27 Hemophagocytic Lymphohistiocytosis

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Keywords
Hemophagocytic lymphohistiocytosis (HLH) · Perforin/granzyme pathway · Familial hemophagocytic lymphohistiocytosis (FHL) · Secondary hemophagocytic lymphohistiocytosis (secondary HLH) · Hyperinflammation

27.1 Case Report

A previously healthy 11-month-old girl was transferred to the emergency room (ER) with a 7-day history of high fever and progressive lethargy. Her parents reported that she had developed mild cough and rhinorrhea 1 week before presentation. These symptoms were followed by severe illness with high fever, distended abdomen, skin rash, and poor oral intake. She was empirically started on broad-spectrum antibiotics at the referring hospital before transfer.

Upon arrival at the ER, the patient appeared acutely ill. She was febrile (39.2 °C), tachycardic, and tachypneic. Physical examination was notable for generalized petechiae and substantial hepatosplenomegaly. A complete blood count test demonstrated pancytopenia (white blood cells, 3290/μL; hemoglobin, 7.9 g/dL; platelets, 34,000/μL; and neutrophils, 691/μL). A peripheral blood smear did not reveal any malignant cells. The patient also had elevated serum levels of aspartate aminotransferase (985 U/L; reference range, 20–60 U/L), alanine aminotransferase (440 U/L; reference range, 5–45 U/L), bilirubin (2.5 mg/dL; reference range, <1.0 mg/dL), triglycerides (372 mg/dL; reference range, <150 mg/dL), ferritin (12,530 ng/mL; reference range, 10–60 ng/mL), and lactate dehydrogenase (620 U/L; reference range, 150–500 U/L). A coagulation profile indicated normal prothrombin time and activated partial thromboplastin time with hypofibrinogenemia (110 mg/dL; reference range, 150–400 mg/dL). Chest radiography revealed bilateral pleural effusions, and computerized tomography (CT) scans of the abdomen and pelvis showed multiple, prominent mesenteric lymph nodes, ascites, and hepatosplenomegaly. A CT scan of the head was normal with no evidence of an intracranial mass or acute bleeding. Bone marrow examination was performed on hospital day 1 and demonstrated hypercellular marrow with 90% cellularity and remarkable hemophagocytosis without any obvious evidence of malignant cells (Fig. 27.1).

The combination of the patient's clinical features (i.e., fever and splenomegaly) and laboratory findings (i.e., pancytopenia, hypertriglyc-

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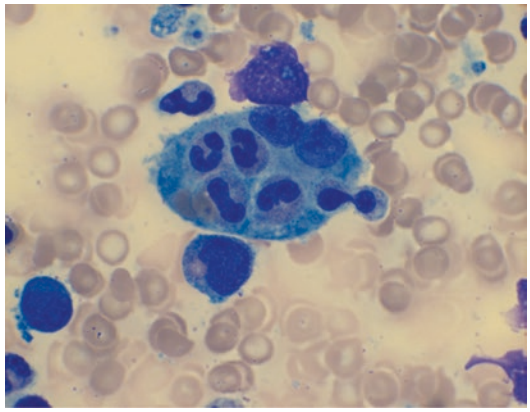

Fig. 27.1 Hemophagocytosis seen in bone marrow aspirates

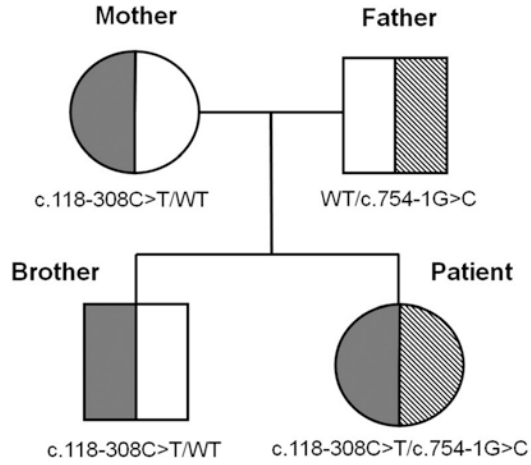

Fig. 27.2 Genetic pedigree of *UNC13D* mutations in the patient and her family

eridemia, hypofibrinogenemia, elevated ferritin level, and hemophagocytosis in the bone marrow) met the revised diagnostic criteria for hemophagocytic lymphohistiocytosis (HLH) proposed by the Histiocyte Society (Henter et al. 2007). The patient was administered dexamethasone (10 mg/m^2), etoposide (150 mg/m^2), and cyclosporine on hospital day 2, according to the HLH-2004 treatment protocol imparted by the Histiocyte Society (Henter et al. 2007).

The results of additional tests were as follows: Serum level of soluble interleukin-2 receptor alpha (IL-2RA) was 22,400 U/mL (reference range, 334–3026 U/mL). Natural killer (NK) cell activity was reportedly decreased. A blood culture was negative for microorganisms. Polymerase chain reaction (PCR) studies of respiratory viruses were positive for parainfluenza virus and rhinovirus. Serologic and/or PCR studies for mycoplasma, Epstein–Barr virus (EBV), and cytomegalovirus were negative. Cerebrospinal fluid (CSF) examination was unremarkable. Brain magnetic resonance imaging revealed no significant findings. Genetic testing for HLH confirmed heterozygous mutations in the *UNC13D* gene (c.118_308C > T and c.754_1G > C). Genetic testing of the parents and a sibling revealed them as heterozygous mutation carriers (Fig. 27.2). After 1 week of treatment, the patient improved in general condition and laboratory findings. After 8 weeks of initial therapy, the patient showed a complete response.

