

REVIEW ARTICLE

Genetics and pathophysiology of haemophagocytic lymphohistiocytosis

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Abstract

Haemophagocytic lymphohistiocytosis (HLH) represents a life-threatening hyperinflammatory syndrome. Familial studies have established autosomal and X-linked recessive causes of HLH, highlighting a pivotal role for lymphocyte cytotoxicity in the control of certain virus infections and immunoregulation. Recently, a more complex etiological framework has emerged, linking HLH predisposition to variants in genes required for metabolism or immunity to intracellular pathogens. We review genetic predisposition to HLH and discuss how molecular insights have provided fundamental knowledge of the immune system as well as detailed pathophysiological understanding of hyperinflammatory diseases, highlighting new treatment strategies.

KEYWORDS

haemophagocytic lymphohistiocytosis, hyperinflammation, immune dysregulation, inborn errors of immunity, macrophage activation syndrome

1 | INTRODUCTION

Haemophagocytic lymphohistiocytosis (HLH) denominates a potentially lethal, systemic hyperinflammatory syndrome. HLH has been linked to intracellular infections ranging from herpesviruses and influenza to visceral leishmaniasis that elicit strong T cell-mediated immune responses. As such, HLH can be viewed as an

exaggerated immune response that in certain settings can be successfully treated with radical immunosuppressive regimens rather than treatments targeting the infection. T cell-mediated immune responses also contribute to control of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, but patients very seldom fulfil HLH criteria. HLH has also associated with malignancies and autoinflammatory syndromes. In addition, iatrogenic HLH

Abbreviations: CHS, Chediak-Higashi syndrome; EBV, Epstein-Barr virus; FHL, familial HLH; GS2, Griscelli syndrome type 2; HLH, haemophagocytic lymphohistiocytosis; IBD, inflammatory bowel disease; IEM, inborn errors of metabolism; IFN, interferon; IL, interleukin; LCMV, lymphocytic choriomeningitic virus; MAS, macrophage activation syndrome; NK, natural killer; PID, primary immunodeficiency diseases; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; TLR, Toll-like receptor; TNF, tumour necrosis factor; XLP, X-linked lymphoproliferative disease.

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has been associated with chimeric antigen receptor T-cell therapies, gene therapies, antibody therapies including rituximab and immune checkpoint inhibitors, as well as allogeneic stem-cell transplantation. Here, we provide a historical perspective on studies that have uncovered genetic factors that predispose to the development of HLH. Importantly, these studies have unravelled how several different immunological pathways can contribute to disease, thereby highlighting new therapeutic targets.

2 | HISTORICAL BACKGROUND

Reports of adults with a rare and invariably fatal condition characterised by relapsing fever, progressive erythropenia, neutropenia, a highly reactive marrow and hepatosplenomegaly are considered the first descriptions in the medical literature of what is currently termed HLH.^{1,2} In 1952, Farquhar and Claireaux described two infant siblings that were presented with familial so-called 'haemophagocytic reticulosis' with a fatal outcome, providing the first indication of a strong genetic contribution to such life-threatening hyperinflammatory syndromes.³ In a seminal study, Fauci and colleagues found that NK cell cytotoxicity was severely impaired in patients with autosomal recessive Chediak-Higashi syndrome (CHS),⁴ which is characterised by defective pigmentation, abnormal leukocyte granulation and increased susceptibility to infections. Affected children, as Filipovich and colleagues observed, develop fatal HLH or alternatively lymphomas in childhood or adolescence.^{4,5} An animal model of CHS, the *beige* mouse, similarly displayed defective NK cell cytotoxicity.⁴ In the mid-1980s, research constellations including Griscelli, Arico and Janka reported defective NK cell cytotoxicity in children with suspected familial HLH and normal pigmentation.⁶⁻⁸ In most of these patients, NK cell cytotoxicity remained persistently impaired irrespective of splenectomy, corticosteroids, chemotherapy or whether their cells were investigated during hyperinflammation or in remission. However, in a few patients, NK cell activity was restored following treatment.⁶ Furthermore, in a key study Henter and colleagues analysed serum from familial HLH patients revealing highly elevated interferon (IFN)- γ , tumour necrosis factor (TNF) and soluble CD8 levels in the patients.⁹ These findings led to the classification of familial HLH as a disease of systemic hypercytokinaemia and suggested an important role for IFN- γ and TNF as well as CD8⁺ T cells in the pathogenesis, in line with histological observations of activated macrophages and CD8⁺ T cells infiltrations in multiple tissues of HLH patients.¹⁰

