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Histiocytic Disorders: Recent Insights into Pathophysiology and Practical Guidelines

Alexandra Filipovich,[1] Kenneth McClain,[2] Alexei Grom[1]

The Histiocytoses are defined as non-malignant disorders due to abnormal accumulation and behavior of cells of the mononuclear phagocytic system. The best known histiocytoses, Langerhans cell histiocytosis (LCH), and hemophagocytic lymphohistiocytosis (HLH), each with an estimated incidence of 1/50,000 to 1/150,000, are sufficiently "common," complex and costly, to constitute an important problem in medical practice. At the same time, LCH, HLH and an array of other and more rare histiocytoses are sufficiently uncommon that most physicians lack the experience to diagnose, let alone care for patients with these conditions. The pathophysiology of most of the histiocytoses is unknown and, in the case of the widely-disseminated and potentially fatal forms, treatments to date have been variably effective and sometimes highly toxic. MAS has been reported to occur in association with almost any rheumatic disease, it is by far most common in the systemic form of Juvenile Idiopthic Arthritis (SoJIA). It is now recognized that MAS bears a close resemblance to Hemophagocytic Lymphohistiocytosis or HLH, and MAS is recognized as the major fatal complication of soJIA.

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OVERVIEW OF THE HISTIOCYTOSES

The mononuclear phagocytic system consists of dendritic cells and monocyte/macrophages, historically referred to as histiocytes. The histiocytoses comprise a wide variety of individually rare and diverse conditions that affect both children and adults, and range from benign skin lesions to rapidly life-threatening systemic disorders. Progress in understanding the molecular and biologic underpinnings of the histiocytoses has been hindered by (1) the rarity of individual disorders, (2) lack of funding for "orphan" diseases, (3) lack of suitable animal models for many of the histiocytoses, and (4) dispersion of patients with histiocytoses among many medical subspecialties.

Mononuclear phagocytes (histiocytes) arise from the myeloid hematopoietic lineage. Their ultimate fate, however, in terms of biologic characteristics and location in the human body, is directed by the cytokine and chemokine environments that are elaborated by other cell lineages. Normal functions of histiocytes, which are a major component of the innate immune system, include phagocytosis (housekeeping and recycling), and antigen presentation and activation of the adaptive immune system through direct contact and cytokine signaling. Histiocytes normally participate in wound and tissue repair and have key roles in initiation, as well as, regulation of inflammation.

The true incidences of histiocytoses are not known, and may vary according to ethnicity and inbreeding (as in the case of the autosomal recessive disease HLH, which is more common in some isolated regions of the world and in populations where consanguinous marriages are practiced).

The histiocytic disorders are generally defined by their constitutive cell type, on the basis of widely recognized pathologic and histologic criteria (Table 1). Pathologists now accept that that these morphologic and biochemical criteria must also be paired with a relevant clinical context, for example, clinicopathologic criteria.

LCH

LCH, published as such in 1868 by the 21-year-old Paul Langerhans, is reported to be the most common of the histiocytoses. Reports of a nonfatal disease with painful skull lesions date back to Hippocrates. Between 1893 and 1920 Hand, Schűller and Christian elaborated the

From the [1]Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; and [2]Texas Children's Cancer Center, Houston, Texas.
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Correspondence and reprint requests: Alexandra Filipovich, MD, Division of BMT & Immune Deficiency, 3333 Burnet Avenue, MLC 7015, Cincinnati, OH 45229 (e-mail: list.filipovich@cchmc.org).