Because familial HLH was diagnosed, the patient and her family were allowed to decide to proceed with allogeneic hematopoietic stem cell transplantation (HSCT). She was kept on continuation treatment with dexamethasone, etoposide, and cyclosporine before receiving HSCT.

She received an allogeneic HSCT from an unrelated donor. The conditioning regimen was reduced-intensity and consisted of fludarabine (150 mg/m^2), melphalan (140 mg/m^2), and alemtuzumab. Cyclosporine and short-term methotrexate were used for graft-versus-host-disease prophylaxis. Allogeneic HSCT was uneventful with successful engraftment. She remains asymptomatic 3 years after the initial presentation with complete donor chimerism.

27.2 Diagnosis

HLH is a disorder of immune cell regulation. It is characterized by distinctive clinical features that are indicative of excessive inflammation and tissue damage and is often associated with genetic defects affecting the cytotoxic function of cytotoxic T cells and NK cells. HLH can be classified into two major forms: primary/genetic and sec-

ondary/acquired. According to the classification of human primary immunodeficiencies by the International Union of Immunological Societies Expert Committee on Primary Immunodeficiency in 2014, primary/genetic HLH is further classified as familial HLH (FHL) syndromes and lymphoproliferative syndromes (Table 27.1) (Al-Herz et al. 2014). FHL syndromes are then further classified into subtypes with and without hypopigmentation. FHL without hypopigmentation, in which HLH is the defining clinical manifestation, has five subtypes (FHL1, FHL2, FHL3, FHL4, and FHL5), which are classified by their causative mutated genes. In FHL with hypopigmentation, HLH is a component of syndromic disease and is usually accompanied by other clinical manifestations, such as defects in pigmentation. This form of HLH includes Chédiak–Higashi syndrome, Griscelli syndrome type 2, and Hermansky–Pudlak syndrome type 2. Lymphoproliferative syndromes can be X-linked (e.g., XLP and XMEN syndrome) or autosomal recessive (e.g., *ITK* deficiency and *CD27* deficiency).

Secondary HLH is not associated with any known genetic abnormality or immunodeficiency syndrome. It is thought to be a kind of reactive phenomenon and can be associated with infection (e.g., infection-associated hemophagocytic syndrome), malignant neoplasms (e.g., malignancy-associated hemophagocytic syndrome), and rheumatic/autoimmune diseases (e.g., macrophage activation syndrome) (Table 27.2).

It is challenging to make a timely diagnosis of HLH in clinical settings because a single diagnostic indicator for HLH other than a genetic diagnosis does not exist. Patients with HLH can present with several clinical features that overlap with those of infection, inflammatory conditions, and malignant neoplasms and are accompanied by various abnormal laboratory tests. Each of these individual findings may not be specific for HLH but collectively can contribute to a diagnosis of HLH. Therefore, diagnostic criteria consisting of clinical and laboratory findings are required to diagnose HLH. The HLH-2004 diagnostic criteria suggested by the Histiocyte Society are most commonly used (Table 27.3) (Henter et al. 2007). Diagnosis of HLH can be established by molecular/genetic findings that are consistent with HLH or when five of the eight criteria (two clinical symptoms, one histopathologic finding, and five laboratory findings) are fulfilled.

The clinical symptoms of HLH can be attributed to severe inflammation, hypercytokinemia, and infiltration of histiocytes into organs. Most patients present with a prolonged and unexplainable fever that is frequently proceeded by suspicious viral infections. Splenomegaly is a direct result of infiltration by activated lymphocytes and macrophages. It is present in 80–100% of patients at diagnosis and is usually progressive (George 2014; Koh et al. 2015).

Hemophagocytosis is a self-destructive phenomenon, consisting of phagocytosis of erythrocytes, leukocytes, platelets, and their precursors in the bone marrow and other tissues by activated histiocytes. Hemophagocytosis is a striking finding in patients with HLH, but it is neither very sensitive nor specific for diagnosis of HLH. Hemophagocytosis is not a defining feature of HLH but is rather a component of the diagnostic criteria. Hemophagocytosis in the bone marrow may not be apparent during the early phase of disease, and therefore the absence of hemophagocytosis does not preclude the diagnosis of HLH. However, bone marrow examination is mandatory not only to search for hemophagocytosis but also to differentiate HLH from hematologic malignancies.

Cytopenias can be attributed to high concentrations of tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ), as well as direct hemophagocytosis (George 2014). Bicytopenia or pancytopenia is observed in most cases of HLH. In some cases, cytopenia may not be present during the early period but eventually appears as the disease progresses.

