

## BASIC SCIENCE FOR THE CLINICIAN

### Pathogenic mechanisms in macrophage activation syndrome

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#### Introduction

In pediatric rheumatology, the term "macrophage activation syndrome" refers to a set of clinical symptoms caused by the excessive activation and proliferation of T-lymphocytes and well-differentiated macrophages. Although MAS is most frequently seen in systemic onset juvenile rheumatoid arthritis (soJRA) [1-8], it can occur in association with almost any rheumatic condition [8-10]. The pathognomonic features of this syndrome in the clinical context of MAS are found in bone marrow aspiration: numerous, well-differentiated macrophages (or histiocytes) actively phagocytosing hematopoietic elements. Such phagocytic macrophages and activated T lymphocytes may infiltrate almost any organ in the body and may account for many of the systemic features of this syndrome including fever, hepatosplenomegaly, lymphadenopathy, severe cytopenias, serious liver disease, coagulopathy, as well as neurological involvement. Thus, post-mortem evaluation of one of the patients with MAS revealed extensive macrophagic infiltration of the heart, adrenal glands, liver, pancreas and meninges. [9]

Since in many cases, macrophage activation syndrome (MAS) is triggered by infections or modifications in the drug therapy, the term "reactive hemophagocytic lymphohistiocytosis" has also been used to classify this condition. [10-12] In turn, "hemophagocytic lymphohistiocytosis" is a more general term which applies to a spectrum of disease processes characterized by accumulations of histologically benign well-differentiated mononuclear cells with a macrophage phenotype [13-14]. Hemophagocytic lymphohistiocytosis (HLH) can be further divided into at least two major groups of conditions which are often difficult to distinguish from each other: primary, or familial hemophagocytic lymphohistiocytosis (FHLH), and secondary. The group of secondary hemophagocytic disorders includes infection associated HLH (IAHLH) and malignancy associated HLH. The familial form is associated with mutations in the perforin gene or in the genes which products are involved with intracellular perforin trafficking [14-16] and both forms are associated with severe defects in NK and cytotoxic T cell function. [17-18] EBV tropism and HLH may represent another mechanism [19]. Although there are many clinical similarities between MAS of soJRA, IAHLH, and FHLH, the exact relationships between these conditions are not understood.

### **Cytokine storm in MAS**

The exact pathogenic mechanisms involved in the development of MAS are not known. It appears that there is an underlying abnormality in immunoregulation that contributes to the lack of control of an exaggerated immune response. [14] Indeed, 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 from activated macrophages and T cells. Indeed, excess of circulating IL-1 $\beta$ , TNF- $\alpha$ , IL-6, IL-18, and IFN- $\gamma$  is likely to contribute to the early and persistent findings of fevers, hyperlipidemia, and endothelial activation responsible in part for the coagulopathy. This storm likely has a role in later sequelae including hepatic triaditis, and central nervous system demyelination. In fact, hemophagocytosis, the pathognomonic feature of the syndrome, is a hallmark of cytokine-driven excess activation of macrophages. [14]