Spurred by a wealth of clinical observations and laboratory findings, Henter and colleagues led initial consensus efforts to define HLH, with the first Histiocyte Society diagnostic guidelines which were published in 1991.¹¹ These guidelines stipulated unremitting fever, splenomegaly, bicytopenia, hypertriglyceridemia/hypofibrinogenaemia and haemophagocytosis as requirements for a diagnosis of HLH. At the time, it was recognised that HLH could present as a primary early-onset, autosomal recessive condition or develop secondary to stimuli that induced a strong activation of the immune system, including infections (particularly by viruses), malignancies and

Key Notes

- Clinical definitions of haemophagocytic lymphohistiocytosis (HLH) have provided a crucial platform for understanding and dissecting life-threatening hyperinflammatory disorders
- Familial HLH is predominately caused by genetic defects affecting lymphocyte cytotoxicity
- Development of HLH is also associated with inborn errors of immunity, inflammation and metabolism, highlighting different paths to disease and revealing novel therapeutic targets

prolonged intravenous administration of soluble lipids.¹¹ In rheumatic diseases, particularly systemic onset juvenile rheumatoid arthritis, the term macrophage activation syndrome (MAS) was increasingly used to describe a constellation of symptoms similar to HLH that also could be treated by immunosuppressive drugs.^{12,13} These first diagnostic guidelines increased awareness, facilitated systematic studies and provided new insights to HLH. Imashuku and colleagues identified high serum ferritin as a hallmark of histiocytosis associated with viral infections as well as malignancy.¹⁴ Soluble CD25 was later described as elevated in HLH patients and recognised as a predictor of poor outcome.¹⁵ Furthermore, it had earlier been noted that haemophagocytosis was not always evident or could be missed in the diagnostic evaluation.¹⁰ According to these insights, the Histiocyte Society diagnostic guidelines for HLH were modified to include hyperferritinaemia, elevated serum CD25 and impaired NK cell cytotoxicity, with a diagnosis based upon fulfilment of five out of a total of eight criteria.¹⁶ These clinical, laboratory and histological criteria have provided a crucial framework to dissect the genetic underpinnings of HLH.

Here, we discuss how these groundbreaking initiatives have led to a rich understanding of the genetic susceptibility as well as the molecular and cellular pathophysiology of HLH. We highlight advances as well as unanswered questions. In addition, we discuss how current knowledge of a spectrum of HLH-related disorders can aid efforts to provide precision medicine for individuals suffering from diverse hyperinflammatory disorders.

3 | GENETIC DISSECTION OF FAMILIAL HLH

3.1 | Initial associations of haemophagocytic lymphohistiocytosis to defective lymphocyte cytotoxicity

Genetic linkage studies were instrumental in identifying the first genes known to cause HLH, benefiting from advances in molecular cloning and sequencing techniques as well as human genome

mapping efforts. In 1996, two groups identified a variant in *Lyst* as causative of the mouse *beige* phenotype by screening of yeast artificial chromosome libraries.¹⁷ Deleterious *LYST* variants were also uncovered in a CHS patient.¹⁷ At the time, it was known that CHS arises from a secretory defect that prevents the exocytosis of perforin-containing cytotoxic granules, a form of specialised secretory lysosome specifically expressed in cytotoxic T cells and NK cells.^{18,19} *LYST* is also required for biogenesis of melanosomes in melanocytes, explaining hypopigmentation in CHS. Perforin is specifically expressed by cytotoxic T cells and NK cells and, together with Fas, represents the major pathway for lymphocyte-mediated target cell killing.²⁰ Upon exocytosis, perforin forms pores in target cell membranes that facilitate entry of pro-apoptotic granzymes, which also are stored in cytotoxic granules.²¹ In 1998, through linkage analyses, variants in *SH2D1A* were identified as a cause of X-linked lymphoproliferative disease type 1 (XLP1),^{22–24} which is characterised by fulminant, life-threatening Epstein-Barr virus (EBV) infections fulfilling HLH criteria, dysgammaglobulinaemia, as well as EBV-related lymphoproliferative disorders, or lymphoma. *SH2D1A* encodes SAP, a signalling protein that binds the phosphorylated cytoplasmic tail of a variety of SLAM family receptors that are widely expressed on hematopoietic cells, thereby preventing recruitment of phosphatases that otherwise convey signals that inhibit cell-cell interactions.