Hypertriglyceridemia occurs secondary to decreased lipoprotein lipase activity, which is initiated by increased TNF-α and IFN-γ levels (George 2014). Activated macrophages secrete plasminogen activators that lead to high plasmin levels, hyperfibrinolysis, and hypofibrinogen-

Table 27.1 The classification of primary HLH according to the updated classification of human primary immunodeficiencies by the International Union of Immunological Societies Expert Committee on Primary Immunodeficiency, 2014

HLH type	Gene	Protein	Function	Inheritance	Clinical features
1. Familial HLH (FHL) syndromes					
(1) FHL syndromes without hypopigmentation					
FHL1	Unknown	Unknown		AR	HLH is the defining feature
FHL2	*PRF1*	Perforin	Cytolytic pore formation	AR	HLH is the defining feature
FHL3	*UNC13D*	Munc13-4	Vesicle priming for fusion	AR	HLH is the defining feature
FHL4	*STX11*	Syntaxin-11	Vesicle fusion with the cell membrane	AR	HLH is the defining feature
FHL5	*STXBP2*	Munc18-2	Vesicle fusion with the cell membrane	AR	HLH is the defining feature
(2) FHL syndromes with hypopigmentation					
Griscelli syndrome, type 2	*RAB27A*	Rab27a	Vesicle docking to the cell membrane	AR	Partial albinism, hepatosplenomegaly, HLH, cytopenias
Chédiak–Higashi syndrome	*LYST*	LYST	Intracellular protein trafficking	AR	Partial albinism, recurrent infections, hepatosplenomegaly, HLH, giant lysosomes, neutropenia, cytopenias, bleeding tendency, neurologic dysfunction
Hermansky–Pudlak syndrome, type 2	*AP3B1*	AP3B1	Intracellular protein trafficking	AR	Partial albinism, recurrent infections, pulmonary fibrosis, increased bleeding, neutropenia, HLH
2. Lymphoproliferative syndrome					
(1) X-linked					
XLP1	*SH2D1A*	SAP	Intracellular signaling	XR	Triggered by EBV infection, HLH, lymphoproliferation, aplastic anemia, lymphoma, hypogammaglobulinemia, absent iNKT cells
XLP2	*BIRC4*	XIAP	Inhibition of apoptosis	XR	EBV infection, splenomegaly, lymphoproliferation, HLH, colitis, IBD, hepatitis, low iNKT cells
XMEN syndrome	*MAGT1*	Magnesium transporter 1	T-cell activation via TCR	XR	Combined immunodeficiency, lymphoma
(2) Autosomal recessive					
ITK deficiency	*ITK*	ITK	TCR-mediated activation	AR	EBV-associated B-cell lymphoproliferation, lymphoma, normal or decreased IgG
CD27 deficiency	*CD27*	CD27	Lymphocyte costimulatory molecule	AR	Triggered by EBV infection, HLH, aplastic anemia, lymphoma, hypogammaglobulinemia, low iNKT cells

AP3B1 adaptor-related protein complex 3 beta 1 subunit; *AR* autosomal recessive; *EBV* Epstein–Barr virus; *FHL* familial hemophagocytic lymphohistiocytosis; *HLH* hemophagocytic lymphohistiocytosis; *IBD* inflammatory bowel disease; *ITK* interleukin-2-inducible T-cell kinase; *LYST* lysosomal trafficking regulator; *iNKT cells* invariant natural killer T cells; *SAP* signaling lymphocytic activation molecule-associated protein; *TCR* T-cell receptor; *XIAP* X-linked inhibitor of apoptosis protein; *XLP* X-linked lymphoproliferative disease; *XMEN* X-linked immunodeficiency with magnesium defect, Epstein-Barr virus infection, and neoplasia; *XR* X-linked recessive

Table 27.2 Subtypes of secondary hemophagocytic lymphohistiocytosis and their associated conditions

1. Infection-associated hemophagocytic syndrome
Virus-associated hemophagocytic syndromes: EBV, CMV, HSV, HIV, influenza virus, parainfluenza virus, etc.
Other infections associated with hemophagocytic syndromes: bacterial, parasitic, fungal
2. Malignancy-associated hemophagocytic syndrome
Hematologic malignancies: lymphoma, leukemia
Solid tumors: prostate cancer, lung cancer, hepatocellular carcinoma, etc.
3. Macrophage activation syndromes (associated with rheumatic/autoimmune disease)
Systemic idiopathic juvenile arthritis
Still disease
Systemic lupus erythematosus
Kawasaki disease
Rheumatoid arthritis

CMV cytomegalovirus, *EBV* Epstein–Barr virus, *HIV* human immunodeficiency virus, *HSV* herpes simplex virus

Table 27.3 Diagnostic criteria of hemophagocytic lymphohistiocytosis proposed in the HLH-2004 treatment protocol by the Histiocyte Society (Henter et al. 2007)

A diagnosis of HLH can be established if one of either parameter (1 or 2) below is fulfilled:
1. A genetic diagnosis consistent with HLH
2. Diagnostic criteria for HLH fulfilled (five out of the eight criteria below):
Clinical criteria
Fever
Splenomegaly
Histopathologic criteria
Hemophagocytosis in the bone marrow, spleen, or lymph nodes with no evidence of malignant neoplasm
Laboratory criteria
Cytopenias (affecting ≥2 of 3 lineages in the peripheral blood): hemoglobin (<9.0 g/dL), platelets (<100,000/μL), neutrophils (<1000/μL) (in infants <4 weeks: hemoglobin <10.0 g/dL)
Hypertriglyceridemia and/or hypofibrinogenemia: fasting triglycerides ≥3.0 mmol/L (i.e., ≥265 mg/dL), fibrinogen ≤150 mg/dL
Low or absent NK-cell activity (according to local laboratory reference)
Ferritin ≥500 ng/mL
Soluble CD25 (i.e., soluble IL-2 receptor) ≥2400 U/mL

HLH hemophagocytic lymphohistiocytosis, *NK* natural killer

emia (Rosado and Kim 2013). Such biochemical dysfunctions are present in 40–70% of cases at diagnosis (Koh et al. 2015; George 2014).