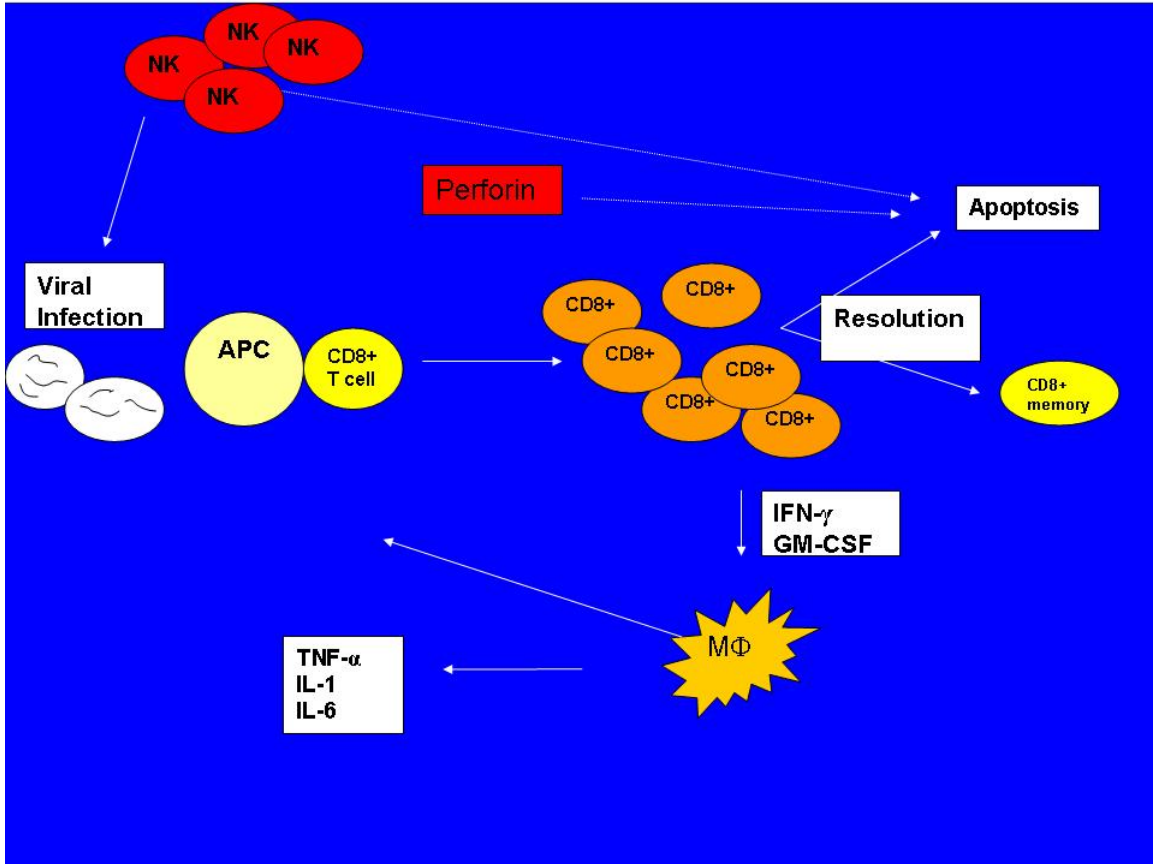
### **Central role for IFN- $\gamma$ producing CD8<sup>+</sup> T cells**

A recent study of liver biopsies in MAS patients demonstrated massive infiltration of the liver by IFN- $\gamma$  producing CD8<sup>+</sup> T lymphocytes and hemophagocytic macrophages producing TNF- $\alpha$  and IL-6. [20] Studies in perforin deficient mice, an animal model of HLH, suggest that these cytotoxic CD8<sup>+</sup> cells producing IFN- $\gamma$  are particularly important in the pathogenesis of excessive macrophage activation. Although perforin-deficient mice do not spontaneously develop MAS/HLH, they manifest many of the features of this syndrome after infection with lymphocytic choriomeningitic virus. Remarkably, the MAS-like symptoms in these animals can be almost completely prevented by elimination of CD8<sup>+</sup> T cells or by neutralization of IFN- $\gamma$ . [21]

In contrast, neutralization of IL-1, TNF- $\alpha$  or IL-6 provides only mild alleviations of the symptoms. Since IFN- $\gamma$  is well known macrophage activator, it has been suggested that IFN- $\gamma$  is critical to the expansion of macrophages in these animals. Consistent with the animal data, the increase in serum IFN- $\gamma$  levels in MAS patients compared to those in patients with active systemic JRA is dramatically higher than the increase in the levels of any other cytokine. [22] Another interesting observation is that the development of MAS is associated with a dramatic increase in the serum levels of sIL2 receptors that are likely to be shed off by highly activated CD8<sup>+</sup> T lymphocytes. [24] Combined these observations suggest that in MAS patients, similar to the animal models, massive activation and expansion of cytotoxic CD8<sup>+</sup> T cell is associated with the production of IFN- $\gamma$  and other macrophage activating cytokines (Figure 1A and 1B).

