-cell-depleted mice indicate that NK cells and perforin-based systems are involved in the down-regulation of immune responses [28–31]. For instance, Su *et al.*, showed that during murine cytomegalovirus infections of immunocompetent mice *in vivo* depletion results in augmented production of interferon gamma by CD4<sup>+</sup> and CD8<sup>+</sup> T cells and augmented expansion of CD8<sup>+</sup> cells [30]. In this system, the increased expansion of CD8<sup>+</sup> cells still can be explained by the failure of NK cells to limit infection during the first days of infection leading to increased viral load on CD8<sup>+</sup> lymphocytes at later stages of the infection. However, similar effects have been demonstrated in the experimental systems in which immune responses were elicited by anti-CD3 antibodies or staphylococcal toxins instead of viruses. For instance, Kagi *et al.* demonstrated that the injection of staphylococcal enterotoxin B into perforin-deficient mice results in dramatically increased selective expansion and prolonged persistence of CD8<sup>+</sup> but not CD4<sup>+</sup> staphylococcal enterotoxin B reactive T cells [31]. These experiments suggest that there likely to be a direct effect of perforin-based systems on the survival of activated lymphocytes. It has been hypothesized by some authors that abnormal cytotoxic cells may fail to provide appropriate apoptotic signals for removal of ac-

tivated T cells after infection is cleared [32]. Such T cells may continue to secrete cytokines, including interferon gamma and granulocyte-macrophage colony-stimulating factor, two important macrophage activators. Subsequently, the sustained macrophage activation results in tissue infiltration and in the production of high levels of tumor necrosis factor alpha, interleukin 1, and interleukin 6, which play a major role in the various clinical symptoms and tissue damage. A report on successful treatment of JRA-associated MAS by cyclosporin A with transient exacerbation of MAS by conventional-dose granulocyte-specific colony-stimulating factor does support this hypothesis [33].

## Conclusions

The hypothesis that impaired cytotoxic functions and the lack of immunoregulatory role of NK cells are relevant to the development of MAS in soJRA still remains to be proven. However, it is becoming clear that the similarities between MAS and HLH syndromes are not limited to only clinical features: both are associated with similar immunologic abnormalities. It also becomes clear that there is significant heterogeneity in terms of mechanisms leading to abnormal cytotoxicity in both HLH and MAS. Based on this, we agree that new terminology may be necessary, but the ideal terms should be defined based on the more precise understanding of such heterogeneity.

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