Ferritin is a clinically useful screen for the early diagnosis of HLH. Ferritin production is upregulated in macrophages in response to inflammatory cytokines (Rosado and Kim 2013). Ferritin is an acute-phase reactant and is nonspecifically elevated in other inflammatory conditions, including infection, cancer, and autoimmune diseases. According to the HLH-2004 criteria, a ferritin value greater than 500 ng/mL is a positive diagnostic parameter. Although this value is highly sensitive, it may be unspecific for HLH. Marked hyperferritinemia (ferritin >2000 ng/mL up to 10,000 ng/mL) was demonstrated to be specific for HLH, especially in pediatric patients (Lehmberg et al. 2014).

Serum levels of soluble IL-2RA (also called soluble CD25) reflect T-cell activation and may be elevated in many clinical conditions, such as autoimmune diseases, malignant neoplasms, and HLH. Soluble IL-2RA can serve as a useful marker for diagnosis and follow-up of HLH because its levels are closely associated with that of disease activity.

NK-cell activity is a marker of cytotoxic function, which is markedly reduced or absent in patients with primary HLH. NK-cell activity normalizes only after allogeneic HSCT. In contrast, patients with secondary HLH who have low NK-cell activity at presentation experience normalized NK-cell activity later. NK-cell activity can be measured by chromium-51 (^{51}Cr) release assays or flow cytometric assays. As ^{51}Cr release assays require the use of radioactive chromium, are cost prohibitive, and often exhibit interlaboratory variability, these assays are not preferred in clinical settings. Flow cytometric assays are a more preferred method to measure NK-cell activity in clinical practice. However, the results from these assays should be interpreted with caution because they may vary, depending on sample quality and the expertise of the examiners.

In addition to the abnormalities listed as components of the HLH diagnostic criteria, many other clinical features and laboratory abnormali-

ties can be observed at presentation. HLH may affect many other organ systems in addition to the hematopoietic system, including the liver, kidneys, heart, and central nervous system (CNS). CNS involvement is especially frequent at presentation. CNS involvement is defined by either the presence of neurologic symptoms such as seizure, inconsolable irritability, and mental status changes or by abnormalities found in CSF examination or neuroimaging. CNS involvement is reported to be one of the most important prognostic indicators of HLH (Koh et al. 2015; Horne et al. 2008). Other laboratory abnormalities include coagulopathy and increased levels of lactate dehydrogenase, bilirubin, and transaminases. Although not a component of the diagnostic criteria of HLH, abnormalities in liver function tests are frequently observed in HLH and are usually progressive if proper management is not initiated (Koh et al. 2015; Jordan et al. 2011). Therefore, HLH should be considered in the differential diagnosis of patients who present with prolonged fever and hepatitis of unknown causes (Ryu et al. 2013).

Other ancillary tests such as flow cytometric assays for intracellular perforin expression, granule release assays for evaluation of granule-mediated cytotoxicity, and assays for expression of SLAM-associated protein (SAP) and X-linked inhibitor of apoptosis (XIAP) can be helpful in the diagnosis of HLH and rapid categorization of HLH subtypes. These assays can also be used to guide the priority of genetic testing (Morimoto et al. 2016; Sieni et al. 2014).

Some tests required for HLH diagnosis are not readily available in individual hospitals, and genetic tests or flow cytometric assays are available only in some tertiary referral hospitals. HLH is often rapidly progressive and fulminant. It is, therefore, very important to make a timely diagnosis in the early stages of the disease. Unexplained cytopenias and hepatitis accompanied by fever and marked elevation of ferritin without evidence of malignant disease can be clinical clues of HLH (Jordan et al. 2011). An algorithmic approach is useful for the prompt suspicion of HLH and proper initiation of early treatment (Fig. 27.3).

27.3 Molecular Pathophysiology

HLH is a hyperinflammatory syndrome due to a highly stimulated but ineffective immune process. Primary/genetic HLH is caused by genetic defects of the effector cell, leading to impaired secretion of cytotoxic granules. A cytotoxic granule is a type of specialized lysosome that acts as a secretory vesicle containing perforin and granzyme, which efficiently kill targeted cells. Several intracellular steps are required for granule-mediated cytotoxic pathways in cytotoxic T cells and NK cells, including the production of perforin and granzyme, the packaging of these molecules into cytotoxic granules, and the intracellular transportation, docking, priming, and fusion of the granules with cell membranes (Ishii 2016; Jordan et al. 2011; Seo 2015). Primary HLH is associated with mutations in the genes that encode the proteins involved in each step of the pathway (Fig. 27.4). During conventional immune regulation, antigen-presenting cells (APCs) activate cytotoxic T cells in response to a viral infection. The activated cytotoxic T cells then secrete inflammatory cytokines, which activate macrophages. In turn, the activated macrophages produce cytokines, which activate additional cytotoxic T cells and themselves. As the cytotoxic T cells become further activated and antigen presentation is no longer required, they selectively eliminate the APCs that continue to present virus-related antigens through granule-mediated cell lysis. However, in HLH, these APCs are not eliminated by cytotoxic T cells because of impaired cytotoxic granule secretion. This produces APCs that continue to present antigens to T cells, resulting in the uncontrolled overactivation of T cells. This leads to the release of large amounts of IFN-γ by the T cells, which in turn continues to activate APCs that release large amounts of cytokines. This vicious cycle of immune dysregulation causes an ineffective and uncontrolled cytokinemia, thereby leading to a systemic hyperinflammatory response in primary HLH (Morimoto et al. 2016). The causative genes and their associated proteins and functions in primary HLH are summarized in Table 27.1.