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Table 1. Classification of Histiocytic Disorders

Benign disorders of varying biologic behavior
a. Dendritic cell related
Langerhans cell histiocytosis
Juvenile xanthogranuloma and related disorders including:
- Erdheim-Chester disease
- Solitary histiocytomas with juvenile xanthogranuloma phenotype
- Secondary dendritic cell disorders
b. Monocyte/macrophage related
Hemophagocytic lymphohistiocytosis
Familial and sporadic
Secondary hemophagocytic syndromes:
- Infection associated
- Malignancy associated
- Autoimmune associated (MAS)
- Other
Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease)
Solitary histiocytoma of macrophage phenotype

LCH triad of skull defects, exophthalmos, and diabetes insipidus. Thus, in the medical literature LCH has been referred to as Hand-Schuller-Christian disease and Histiocytosis "X" (denoting the uncertainty about the disease and its pathogenesis).

It is now well recognized that LCH can present at any age from the neonatal period to old age. The spectrum of clinical presentations is broad and protean. LCH lesions may spontaneously regress, or repeatedly "reactivate" contributing to long-term disabilities such as diabetes insipidus and neurodegenerative disease. Life-threatening forms of LCH, previously referred to as Letterer-Siwe disease, typically present in infancy and clearly require intensive therapy and, sometimes, salvage therapy such a allogeneic bone marrow transplantation to cure the children.

Clinical involvement with LCH can be highly variable, but most often involves bony lesions (present in about 80% of cases), which may be painful or painless, as is common with skull lesions. Skin involvement, typically papulosquamous lesions, often affecting the scalp (and frequently mistaken for cradle cap, seborrhea) is reported in 30% to 60% of patient series. Soft tissue swelling, often in proximity to bony lesions, external ear drainage, enlargement of lymph nodes and thymus, and gum hypertrophy with premature eruption of baby teeth, represent the constellation of symptoms that are relatively easily treated. More serious systemic involvement (eg, as described for Letterer-Siwe disease) includes fevers, hepatosplenemagaly, liver dysfunction, and hematopoietic failure ± intestinal involvement. The latter findings are typical of the widely disseminated form of LCH seen in young infants, which carries a high mortality rate.

Etiopathogenesis of LCH

The name LCH suggests that the dendritic Langerhans cell usually located in the dermal/epidermal border of the skin is the key cell of the disease. To date, this has not been proven. Over the years LCH has been classified as a neoplasm, a reactive disorder or an aberrant immune response. Associated genetic defects, if any, have not been described. LCH cells are considered pathologic in that they bear heterogeneous characteristics not normally identified in the Langerhans cells, which are typically resident to the skin.The granulomatous lesions of LCH, which can be found in nearly any organ, represent an accumulation of normal inflammatory cells including eosinophils, lymphocytes (especially T cells) and macrophages in addition to the LCH cells. Although clonal accumulations of LCH cells have been described, current thinking suggests that LCs home to the skin during embryonic development and, like segments of skin within which they reside, naturally expand from single progenitor cells rather than through neoplastic proliferation.

Although there is ample evidence that LCH cells react to the local cytokine and chemokine environment, and may be particularly susceptible to signals from local T cells, the pathogenesis of LCH remains elusive. In the past, studies of LCH biology have focused largely on histochemistry to characterize abnormalities in LCH cells versus normal Langerhans cells. Such approaches have been limited by the specific tools used and the specific hypotheses that were being tested.

More recently, the genome wide discovery approach has been used to identify the key active molecules and pathways in human LCH samples (C. Allen and K. McClain, Texas Children's Hospital, largely unpublished). Purified $CD207^+$ (langerin$^+$) cells and $CD3^+$ cells isolated from LCH lesions have been compared to their counterparts from normal tissues. Microarray experiments yielded some expected results, but also some surprising results. The 29 genes upregulated more than 4-fold in the pathologic LCH LCs included some genes previously described as differentially expressed, including E-cadherin and RANTES. Additionally, some genes not previously associated with LCH that regulate lymphocyte activation and migration, including osteopontin, vanin-1, and LAMP-3, were upregulated in LCH LCs. However, failure to identify differences in expression in specific genes previously published to be associated with these functions was unexpected, including CCR6 and CCR7, cell surface markers involved in T cell activation (CD40, CD80, CD86), pro-inflammatory cytokines (tumor necrosis factor [TNF]-α, interferon [IFN]-γ, interleukin [IL]-1α, IL-1β, leukemia inhibitory factor [LIF], IL-4, IL-10, tumor growth factor [TGF]-β), genes involved in regulation of apoptosis (Flt-3, Flt3-ligand, bcl-2), or markers of cell proliferation (Ki67, PCNA, p53). The comparative gene expression profile observed does not appear consistent with one of the prevailing models of LCH cells as (possibly malignant), activated, immature dendritic cells that independently elaborate a "cytokine storm." So, back to "Histiocytosis X."