These early discoveries thus implicated impaired perforin-mediated cytotoxicity and dysregulated lymphocyte interactions in susceptibility to severe EBV infections and development of HLH. They paved the way for a detailed molecular understanding of genetic predisposition to HLH.

3.2 | Identification of autosomal recessive genes causative of familial HLH

In 1999, performing linkage analyses of consanguineous families, Kumar and colleagues uncovered autosomal recessive variants in *PRF1*, encoding perforin, as a cause of familial HLH.²⁵ Notably, they concluded that perforin-based effector systems are involved not only in the killing of abnormal cells but also in the down-regulation of cellular immune activation.²⁵ Perforin deficiency is termed familial HLH type 2 (FHL2). Shortly thereafter, de Saint Basile and colleagues showed that variants in *RAB27A* cause Griscelli syndrome type 2 (GS2),²⁶ an autosomal recessive disorder of pigmentation also characterised by development of HLH. *RAB27A* encodes a small GTP-binding protein preferentially expressed by melanocytes and hematopoietic cells which is required for cytotoxic granule exocytosis by lymphocytes.²⁶ In subsequent linkage studies of other familial HLH pedigrees, autosomal recessive variants in *UNC13D*, *STX11* and *STXBP2* were identified as causative of FHL3-5.^{27–30} These genes encode the cytosolic proteins Munc13-4, syntaxin-11 and Munc18-2, respectively. They display high sequence homology to the presynaptic neuronal proteins that regulate SNARE-complex mediated neurotransmitter release but are instead expressed in lymphocytes and other hematopoietic cell lineages. Munc18-2 is also expressed in

epithelial cells of the gut, potentially explaining the gastrointestinal manifestations that are a particular feature of Munc18-2 deficient patients.^{31,32} Importantly, studies of lymphocytes from patients with biallelic nonsense variants in these genes have established that their protein products are required for lymphocyte cytotoxic granule exocytosis, explaining why such variants give rise to syndromes that clinically phenocopy perforin-deficiency (Figure 1).^{27,29,30,33} Retrospective analyses have demonstrated that patients with biallelic nonsense variants in *PRF1* or *UNC13D* invariably present with HLH within their two first years of life.^{34–36} Nonsense variants in *STXBP2*, *RAB27A* and in particular *STX11* may present later in childhood, which can be explained by the fact that cytokine stimulation of cytotoxic lymphocytes can restore exocytosis by syntaxin-11 deficient cells.³³

Altogether, a number of genetic studies determined that defective lymphocyte cytotoxicity is a frequent cause of familial HLH, highlighting how lymphocyte cytotoxicity is crucial for maintenance of immune homeostasis. They also illustrated how rare human diseases, characterised by genetic variants and frequently triggered by infectious agents, can provide molecular and pathophysiological insights that are not easily uncovered in animal model systems.³⁷