**Figure 1A. Pathogenesis of macrophage activation in hemophagocytic syndromes.**

**A normal immune response induced by a viral infection.** NK cells provide the first line of defense by inducing lysis of virally infected cells, thus, limiting the extent of viral replication during the first 2-3 days of infection. Antigen-specific CD8<sup>+</sup> cells become important at later stages. They efficiently eliminate infected cells and secrete pro-inflammatory cytokines such as IFN- $\gamma$  that activate other immune cells including macrophages. After infection is cleared, such cytotoxic CD8<sup>+</sup> cell become pathogenic due to their pro-inflammatory activities. They are eventually eliminated through mechanisms that presumably involve NK cells and/or perforin. Few of them survive as memory cells.

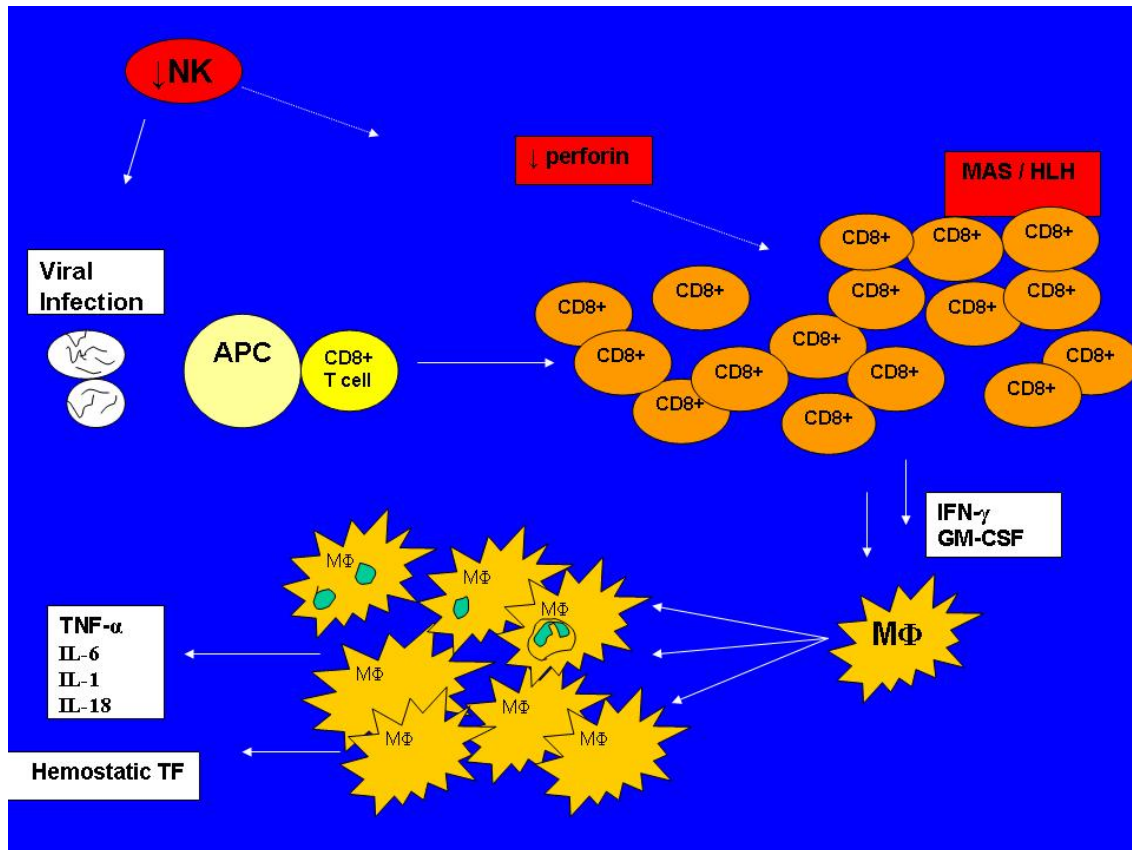


**Figure 1B. Pathogenesis of macrophage activation in hemophagocytic syndromes.**

**An immune response in MAS triggered by a viral infection.** NK cells fail to limit the extent of viral replication at early stages of infection leading to increased viral load and more massive expansion of cytotoxic CD8+ cells. At later stages due to perforin deficiency and/or poor NK cell function, cytotoxic CD8+ cells are not eliminated even if infection is cleared. They continue to survive and secrete proinflammatory cytokines including IFN- $\gamma$ . Prolonged stimulation of macrophages with cytokines leads to their excessive activation and proliferation associated with hemophagocytic activity and

production of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1, and IL-6.