Diagnosis and Treatment of LCH

The diagnosis of LCH is based on the histologic and immunophenotypic examination of lesional tissue. The primary diagnostic feature is the morphologic identification of characteristic LCH cells. Additionally, positive staining of the lesional cells with CD1a and/or Langerin (CD207) is required for definitive diagnsosis.

Guidelines for treatment of LCH have been elaborated by the LCH working party of the Histiocytosis Society, and are available on the Web pages of that organization. The recommendations are briefly summarized here. The goal of therapy in LCH is to decrease the activity and proliferation of histiocytes , lymphocytes, and other immune cells that contribute to disease activity.

Patients with disease that is localized to skin, bone, and lymph nodes (defined as "nonrisk" organs) generally have a good prognosis and require minimal treatment. However, patients with lesions in "risk" organs (liver, spleen, lung, bone marrow) have a worse overall prognosis in terms of morbidity and mortality. Patients with LCH in the central nervous system (CNS), vertebrae, facial bones, or bones of the anterior or middle cranial fossa are at higher risk for morbidity and recurrent disease. LCH in the orbit, mastoid, or temporal skull regions are classified as "CNS risk" because of increased frequency of associated diabetes insipidus (DI) and other endocrine or parenchymal brain lesions. LCH in only 1 bone of "nonrisk" location is generally benign, and the disease responds well to several treatment modalities including observation, surgical excision, steroid injection, or indomethacin therapy.

As a result of evaluation of several sequential treatment trials, the Histiocyte Society has stratified patients with LCH into "low" (LR) and "high" (HR) risk groups based on outcomes related to extent and location of the LCH lesions. LR patients include patients with skin, bone, lymph node, and pituitary involvement. Patients with lung, liver, spleen, and bone marrow involvement usually have a worse prognosis and are considered higher risk. However, children with lung LCH without involvement of other HR organs respond to treatment much better than those with other HR organs [1].

Preliminary results from the LCH-III study show that HR patients treated with velban/prednisone/mercaptopurine did as well as those randomized to also receive intravenous methotrexate during induction and oral methotrexate during continuation treatment. When patients do not respond adequately by the sixth week of treatment it is recommended that they now be switched to a salvage protocol (LCH-S-2005) with high doses of cladribine and cytarabine [2]. The LR patients treated with velban and prednisone for 12 months had fewer relapses than those treated for 6 months. Further follow-up data is needed to confirm this.

Retrospective analysis of "CNS-risk" patients treated with only surgery, steroid injection, radiation therapy, or a single chemotherapy drug have a 40% incidence of DI [3]. If they receive velban and prednisone for 6 months the incidence of DI is 20% [4]. Complications of developing DI include a 50% incidence of anterior pituitary hormone deficiencies and 50% incidence of developing radiographic or clinical evidence of a neurodegenerative syndrome (ND-CNS-LCH) [5]. Twenty-five percent of patients diagnosed with ND-CNS-LCH with the radiographic abnormalities of T2 hyperintense signals in the cerebellum, basal ganglia, or pons have clinical symptoms that include ataxia, dysarthria, dysmetria, learning, and behavior difficulties [5]. Treatment with intravenous immunoglobulin or *trans*-retinoic acid resulted in stabilization, but no improvement of the symptoms. A recently published study describes the efficacy of cytarabine treatment of patients with ND-CNS-LCH [6].