3.3 | Non-coding variants as a cause of familial HLH

The incidence of autosomal recessive familial HLH in infancy and childhood has been estimated as 1/50 000 live-births,³⁸ which is, for example comparable to that of severe combined immunodeficiency.³⁹ Great advances in sequencing technologies have facilitated a molecular diagnosis in a large number of patients and unravelled many new primary immunodeficiency diseases.⁴⁰ Nonetheless, high-throughput sequencing efforts currently focus on analyses of the coding regions of genes and may fail to identify non-coding disease-causing variants in a majority of patients with suspected Mendelian inheritance.⁴¹ Meticulous clinical, functional and genetic analyses of patients with early-onset HLH and defective lymphocyte cytotoxicity have revealed that non-coding variants can explain more than 50% of early-onset HLH cases, at least in some geographical regions.^{42–45} These studies have revealed a lymphocyte-specific intronic enhancer and alternative transcriptional start site of *UNC13D* controlled by ETS family transcription factor binding.⁴⁶ In the same intron, another variant impairing NFκB transcription factor binding has been associated with reduced *UNC13D* transcription in a patient diagnosed with recurrent MAS.⁴⁷ Moreover, deletions of the *RAB27A* promoter have been reported in GS2,^{48,49} in some cases deleting a lymphocyte-specific promoter that thereby impairs lymphocyte cytotoxicity without affecting the pigmentation of melanocytes.

Future efforts promise to unravel many more disease-causing variants in non-coding gene regulatory elements, which can be facilitated by analyses of evolutionary conservation and knowledge of non-coding elements that may regulate HLH-associated genes.^{42,50} Importantly, sensitive functional assays and determination of protein

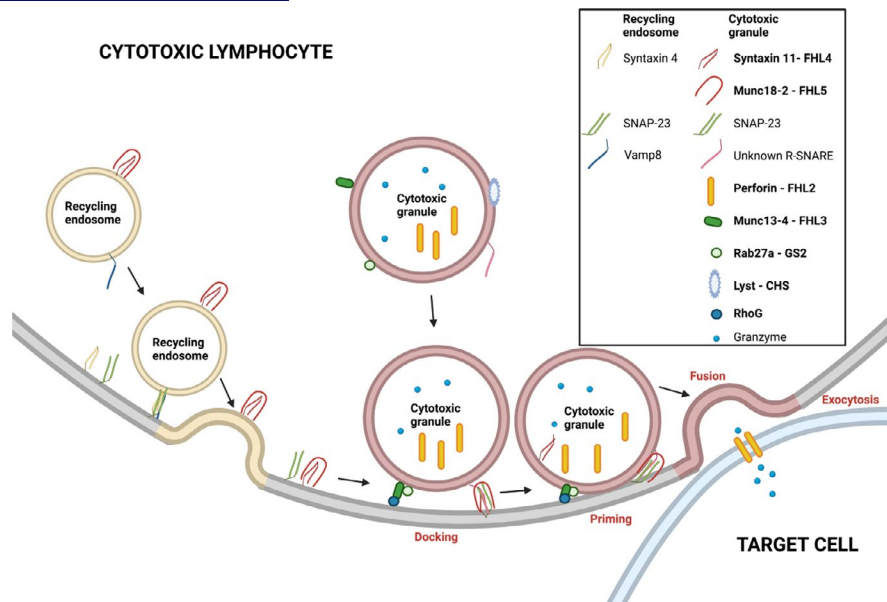


FIGURE 1 Schematic illustration of targeted secretion of cytotoxic granules at the immune synapse in cytotoxic lymphocytes. Target cell recognition initiates signalling by activating receptors and a cascade of biochemical events culminating in VAMP8-dependent fusion of recycling endosomes with the plasma membrane likely through forming a SNARE complex with Stx4 and SNAP-23, thereby depositing Stx11 and Munc18-2 that are required for cytotoxic granule fusion. Activating signals also recruit Munc13-4 to cytotoxic granules where it interacts with Rab27a and promotes docking of the cytotoxic granules at the immune synapse through binding of RhoG, a protein that can provide membrane anchoring through covalent lipid modifications. Stx11 interacts other SNARE proteins, mostly likely plasma membrane SNAP-23 and a vesicular SNARE, to drive the final step of cytotoxic granule fusion and exocytosis that mediates the release of perforin and granzymes into the immune synapse. Perforin multimers can form pores in the target cell membrane that facilitate entry of granzymes into the cytosol of the target cell where they promote apoptosis. Proteins encoded by genes associated with primary HLH are highlighted in bold

expression complement to high-throughput genetic analyses, as they can direct analyses for as well as determine the functional impact of rare, non-coding variants.⁵¹⁻⁵³ Such efforts can hopefully substantially increase the proportion of patients that obtain a molecular diagnosis.