Hemophagocytosis of blood elements in the bone marrow leads to peripheral cytopenias. Production of pro-coagulant tissue factor (TF) combined with the TNF- $\alpha$  effects on vascular endothelial cells contribute to the development of coagulopathy.



This leads to subsequent activation and expansion of macrophages. The activated macrophages, in turn, exhibit hemophagocytic activity and secrete pro-inflammatory cytokines including IL-1, TNF- $\alpha$ , IL-6 and IL-18 which are responsible for many of the clinical manifestations of MAS.

### Upstream events

The events that cause the expansion of T lymphocytes and macrophages are less clear. In at least some FHLH patients, the development of the symptoms has been linked to the mutations in the gene encoding perforin. Perforin is a protein that NK cells and cytotoxic CD8<sup>+</sup> cells utilize to kill tumors or cell infected by intracellular microbes such as viruses. Patients with Virus-Associated HLH also have very low or absent cytolytic NK cell activity. However, in contrast to FHLH, 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 .[18] It appears that NK function may completely recover in some of these patients after the resolution of the acute phase of the syndrome.

### Perforin expression and NK function in soJRA and MAS

Increasing evidence suggests that depressed NK activity often associated with abnormal perforin expression may be important to the pathogenesis of macrophage activation syndrome in soJRA as well.

One recent study demonstrated reduced perforin expression in two subsets of cytotoxic CD8<sup>+</sup> T 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 juvenile rheumatoid arthritis and healthy controls. [25] Interestingly, perforin expression returned to the normal levels after autologous hematopoietic stem cell transplantation performed in four patients. Based on the similarities with FHLH, the authors suggested that low perforin expression might be responsible for the increased incidence of MAS in soJRA.

Another 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 samples collected during the acute stage and after the resolution of MAS was profoundly depressed in all patients. In some patients, low NK activity was associated with very low numbers of NK cells but mildly increased levels of perforin expression in NK T cells and cytotoxic CD8<sup>+</sup> T lymphocytes. This is a pattern somewhat similar to that in virus-associated HLH. In contrast, in other 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, a pattern indistinguishable from that in carriers of FHLH. Remarkably, most of the patients with low perforin expression had a history of multiple previous episodes of MAS. [26]

It has also been reported that decreased absolute numbers of NK cells and/or depressed NK cell cytolytic activity might be a feature that distinguishes the patients with systemic juvenile rheumatoid arthritis from those with other forms of juvenile rheumatoid arthritis. [27-28] This observation may be another clue to the understanding of the reasons for increased incidence of MAS in soJRA. These data also suggest that the pathway common to both MAS and HLH is likely to be associated with abnormal cytolytic functions.

### **Cytotoxic cell function and cellular immune responses**

The exact mechanisms that would link deficient NK cell and cytotoxic T lymphocyte functions with expansion of activated macrophages are not clear. Two alternative explanations have been suggested in the literature. One is related to the fact that HLH patients appear to have diminished ability to control some infections. [24] More specifically, NK cells and cytotoxic T lymphocytes fail to kill infected cells and, thus, to remove the 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. The fact that MAS episodes are often triggered by the viruses from the Herpes group does support this hypothesis. The Herpes viruses, including CMV and EBV, have evolved evasion mechanisms to down-regulate or sequester MHC Class I molecules, thus preventing efficient cytolytic CD8<sup>+</sup> T cell responses. In contrast, such down-regulation of the expression of MHC Class I molecules serves as an activating signal for NK cells that triggers their cytolytic activity against infected cells. Therefore, NK cells become particularly important in the defense against the Herpes group viruses. Consistent with this idea, infection of NK-depleted or perforin deficient mice with Murine

Cytomegalovirus (MCMV) does result in an exaggerated immune response associated with more persistent expansion of T cells that secrete IFN- $\gamma$ , an important macrophage activator. [28-29]. As mentioned previously, viral tropism such as in EBV-HLH, may provide another mechanism important in MAS. [19]