LCH cells express CD1a and CD52—potential targets for treatment with monoclonal antibodies anti-CD1a, which is under development, and anti-CD52 (Campath) [7]. Some LCH patients have insufficient responses to the salvage therapy and are candidates for stem cell transplant. Patients with severe hematologic dysfunction, in particular, may be directed to hematopoietic cell transplant (HCT).

HCT for LCH

HCT is a theoretically attractive option for management of refractory LCH because of its immunomodulatory potential. Many published reports have described 1 or a few cases, and date back more than 20 years. The collected experience with HCT for LCH in the literature up until 2008 was recently compiled [8]: 44 pediatric cases, of which the vast majority presented in infancy. The majority of patients in that review received myeloablative conditioning, typically consisting of combinations of Busulfan, Cytoxan, VP-16, and/or TBI. Survival data was available for 34/44 cases indicating 59% 5-year overall survival (OS) for patients with allogeneic donors, compared with 25% OS with the use of autologous HCT. Infections were the major cause of death in this historic review, followed by regimen related toxicity. Importantly, 24 of 26 patients were disease free at last follow-up. The most recently published series from Japan described 15 children transplanted at a median age of 23 months since 1995. Ten of 15 HCT utilized allogeneic cord blood donors. Ten-year OS in this group was 73%, but only 55% among the 9 patients who had LCH "risk organ" involvement at the time of HCT [9].

There has been increased interest in the use of reduced-intensity conditioning (RIC) for these cases [10]. The largest single-center experience with RIC

HCT for LCH (9 patients) was reported from Vienna. All patients received Fludarabine in their pre-HCT regimen, 8 received Melphalan; additionally total lymphoid irradiation (TLI) or total-body irradiation (TBI), antithymocyte globulin (ATG), or Campath were added in a varying number of this patient group. Seven of 9 are long-term disease-free survivors [11]. Despite these more encouraging results, allogeneic HCT for HR LCH has been associated with significant mortality. HCT is a reasonable option for this patient population but the optimal timing, preparative modalities, and donor source(s) remain unclear.

HLH

HLH is a collection of nonmalignant, but frequently life-threatening disorders, associated with an ever-growing list of genetic and acquired causes. The predominant clinical findings of HLH are fevers (often hectic and persistent), cytopenias, hepatitis, and splenomegaly.

Until recently, it was widely believed that symptoms of HLH because of genetic causes arose during infancy and early childhood. With the more widespread availability of genetic testing, it is apparent that the first significant episode of HLH can occur throughout life from prenatal presentations through the seventh decade. Distinctions between primary (genetically determined) and secondary (acquired) forms of HLH become increasingly blurred together as new genetic causes are identified and patients who develop HLH beyond early childhood or in the contexts of EBV infection or autoimmune disease are being found to share some of the same genetic etiologies.

Etiopathogenesis of HLH and Related Disorders

Hemophagocytic lymphohistiocytosis (HLH) results when critical regulatory pathways responsible for the natural termination of immune/inflammatory responses are disrupted or overwhelmed. In HLH, pathologic genetic defects alter normal crosstalk between innate and adaptive immune responses in a manner that compromises homeostatic removal of cells which are superfluous or dangerous to the organism. The result is excessive and persistent activation of antigen presenting cells (histiocytes) and T lymphocytes. The clinical findings associated with systemic inflammation such as prolonged high fevers and hepatitis reflect this immunologic perturbation.

Currently, HLH (FHL:OMIM 267700, 603553) includes the autosomal recessive genetic disorders as well as the "secondary" forms. In recent years, many more cases of hemophagocytic disorders are being diagnosed, especially in the context of severe inflammatory reactions to viral exposure including, in addition to Epstine-Barr virus (EBV)-HIV and Avian influenza.