3.4 | Molecular pathophysiology of familial HLH, new genetic susceptibilities and future prospects

The genetic dissection of familial HLH has provided a platform for molecular understanding of lymphocyte exocytosis. At transient immune synapses between cytotoxic lymphocyte and target cells, syntaxin-11 is bound by Munc18-2. Upon activating receptor signals, syntaxin-11 is recruited to the synapse through the directed fusion of recycling endosomes with the plasma membrane.⁵⁴ Signals from activating receptors also recruit Munc13-4 to cytotoxic granules,^{55,56} where it interacts with Rab27a and promotes cytotoxic granule docking at the plasma membrane.^{57,58} In neurons, Munc13-1, a close homologue of Munc13-4, gauges synaptic activity and orchestrates the formation of SNARE complexes by forming supramolecular assemblies on the presynaptic membrane, binding plasma membrane phospholipids via N-terminal C₁-C₂B domains, bridging the vesicle to the plasma membrane and helping to open syntaxin-1 in the so-called priming process that confers a fusion

competent SNARE complex that drives membrane fusion.⁵⁹ In contrast to neuronal Munc13 isoforms, Munc13-4 uniquely lacks the archetypical N-terminal C₁ domain.⁶⁰ How Munc13-4 facilitates docking of cytotoxic granules to the plasma membrane for lymphocyte exocytosis has therefore been enigmatic. Furthermore, while dynamic clearance of cortical actin at immune synapses is necessary for cytotoxic granule exocytosis,⁶¹ it has been unclear how activation of the exocytosis machinery and the remodelling of the actin cytoskeleton is coordinated. Recently, deleterious biallelic variants in *RHOG*, encoding the small GTPase RhoG, were identified in a patient exhibiting characteristic clinical features of HLH and defective cytotoxic lymphocyte exocytosis.⁶² Cellular studies confirmed a pivotal role for RhoG in lymphocyte exocytosis and uncovered interactions with Munc13-4 that facilitated docking of Munc13-4-positive cytotoxic granules to the plasma membrane, subsequent membrane fusion and release of cytotoxic granule content.⁶² RhoG also interacts with anillin, a scaffold proteins that links actin, RhoA and myosin, and thereby can coordinate actin cytoskeleton reorganisation at dynamic, transient immune synapses. Further studies can therefore shed light on the association between RhoG deficiency and HLH, clinical penetrance, as well as the wider spectrum of manifestations of RhoG deficiency. Furthermore, the molecular understanding of how exocytosis in lymphocytes is regulated and effected remains incomplete. How activating receptor signalling spatiotemporally instigates exocytosis is not well understood. While studies of familial

HLH have established a requirement for syntaxin-11 in lymphocyte exocytosis, the SNARE protein complex partners have not been unequivocally defined. For example, it is not clear whether VAMP2, VAMP7, VAMP8 or another R-SNARE on cytotoxic granules mediates fusion and why there may be differences between species.^{63–65}

Further mechanistic studies of lymphocyte cytotoxicity in cells from patients as well as in experimental systems can provide important insights into HLH susceptibility. It is possible that genetic variants in other more ubiquitously expressed proteins involved in SNARE-mediated lymphocyte exocytosis also contribute to HLH predisposition, accounting for additional patients.

4 | OTHER GENETIC CONTRIBUTIONS TO HLH SUSCEPTIBILITY

In 2006, Latour and colleagues identified hemizygous loss of function variants in *XIAP* as the cause of XLP2, with patients often presenting with EBV infection and 92% (11/12) of patients fulfilling HLH criteria.⁶⁶ A more comprehensive review of patients with *XIAP* deficiency reported that 54% of patients manifest with HLH during the course of disease.⁶⁷ In contrast to XLP1, no increased risk of lymphoma has been reported. Rather, patients may present clinically with splenomegaly or inflammatory bowel disease (IBD).⁶⁷ *XIAP* is a ubiquitously expressed inhibitor of apoptosis but also regulates inflammasome activity.⁶⁸ In this context, *de novo* activating *NLR4* variants have been shown to cause severe, early-onset HLH.⁶⁹ Dysregulation of the actin cytoskeleton has also been linked to inflammasome activation and HLH predisposition. Patients with *de novo* variants in *CDC42*, encoding Cdc42, a protein which is a small Rho family GTPase that regulates actin dynamics, have been described with HLH in the absence of infections.⁷⁰ Similar to patients with *NLR4*-related autoinflammatory disease, interleukin 18 (IL-18) was elevated in patients with *de novo* *CDC42* mutations. Thus, IL-18 may co-stimulate IFN- γ production for development of HLH in a mechanism distinct from antigen-driven activation in patients with mutations in genes required for lymphocyte cytotoxicity. Disease-associated variants in other genes are further substantiating the links between actin dysregulation, inflammasome activation and HLH.