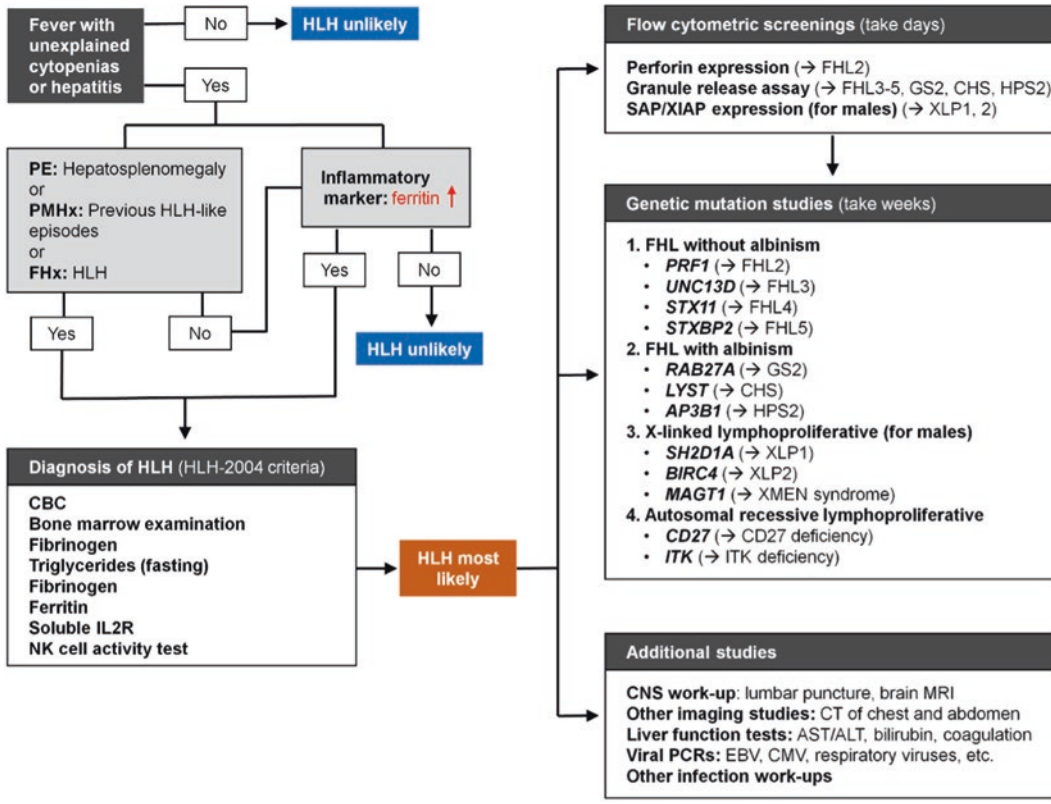

Fig. 27.3 The HLH diagnostic algorithm. It is important to recognize clinical features suggesting HLH (fever with unexplained cytopenias or hepatitis accompanied by hepatosplenomegaly) to make a timely diagnosis of HLH. Ferritin is a very useful screen for early diagnosis of HLH. Flow cytometric assays are helpful in the rapid diagnosis of HLH and can guide the priority of genetic testing. Genetic testing should be recommended for all patients meeting HLH diagnostic criteria, irrespective of age and clinical presentation

Although both primary and secondary HLH are characterized by uncontrolled hypercytokinemia, secondary HLH is not associated with any obvious genetic abnormality. The pathophysiology of secondary HLH is not fully defined. Historically, primary and secondary HLH were considered two distinct syndromes. However, recent understanding of HLH pathogenesis has blurred this distinction, suggesting that the clinical spectrum of HLH occurs across a continuum of genetic risk. Severe (null) mutations, mild hypomorphic (usually missense) mutations/polymorphisms, and even complex polygenic traits appear to determine an individual's risk for developing HLH in response to immune stimuli (Risma and Jordan 2012). Specifically, severe mutations underlie early-onset, primary HLH, and polygenic traits are more likely to be associated with secondary HLH.

27.3.1 Familial HLH Syndromes Without Hypopigmentation

The FHL syndromes without hypopigmentation are often referred to simply as familial HLH. They are inherited in an autosomal recessive manner and comprise five subtypes (FHL1, FHL2, FHL3, FHL4, and FHL5). Approximately 70% of FHL cases result from mutations in two genes, *PRF1* and *UNC13D*, which define the FHL2 and FHL3 subgroups, respectively.