HLH is characterized by multisystem inflammation—a reactive process resulting from prolonged and excessive activation of antigen presenting cells (macrophages, histiocytes) and $CD8^+$ T cells, and excessive proliferation and ectopic migration of T cells.

Abnormalities in the function (and sometimes quantity) of NK cells have been observed in a proportion of patients with all forms of HLH. Natural killer (NK) and natural killer T (NKT) cells, in particular, play a major role in maintaining a healthy threshold of immune responsiveness to noxious external stimuli, and are critical to prevention and control of autoimmune conditions and severe reactions to viral infections. NK cells (using their cytotoxic and cytokine-mediated mechanisms) form a front line of defense against intracellular pathogens, such as viruses, which infect nonlymphoid tissues early upon entry into the organism. NK cells modulate the initial responses of antigen-presenting cells to incoming pathogens (likely through cytokine signaling), thus attenuating the signals subsequently communicated to antigen-specific T cells. NK cells likely also play a role in culling activated T cells and histiocytes in later stages of antigen-driven activation, contributing to the natural contraction of immune responses.

Also critical to the contraction process of active T cell populations is the mechanism of activation-induced apoptosis, recently shown to be defective in X-Linked Lymphoproliferative syndrome type1 (XKLP1) [11]. As with NK cell cytotoxicity, this process is driven by granule-mediated cytotoxicity .

Studies of cytokine levels in blood and tissues have indicated persistently elevated circulating levels of multiple proinflammatory cytokines during symptomatic disease. It is currently believed that "hypercytokinemia" generated by uncontrolled activation of histiocytes and T cells underlies the progressive organ dysfunction that can eventually lead to death. These symptoms and signs include fevers, hyperlipidemia, endothelial activation/coagulopathy, hepatitic triaditis, central nervous system (CNS) vasculitis and demyelination, inflammatory lung disease with acute respiratory distress syndrome (ARDS), and marrow hyperplasia or aplasia. Hemophagocytosis, for which finding the disorder was named, is a hallmark of activated macrophages/histiocytes.

Genetic Causes of HLH and Related Disorders

To date, autosomal recessive genetic defects associated with HLH are related to one another along the pathway of granule-mediated cytotoxicity. These genetic defects interrupt mechanisms responsible for triggered apoptosis (mediated by cytotoxic cells upon the target cell). Other causal defects interrupt activation induced apoptosis (putative suicide of activated T cells). The first gene reported in 1999 to be a cause

of HLH was perforin, FHL2 [12] , a soluble, pore-forming cytolytic protein synthesized in cytotoxic lymphocytes and sequestered, along with granzyme serine proteases, in secretory cytotoxic granules. When cytotoxic cells contact their targets, the intracellular cytoskeletal scaffold (the microtubule organizing center [MTOC]) is rotated to focus on the contact site where the cytotoxic immunologic synapse forms. Cytotoxic granules are carried along the MTOC toward the immunologic synapse where they degranulate allowing perforin and Granzyme B to enter the contact zone, permeabilize the target cell membrane, and enable delivery of Granzyme B into the target cell. Once internalized, Granzyme B initiates both caspase-dependent and caspase-independent apoptotic pathways, killing the target cell.

A second gene responsible for HLH (FHL3) was reported in 2003: MUNC 13-4. MUNC 13-4 was described as essential for cytolytic granule fusion with other structures related to the cytoplasmic membrane in the process of degranulation [13]. The gene defect responsible for FHL4 is Syntaxin 11 [14], which has been shown, as in MUNC 13-4 deficiency, to result in defective degranulation. Syntaxin 11 is a member of the SNARE protein family, which facilitates fusion in intracellular membrane trafficking events. More recently, mutations in the syntaxin binding protein, MUNC 18-2, have been identified to be associated with HLH (tentatively named FHL5) [15].