An association between EBV and genetic susceptibility to HLH was highlighted with the identification of variants in *SH2D1A* as a cause of XLP1.^{22–24} HLH is the presenting feature of approximately 40% of XLP1 patients.⁷¹ High-throughput sequencing efforts have associated autosomal recessive variants in *CD27*, *CD70*, *CORO1A*, *CTPS1*, *ITK*, *RASGRP1*, *TNFSFR9* (encoding CD137/4-1BB) and X-linked variants in *MAGT1*, with primary immunodeficiency diseases (PID) characterised by life-threatening EBV infections.^{72,73} Several of these PIDs have been associated with development of HLH. Furthermore, while studies of familial HLH patients and perforin-deficient animal models have indicated a critical role for CD8⁺ T cell-mediated target cell killing and IFN- γ in the pathogenesis of familial HLH,^{9,74} a number of associations between HLH and congenital

diseases other than defects in lymphocyte cytotoxicity provide a more complex pathophysiological picture. HLH has been reported in HLH patients virtually lacking T cells.⁷⁵ Moreover, reports of patients with autosomal recessive variants in *IFNGR1*, *IFNGR2* and *STAT1* indicate that HLH can develop upon severe infections yet in the absence of IFN- γ signaling.^{76–78} These PID patients illustrate how clinical features of HLH can develop without CD8⁺ T cell-mediated target cell killing or IFN- γ .

More than 30 years ago, it was recognised that HLH may occur in patients with prolonged intravenous administration of soluble lipids.¹¹ Since then, HLH has been associated with a number of inborn errors of metabolism (IEM), including Wolman disease, Niemann-Pick disease, Gaucher disease, lysinuric protein intolerance, multiple sulfatase deficiency, galactosaemia, Pearson syndrome, galactosialidosis, propionic acidaemia, methylmalonic acidaemia, biotinidase deficiency, cobalamin C deficiency, long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency and disorders of glycosylation (COG6 deficiency).⁷⁹ These genes typically encode ubiquitously expressed proteins. Thus, clinical presentation may involve different organs and tissues, directly or indirectly affecting the immune system. Cytopenias and organomegaly are common pathophysiological features of IEMs. IEM patients that fulfil HLH-2004 criteria generally differ from other forms of HLH by being more commonly associated with a failure to thrive, vomiting, muscular hypotonia and acidosis as well as a lack of fever.⁸⁰

Altogether, these findings greatly expand the spectrum of gene variants that can predispose to HLH, highlighting the importance of inflammatory pathways other than those mediated by over-activated CD8⁺ T cells. The involvement of the inflammasome and of several pro-inflammatory innate cytokines in HLH development provides new therapeutic targets.

5 | NEW PATHOPHYSIOLOGICAL INSIGHTS TO HLH AND NOVEL THERAPEUTICS

Current HLH criteria were created to identify patients with familial HLH in immediate need of immunosuppressive therapy. However, these criteria also include patients with pathophysiological mechanisms besides defects in lymphocyte cytotoxicity, *that is* impaired pathogen control, dysregulated inflammasome activation and metabolic disorders. An era of biological therapies targeting different facets of the immune system offers new possibilities for personalised treatment of treatment of HLH and related hyperinflammatory disorders.⁸¹