FHL1: The mutation driving FHL1 occurs in chromosome 9 (9p21.3-22), although the exact protein and gene remain to be identified.

FHL2: The mutation driving FHL2 resides in the *PRF1* gene on chromosome 10 (10q21-22). It

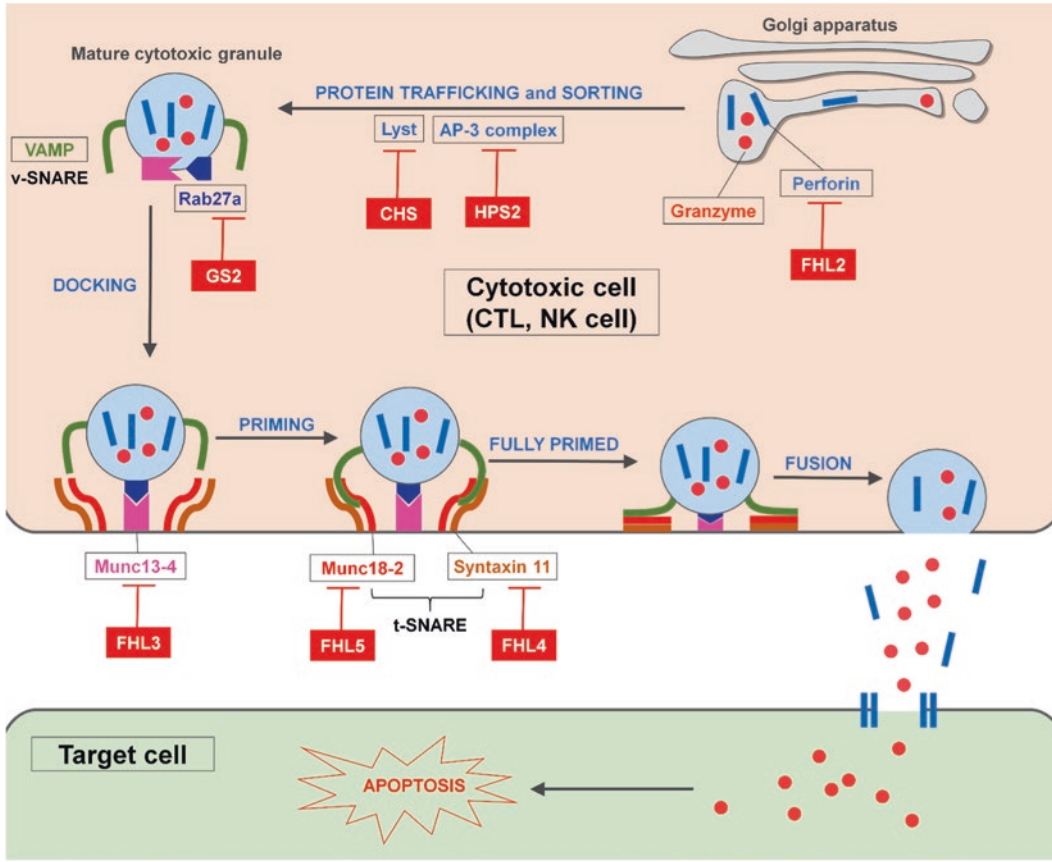

Fig. 27.4 Schematic overview of the genetic defects and pathway of granule-mediated cytotoxic events that cause primary HLH. Several intracellular steps are required for granule-mediated cytotoxicity in cytotoxic T cells and NK cells, including production of cytotoxic granules, intracellular transportation of granules, docking, priming, and fusion with the plasma membrane. Primary HLH is associated with mutations in the genes that encode the proteins involved in each step of the pathway. Abbreviations: *AP-3* adaptor protein 3, *CHS* Chédiak–Higashi syndrome, *CTL* cytotoxic T lymphocyte, *FHL* familial hemophagocytic lymphohistiocytosis, *GS2* Griscelli syndrome type 2, *HPS* Hermansky–Pudlak syndrome, *LYST* lysosomal trafficking regulator, *NK* natural killer, *SNARE* soluble N-ethylmaleimide-sensitive factor attachment protein receptor, *v-SNARE* vesicle membrane SNARE, *t-SNARE* target membrane SNARE, *VAMP* vesicle-associated membrane protein

accounts for 20–50% of FHL cases, depending on the study cohort (Sieni et al. 2014; Ishii 2016). The *PRF1* gene encodes the cytolytic, pore-forming protein perforin, which is synthesized and stored in cytotoxic lymphocytes and NK cells along with granzyme. Perforin monomers form a polymeric pore structure in target cell plasma membranes, through which granzymes enter and induce cell death. Mutations in *PRF2* result in reduced, absent, or functional impairment of perforin (Sieni et al. 2014; Zhang et al. 2014).

FHL3: FHL3 is caused by mutations in the *UNC13D* gene. It accounts for 30–40% of FHL cases but varies widely depending on the geographic area and ethnic group (Sieni et al. 2014; Koh et al. 2015). *UNC13D* encodes Munc13-4, which is involved in the priming of cytotoxic granules and their fusion with cell membranes. Priming is an additional biochemical event that enables granules to fuse with the plasma membrane. Therefore, Munc13-4 loss of function results in the impaired release of perforin and granzyme to

the target cell (Sieni et al. 2014; Zhang et al. 2014).