Related hemophagocytic disorders occur with significant frequency in 5 other genetic diseases, several of which have been linked with defective cytotoxic function. Three distinct immunodeficiencies that are typically associated with pseudoalbinism because of defects in lysosomal trafficking have been associated with life-threatening episodes of HLH: Chediak Higashi syndrome (LYST, or CHS1), Griscelli syndrome (Rab27A), and Hermansky-Pudlak syndrome type II (AP3B1). Rab27a, a small Rho GTPase, interacts directly with MUNC 13-4 and is thought to play a role in docking of the cytotoxic granules on the MTOC.

HLH following exposure to EBV and less commonly other viruses, termed fulminant infectous mononucleosis, is the most frequent life-threatening complication of XLP1. XLP1 is caused by hemizygous mutations in SH2D1A encoding SAP (SLAM Associated Protein), which lead to abnormal NK cell responses and iNKT cell deficiency. Recent research suggests that lymphocytes from patients with XLP1 demonstrate decreased activation-induced apoptosis, which contributes to the lymphoproliferative clinical phenotypes. XLP2, caused by hemizygous mutations in XIAP, or BIRC4, has been described in males who develop sporadic as well as EBV-associated HLH [16]. Patients with XLP1 and XLP2 may survive into adulthood in good health before succumbing to a life-threatening complication of their underlying disease. Thus, lack of prior significant medical history should not exclude consideration of these diagnoses.

Taken together, the 9 genetic disorders described above still account for less than half of the diagnosed cases of HLH in children, including many familial cases still awaiting molecular defiinition.

Diagnosis of HLH

The first significant episode of FHL can occur throughout life, including in utero. Despite attempts to differentiate primary from secondary or reactive forms of HLH, the symptomatic presentations are highly overlapping, and a significant proportion of patients diagnosed with "secondary" HLH are at risk of dying from the disease. In the most typical form of FHL, the clinical course is characterized by prolonged fevers and hepatosplenomegaly. Neurologic symptoms may dominate the initial clinical course with seizures and/or ataxia. Neurologic findings may be highly variable. Standard blood testing typically reveals cytopenias—especially anemia and thrombocytopenia, liver dysfunction, hypofibrinogenemia, hypertriglyceridemia, hypoalbuminemia, and hyponatremia. In the early days to months of the disease, symptoms may improve spontaneously, followed by clinical exacerbations. Importantly, hemophagocytosis may not be obvious on bone marrow biopsy examination early in the course of the disease.

To assist with the rapid diagnosis of HLH, the Histiocyte Society has developed a set of diagnostic guidelines that encompass both clinical and laboratory findings [17]. With additional experience these diagnostic criteria have been modestly modified, as shown in Table 2. A constellation of these features in the absence of a family history or specific genetic diagnosis can contribute to a provisional diagnosis of HLH and support the need for initiation of HLH-specific therapy.

Additional criteria for provisional diagnosis of HLH include elevated levels of ferritin [18] and soluble IL2Rα (sCD25) [19]—both markers of generalized inflammation. Ferritin is induced during the protective anti-inflammatory process of macrophage scavenging of heme through the CD163 receptor, as is IL-10. In the majority of FHL cases, NK function is low or absent, although the number of circulating NK cells ($CD56^+/16^+$) are generally normal. However, the

Table 2. Diagnostic Criteria

A molecular diagnosis consistent with HLH (see above) or at least 5 of 8 criteria listed below
- Fever, typically persistent daily
- Splenomegaly
- Bicytopenia: Hb <9 g/dL; platelets $<100 \times 10^9$/L; neutrophils $<10^9$/L
- Hypertriglyceridemia and/or hypofibrinogenemia
- Hemophagocytosis in bone marrow (BM), spleen, lymph node, or CSF
- Low or absent NK function (note: only present in approximately 50% of HLH patients)
- Elevated ferritin (>500 μg/L)
- Soluble CD25 (IL2Ra) above normal limits for age.

finding of NK function within normal limits, especially during active symptomatic disease, should not preclude a diagnosis of FHL or secondary HLH.