The genetic dissection of familial HLH has enabled efforts to dissect the cellular components driving HLH pathophysiology in the context of defective lymphocyte cytotoxicity in animal gene knockout models (Figure 2). Jordan and colleagues infected *Prf1*^{-/-} mice with lymphocytic choriomeningitic virus (LCMV), providing a model that recapitulated key clinical features of familial HLH.⁷⁴ In line with early discoveries suggesting an important CD8⁺ T cells as well as IFN- γ in

the pathogenesis of HLH,⁹ infection of *Prf1*^{-/-} mice has demonstrated that antibodies depleting or neutralising CD8 and IFN- γ but not TNF, can ameliorate disease and reduce fatality.⁷⁴ Based on these observations, emapalumab, an anti-IFN- γ antibody, has been tested and shown efficacious for the treatment of familial HLH.⁸² Importantly,

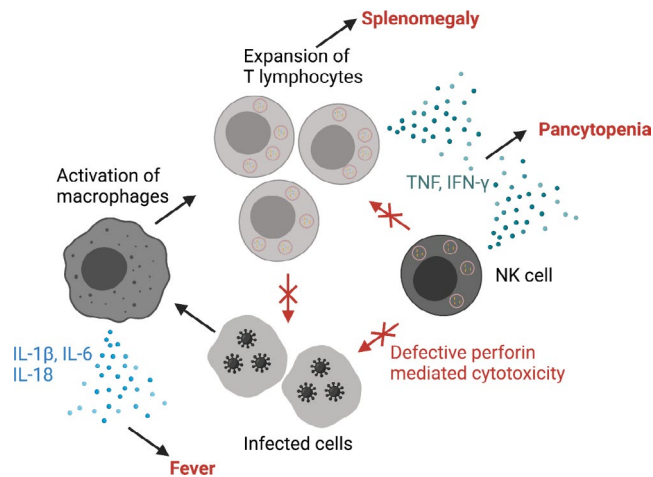


FIGURE 2 Schematic illustration of the pathophysiology in familial HLH caused by defective perforin-mediated lymphocyte cytotoxicity. A triggering factor such as target cell infection induces activation of macrophages and expansion of activated T lymphocytes. Cytotoxic T lymphocytes and NK cells are important for killing of infected cells as well as in the immunoregulatory down-modulation of the immune response. In patients with defective lymphocyte cytotoxicity, this immunoregulation is defective allowing excessive expansion of immune cells and secretion of pro-inflammatory cytokines that lead to hallmarks of HLH with persisting fever, splenomegaly and cytopenia

IFN- γ independent pathways of HLH development exist, as exemplified by patients with genetic defects in the IFN- γ pathway.^{76,78} *Prf1*^{-/-} and *Ifng*^{-/-} double knockout mice can develop HLH upon infection with LCMV.⁸³ Furthermore, in a study where HLH-like disease was triggered upon murine cytomegalovirus infection of *Prf1*^{-/-} or *Gzma*^{-/-} *Gzmb*^{-/-} mice, TNF rather than IFN- γ neutralisation ameliorated disease.⁸⁴ IFN- γ and TNF may actually synergistically induce cell death as well as cytokine-storm-related mortality in mice.⁸⁵ In a SARS-CoV-2 mouse model, combined neutralisation of IFN- γ or TNF was required to fully protect from lethality.⁸⁵ Thus, the efficacy of blocking IFN- γ for broadly treating HLH needs further exploration. In mouse models, distinct viruses cause immunopathology with different dependencies on IFN- γ and TNF. These observations provide a rationale for multi-modal targeting of inflammatory cytokines in HLH and other cytokine storm-related syndromes.

The observation that HLH can develop in a lymphocyte-independent manner by macrophage activation and release of inflammatory cytokines, *for example* as seen in severe combined immunodeficiency patients with undetectable numbers of T cell and NK cells, or mouse models of MAS with repeated administration of TLR ligands together highlight the importance of innate immune cytokines in the pathophysiology of HLH.^{75,86} A promising therapy is inhibition of JAK1/2 kinases with, *for example* the small molecular inhibitor ruxolitinib. JAK1 and JAK2 are required for signalling by multiple cytokines, and inhibitors can thereby target IL-2 and other γ -chain-dependent, IL-6, IL-10 family, IL-12 family, as well as type I and II IFN receptors. Ruxolitinib reduces clinical manifestations of HLH-like disease in mouse models of familial HLH triggered by LCMV infection as well as MAS models with repeated TLR ligand stimulation.^{87,88} Moreover, ruxolitinib has shown promising results for the treatment of HLH patients.⁸⁹