FHL4: Approximately 10–20% of FHL cases include mutations in the *STX11* gene, which encodes syntaxin-11. This protein belongs to the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) family of vesicular proteins. The two types of SNARE proteins include vesicle membrane SNAREs (v-SNAREs) found on the surface of transport vesicles and target membrane SNAREs (t-SNAREs). SNARE proteins act like ropes that facilitate the transport of cytotoxic granules to their destination by binding tightly to the plasma membrane, thus assisting vesicle fusion with cell membranes. Syntaxin-11 is a t-SNARE, and mutations in *STX11* result in decreased or absent expression, leading to defects in vesicle endocytosis and exocytosis (Ishii 2016; Sieni et al. 2014; Zhang et al. 2014).

FHL5: FHL5 is caused by mutations in the *STXBP2* gene, which encodes syntaxin-binding protein 2 (also called Munc18-2). This protein binds to syntaxin-11 to form a t-SNARE complex, thus regulating vesicle membrane fusion. Mutations in *STXBP2* result in impaired cytotoxic function, similar to that observed in FHL4 (Ishii 2016; Sieni et al. 2014).

27.3.2 Familial HLH Syndromes with Hypopigmentation

Griscelli syndrome type 2 (GS2): GS2 is characterized by immune system abnormalities, in addition to hypopigmented skin and hair. Patients with GS2 carry mutations in the *RAB27A* gene. The Rab-27A protein product belongs to the Rab family, which is part of the Ras superfamily of small GTPases. Rab-27A interacts with Munc13-4 during the docking of cytotoxic granules to the plasma membrane. Mutations in *RAB27A* disable this function, resulting in severe degranulation defects in NK and cytotoxic T cells (Sieni et al. 2014; Zhang et al. 2014).

Chédiak–Higashi syndrome (CHS): CHS is characterized by oculocutaneous albinism, neuropathy, coagulopathy, and immune dysregulation. CHS is caused by mutations in the *LYST* gene, which encodes the lysosomal trafficking regulator LYST. *LYST* mutations cause defects in the production of cytotoxic granules, leading to the appearance of dysfunctional giant lysosomes (Sieni et al. 2014; Zhang et al. 2014). Therefore, exocytosis of cytotoxic granules is impaired in CHS, resulting in defective cytotoxic function.

Hermansky–Pudlak syndrome type 2 (HPS2): Hermansky–Pudlak syndrome includes a clinically homogeneous but genetically heterogeneous group of nine autosomal recessive genetic disorders. They share the clinical features of partial oculocutaneous albinism and bleeding disorders. Among these disorders, HPS type 2 is the only subtype that includes both the congenital neutropenia and impaired cytotoxic activity associated with HLH. HPS2 is caused by mutations in the *AP3B1* gene, which encodes the beta-3A subunit of the adaptor protein 3 (AP-3) complex. The AP-3 complex controls trafficking of proteins from the Golgi apparatus to lysosomes. Defects in the AP-3 complex result in the missorting of proteins, thereby impairing the function of secretory lysosomes (Sieni et al. 2014; Zhang et al. 2014).

27.3.3 X-Linked Lymphoproliferative Disease (XLP)

XLP is an X-linked inherited immune disorder that conveys a high risk of developing HLH, which is triggered by EBV infection in most cases. The two types of XLP, XLP1 and XLP2, are caused by different mutations.

XLP1: XLP1 results from loss-of-function mutations in the *SH2D1A* gene. The gene encodes the SAP protein, named for its association with SLAM proteins, which refer to signaling lymphocytic activation

molecules. SAP is an adaptor protein that interacts predominantly with the NK-cell receptor 2B4, which is a member of SLAM family. SAP operates as a molecular switch that converts 2B4 from an inhibitory receptor into an activating one. Thus, in the absence of SAP, 2B4 delivers inhibitory instead of activating signals, resulting in impaired cytotoxicity (Zhang et al. 2014; Sieni et al. 2014).

XLP2: Patients with XLP2 carry mutations in the *BIRC4* gene, which encodes the XIAP protein. XIAP belongs to a family of apoptotic suppressor proteins. *BIRC4* mutations are purported to increase the susceptibility of lymphocytes to activation-induced cell death, thereby reducing the size of the active cell population. This appears inconsistent with the paradigm of HLH, which emphasizes the role of overactivated T cells. Nevertheless, patients with XIAP deficiency can exhibit defects in cytotoxicity similar to that of patients with XLP1 resulting from SAP deficiency (Sieni et al. 2014). The pathophysiologic mechanism that accounts for the occurrence of HLH in XLP2 is not fully understood.

27.4 Treatment

The initial goals of HLH treatment are to suppress the life-threatening inflammatory process that underlies HLH and to kill pathogen-infected APCs, thereby eliminating the stimulus for the ongoing but ineffective activation of cytotoxic cells. In addition, a curative HSCT should be provided to patients with primary or relapsed/refractory HLH to exchange the defective immune system with appropriately functioning cells. To achieve these goals, prompt initiation of immunosuppressive and proapoptotic chemotherapy is mandatory. Because initial treatment is similar in patients with both primary and secondary HLH and the disease is rapidly progressive, treatment should be promptly started for patients who are suspected to have HLH, rather than waiting for the results of genetic testing.