Symptoms of CNS dysfunction, cerebral spinal fluid (CSF) pleocytosis, or findings of foci of inflammation by CNS magnetic resonance imaging (MRI) scanning, are found in nearly half of patients with HLH during the first few months after initial diagnosis.

Treatment of HLH

Because HLH can be rapidly fatal without specific intervention, it is recommended that treatment be started when there is a high clinical suspicion, even when results of some diagnostic studies are still pending. Retrospective review of FHL 25 years ago described mean survival of less than a month after symptomatic onset and 5% overall survival at 1 year after diagnosis. Today, effective initial therapy HLH (FHL) consists of combinations of proapoptotic chemotherapy and immunosuppressive drugs targeting the hyperactivated T cells and histiocytes. Commonly used and effective agents include: Etoposide (VP-16), steroids, and ATG antibodies.

HCT for HLH

Definitive treatment and potential cure of FHL is only achieved by HCT. Projected survival rates 5 years from diagnosis range from 50% to 70% [20]. The best results following myeloablative conditioning have been achieved when HLA-matched related or unrelated donors were used, and CNS disease was absent or quiescent at the time of HCT. The largest series in the literature using unrelated donors reported outcomes of 91 patients transplanted in the United States between 1989 and 2005. Most patients were conditioned with Busulfan, cytoxan, and VP-16 with or without ATG. Early mortality rates after HCT were unacceptably high: 35% at day 100; projected "cure" for approximately 50% of patients. Early deaths were attributable to infection, graft-versus-host disease (GVHD), nonengraftment, venoocclusive disease, and pneumonitis. In addition a significant number of deaths were attributed to HLH reactivation. In contrast, patients who survived beyond day 100 with durable engraftment remained free of HLH at last follow-up [21]. Very similar results were reported for 61 patients who underwent HCT over a 17 year period in Italy [22] and 43 patients with familial HLH in Japan [23]. The Japanese series also included 14 cases of EBV-associated HLH who experienced a somewhat better long-term survival: 86%.

More recently, use of RIC prior to HCT has been investigated [24]. To date, most cases of RIC HCT for HLH have employed Campath, which specifically targets the T cells and antigen-presenting cells (APCs) involved in HLH pathogenesis. Experience at several centers is consistent with superior early posttransplant survival. Approximately half of patients with HLH treated with RIC, however, experience mixed donor chimerism, which may be unstable in the early months posttransplant, and raises concerns for subsequent HLH relapse.

Macrophage Activation Syndrome (MAS)

MAS is a severe, potentially fatal condition associated with excessive activation and expansion of macrophages and T cells leading to an overwhelming inflammatory reaction. The main manifestations of MAS include fever, hepatosplenomegaly, lymphadenopathy, severe cytopenias, serious liver disease, and coagulopathy consistent with disseminated intravascular coagulation [25]. The pathognomonic feature of MAS is often found in bone marrow: hemophagocytosis. Although MAS has been reported to occur in association with almost any rheumatic disease, it is by far most common in the systemic form of Juvenile Idiopthic Arthritis (SoJIA). Systemic Lupus Erythematosus and Kawasaki disease are 2 other examples of conditions in which MAS appears to occur somewhat more frequently than in other rheumatologic diseases [26,27]. It is now recognized that MAS bears a close resemblance to HLH. The true incidence of MAS may be underestimated because relatively mild cases of MAS often remained unrecognized. Indeed, despite the lack of diagnostic criteria, because of increasing awareness of MAS, this syndrome is being diagnosed more and more frequently. Recent evidence suggests that mild subclinical MAS might be occurring in as many as one-third of patients with active systemic disease, and may be the first manifestation of soJIA. Infections or change in medications may precede the diagnosis of MAS; in most patients MAS is triggered by a flare of the underlying rheumatologic disease.