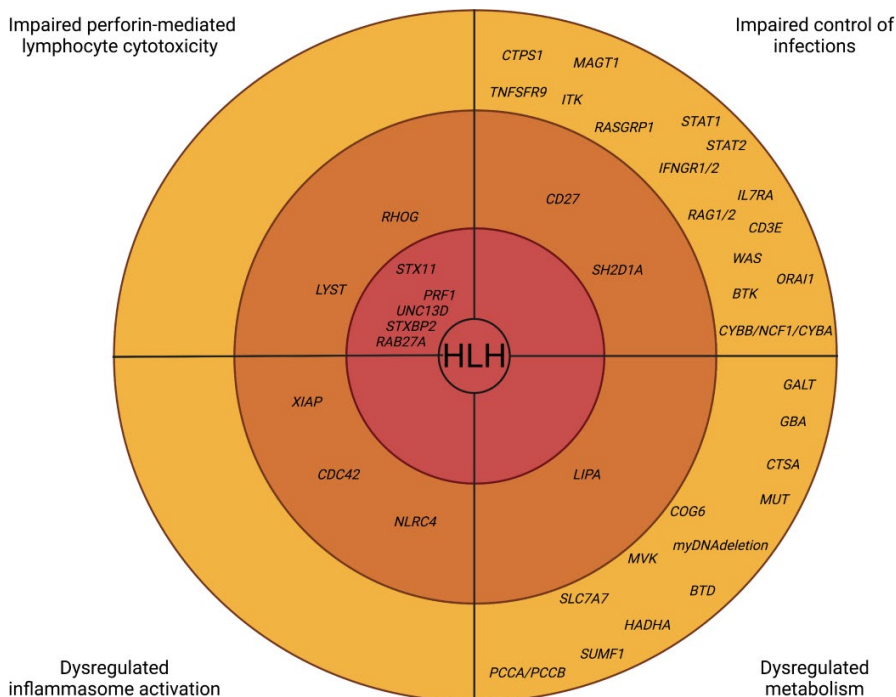


FIGURE 3 Diagram summarises genes that have associated with HLH and indicate to which degree mutations predispose to HLH (red—high likelihood, orange—intermediate, yellow—low likelihood). Patients with nonsense variants in genes required for lymphocyte cytotoxicity invariably present with HLH their first years of life. Patients with inborn errors of immunity may develop HLH in settings of fulminant infection with high load of pathogen associated molecular patterns. A range of metabolic disorders have been associated with development of HLH, but it remains unclear to what extent infections contribute to triggering and pathogenesis

Thus, several different of biological therapies and small molecules hold promise for treatment of HLH. Combinations of these may be beneficial, but trials are needed to determine their efficacy in different forms of HLH. Small molecules that broadly inhibit cytokine signalling are an attractive option, as they can block multiple pathways implicated in lymphocyte as well as inflammasome-driven forms of HLH.

6 | CONCLUSION

Over the last 30 years, the Histiocyte Society's clinical definition of HLH has been a cornerstone for defining hyperinflammatory patients with poor outcome. This definition has inspired and fuelled a large number investigators to identify the underlying causes of HLH, uncovering a range of genetic syndromes that to a varying degree, and potentially triggered by different spectrums of infections, predispose to life-threatening disease (Figure 3). Ultimately, these investigations have provided mechanistic understanding of different pathways that can promote inflammation and immunopathology, providing fundamental understanding of the immune system and novel insights that drastically can improve the treatment of HLH patients. With an increasingly rapid turnover of genome-wide sequencing, novel biomarkers that can differentiate different categories of HLH pathogenesis, and an array of therapeutic options, precision medicine of HLH as well as related inflammatory disorders is achievable.

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We dedicate this review to Jan-Inge Henter who fostered our interest in hyperinflammatory disorders. He has always enthusiastically shared his wealth of knowledge, collaborated across borders and oceans, worked tirelessly and inspired countless scientists to work together to understand and find cures to HLH.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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