However, in many cases of MAS, vigorous attempts to identify an infectious trigger are not successful, and some episodes appear to be triggered by modifications in the drug therapy rather than infection. Furthermore, the importance of NK cells and perforin based systems in the down regulation of the cellular immune responses, have been demonstrated in the experimental animal systems where immune responses were elicited by anti-CD3 antibodies or staphylococcal toxins instead of viruses. [30-31] For instance, Kagi et al. demonstrated that the injection of staphylococcal enterotoxin B (SEB) into perforin-deficient mice results in dramatically increased selective expansion and prolonged persistence of CD8<sup>+</sup> but not CD4<sup>+</sup> SEB-reactive T cells. [32] These experiments suggest that there is likely to be a more 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 the antigen-presenting cells and/or activated T cells after infection is cleared. [32] Such T cells may continue to secrete cytokines including IFN- $\gamma$  and GM-CSF, two important macrophage activators. [33] Subsequently, the sustained macrophage activation results in tissue infiltration and in the production of high levels of TNF- $\alpha$ , IL-1, and IL-6 which play a major role in the various clinical symptoms and tissue damage. A report on successful treatment of juvenile rheumatoid arthritis associated MAS by cyclosporin A with transient exacerbation of MAS by conventional-dose G-CSF, does support this hypothesis. [34]

### **Coagulation abnormalities**

A DIC-like picture is a cardinal feature of MAS that is major contributor to the morbidity and mortality in this syndrome. Therefore, the development of coagulopathy in MAS deserves a separate discussion. In the early reports, the coagulation abnormalities observed in this syndrome have been interpreted by several authors as "consumption coagulopathy" triggered by the vasculitic component of the disease. [3] Others have proposed that severe liver dysfunction induced by macrophages infiltrating the liver parenchyma is central to its pathogenesis. [5] Indeed, liver disease may result in a complex coagulopathy caused by decreased synthesis of clotting factors, such as fibrinogen. The liver also produces inhibitors of coagulation such antithrombin III, protein C and S, and is the clearance site for activated coagulation factors and fibrinolytic enzymes. Thus, patients with severe liver dysfunction are "hypercoagulable" and predisposed to developing DIC and may develop systemic pathological fibrinolysis. For these reasons, coagulation defects in advanced liver disease are often difficult to distinguish from those in DIC.

A MAS patient reported by Prahalad and colleagues, however, developed severe acute hemorrhagic diathesis in the absence of significant liver dysfunction. The vasculitic component in the skin

lesions was not prominent. [7] The most striking feature was the intense perivascular infiltration of the dermis with activated macrophages and T cells. There is ample evidence that activated macrophages themselves may have significant procoagulant activity. Thus in an inflammatory response, they can be induced to produce fibrin stabilizing factor XIIIa and hemostatic tissue factor. Tissue factor expressed on monocytes and on TNF- $\alpha$ -stimulated vascular endothelial cells, has been shown to be central to the pathogenesis of DIC accompanying septicemia. [35] Massive accumulation of activated macrophages in the skin lesions in the patient described by Prahalad et al suggests that similar mechanisms may be relevant to the development of coagulation abnormalities in MAS as well.

A possible role for TNF- $\alpha$  in the development of coagulopathy in MAS has been suggested in many reports. Indeed, increased serum levels of TNF- $\alpha$ , a major macrophage-derived pro-inflammatory cytokine, have been demonstrated in several cases of MAS. [36] Furthermore, excessive amounts of TNF- $\alpha$  have been implicated in the pathogenesis of disseminated intravascular coagulation. [37] The importance of TNF- $\alpha$  in the development of coagulation abnormalities in soJRA was also stressed by de Benedetti et al. in a study that demonstrated strong correlation between the serum levels of soluble TNF receptors and prolongation of PTT and decrease in prothrombin activity. [38]

## **Conclusion**

The hypothesis that impaired cytotoxic functions and the lack of immunoregulatory role of NK cells are relevant to the development of MAS in systemic onset juvenile rheumatoid arthritis still remains to be proven. It is becoming increasingly clear, however, that the understanding of MAS pathophysiology may not only define better treatment and improve the outcome in this syndrome, but also provide important clues to the discovery of new pathways involved in the down-regulation of cellular immune responses in humans.

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