Published observations suggest that, as in HLH, MAS patients have profoundly depressed NK cell function, often associated with abnormal perforin expression [28-30], and these abnormalities are often associated with specific perforin [31] and MUNC13-4 [32,33] polymorphisms.

Diagnosis and Treatment of MAS

There are no validated diagnostic criteria for MAS, and early diagnosis is often difficult. Thus, in a patient with persistently active underlying rheumatologic disease, a fall in the erythrocyte sedimentation rate (ESR) and platelet count, particularly in a combination with persistently high C-reactive protein (CRP) and increasing levels of ferritin, should raise a suspicion of impeding MAS. The diagnosis of MAS is usually confirmed by the demonstration of hemophagocytosis in the bone marrow. Assessment of the levels of sIL2Rα and sCD163 in serum may help with the timely diagnosis of MAS. Although mild elevation of

sIL2Rα has been reported in many rheumatic diseases including JIA and SLE [34], a several-fold increase in the levels of sIL2Rα in these diseases is highly suggestive of MAS [35].

The application of the HLH diagnostic criteria to systemic JIA patients with suspected MAS is problematic. Some of the HLH markers such as lymphadenopathy, splenomegaly, and hyperferritinemia, are common features of active systemic JIA itself, and therefore, do not distinguish MAS from a conventional systemic JIA flare. Patients with systemic JIA often have increased white blood cell and platelet counts as well as serum levels of fibrinogen as a part of the inflammatory response seen in this disease. Therefore, when they develop MAS, they reach the degree of cytopenias and hypofibrinogenemia seen in HLH only at the late stages of the syndrome when medical management becomes challenging. This is even more problematic for the diagnosis of MAS in patients with sytematic lupus erythematosus (SLE) in whom autoimmune cytopenias are a common and difficult to distinguish from those caused by MAS.

Early recognition of this syndrome and immediate therapeutic intervention to produce a rapid response are critical. Prompt administration of more aggressive treatment in these patients may, in fact, prevent development of the full-blown syndrome. To achieve rapid reversal of coagulation abnormalities and cytopenias most clinicians start with intraveneous methylprednisolone pulse therapy (30 mg/kg for 3 consecutive days) followed by 2 to 3 mg/kg/day divided in 4 doses. After normalization of hematologic abnormalities and resolution of coagulopathy, steroids are tapered slowly to avoid relapses of MAS. Not uncommonly, however, MAS appears to be corticosteroid resistant, with deaths being reported even among patients treated with massive doses of steroids.

Parenteral administration of cyclosporine A has been shown to be highly effective in patients with corticosteroid-resistant MAS [36]. The utility of biologic drugs in MAS treatment remains unclear. Although TNF inhibiting agents, biologics that neutralize IL-1, and IL-6 have been reported to be effective in occasional MAS patients [37]; other reports describe patients in whom MAS occurred while they were receiving these agents [38].

Based on some success with intravenous immunoglobulin (IVIG) administration in virus-associated reactive HLH, this treatment might be effective in MAS triggered by viral infection. If MAS, however, is driven by EBV infection, Rituximab, a monoclonal antibody that depletes B lymphocytes, the main type of cells harboring EBV virus should be used [39].

HCT for MAS

No concerted effort to apply allogeneic HCT for definitive treatment of MAS has yet been made. However, it is likely that some patients, defined as HLH and/or with associated genetic defects, have undergone HCT. Given the high mortality associated with MAS as it is currently managed, the option of allogeneic HCT using less intensive conditioning protocols, is reasonable to consider, especially in cases of severe or recurrent MAS episodes. Indeed, sometimes fatal, MAS was observed as a complication of prolonged T cell immunodeficiency in early trials of autologous HCT for severe progressive systemic or polyarticular juvenile idiopathic arthritis. These experiments of nature suggested a failure to control the underlying condition given the patient's genetically predisposed hematopoietic cells [35].

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