The HLH-94 and subsequent HLH-2004 protocols proposed by the Histiocyte Society are the most commonly used treatment protocols (Fig. 27.5) (Henter et al. 2007). The HLH-94 protocol recommended an 8-week induction therapy with dexamethasone, etoposide, and intrathecal methotrexate. After induction therapy, patients with resolved secondary HLH were weaned off

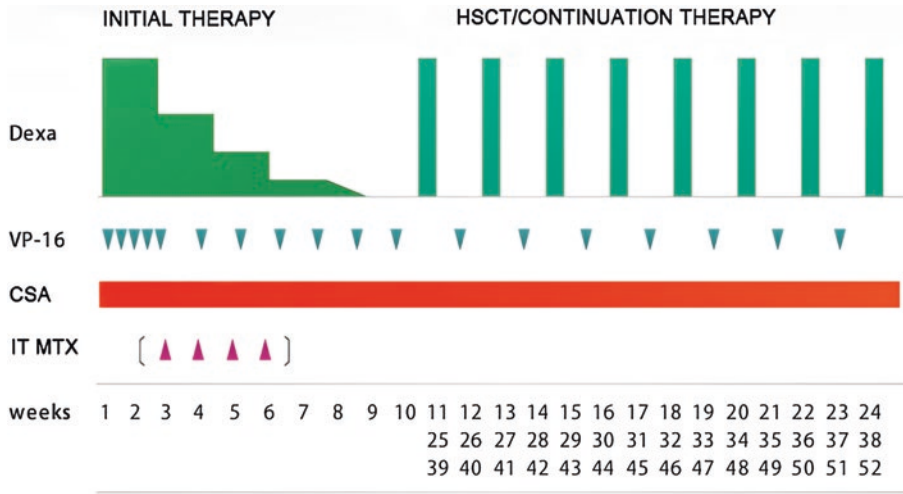

Fig. 27.5 Overview of therapeutic guidelines from the HLH-2004 treatment protocol recommended by the Histiocyte Society

therapy, and patients with primary HLH or persistent secondary HLH were directed to continue therapy as a bridge to allogeneic HSCT. In the HLH-2004 trial, the protocol was modified to achieve a better remission rate by using cyclosporine during the induction therapy phase and by adding hydrocortisone to intrathecal therapy. The HLH-94 trial resulted in an estimated 5-year survival of 54% ± 6%, which was a remarkable improvement for an otherwise fatal disease (Trottestam et al. 2011). The results of the HLH-2004 trial have not yet been published.

An alternative protocol combining antithymocyte globulin (ATG) as a frontline therapy with corticosteroids, cyclosporine, and intrathecal therapy showed promising results (Mahlaoui et al. 2007). On the basis of these results, a phase II clinical trial testing the efficacy of ATG in combination with dexamethasone and etoposide was recently opened in the United States (Filipovich and Chandrakasan 2015).

Allogeneic HSCT is generally recommended as a curative option in patients with genetically verified disease, a family history of HLH, or persistent or reactivated HLH (Seo 2015). In the HLH-94 and HLH-2004 protocols, busulfan-based myeloablative chemotherapy was suggested as a conditioning regimen. However, this conditioning regimen resulted in extensive transplant-related mortality (TRM). Recently, a reduced-intensity conditioning regimen using alemtuzumab (a monoclonal antibody targeting CD52 on mature lymphocytes), fludarabine, and melphalan has improved outcomes and reduced TRM (Marsh et al. 2010).

Some patients with refractory, progressive disease may require salvage or alternative therapeutic approaches. However, the evidence supporting effective salvage therapy is minimal (Marsh et al. 2016). ATG can be used as either a first-line or second-line therapy. Alemtuzumab is suggested to play a therapeutic role in the treatment of refractory HLH. Rituximab, which targets $CD20^+$ B cells, is reportedly efficacious in EBV-associated HLH and XLP. The use of anakinra (a recombinant human interleukin-1 receptor antagonist) is also reported in patients with rheumatologic/autoimmune disorder-associated HLH or macrophage activation syndrome. The IFN-γ blocking monoclonal antibody emapalumab is a promising novel agent that is expected to have a putative therapeutic role as frontline therapy or salvage therapy (Filipovich and Chandrakasan 2015).

In summary, HLH is a hyperinflammatory syndrome that can be caused either by known genetic abnormalities (primary HLH) or may be triggered by infection, cancer, or autoimmune disease in patients with no known genetic cause (secondary HLH). Because initial presentations may be nonspecific, high-suspicion and systematic clinical, immunologic, and genetic work-ups are required. Prompt initiation of proper treatment is mandatory for survival. Considerable progress has been made exploring the molecular/immunologic drivers of HLH pathophysiology and managing HLH treatment. Future directions include identifying improved biomarkers or diagnostic/prognostic markers, establishing better methods of genetic screening, and introducing novel targeted agents.

End-of-Chapter Questions

1. Describe the diagnostic criteria of HLH, and explain why each component occurs in patients with HLH.
2. Describe the perforin/granzyme pathway of immune effector cells and the genetic mutations that cause primary HLH.
3. What kind of triggers or conditions can be associated with secondary HLH?
4. Explain the goals of HLH treatment according to the pathophysiology of HLH.
5. What are the differences in the treatment strategies between primary and secondary HLH?

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