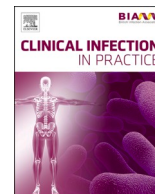




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Hemophagocytic lymphohistiocytosis in adults: A key issue in the COVID-19 era

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ABSTRACT

Hemophagocytic lymphohistiocytosis (HLH) is a syndrome characterized by persistent activation of the mononuclear phagocytic system, systemic inflammation, and hypercytokinaemia, which can lead to liver failure, “sepsis-like syndrome” and ultimately, to multiple organ failure and death. These disorders can be divided into primary and secondary (or reactive), the first, also known as familial HLH, is a genetic condition of childhood, which affects the function of TCD8 and NK cells, and usually presents in the first year of life. The secondary HLH affects mainly adults and adolescents and, it's more related to dysregulation of the immune system. In face of the COVID-19 pandemic and several reports of HLH by SARS-CoV-2, it is necessary to discuss the pathophysiology of HLH in adults more clearly. Thus, we present, for the first time, a didactic approach using illustrations and tables, compiling the most recent and relevant information to better understand this entity.

Introduction

Inspired by the case described by Riley M. *et al.*, of a 51-year-old man who developed severe coronavirus disease 2019 (COVID-19) complicated by suspected hemophagocytic lymphohistiocytosis (HLH), we decided to present an illustrated current view of HLH (Riley *et al.*, 2020).

The hemophagocytic lymphohistiocytosis (HLH) along with other hyperinflammatory and hyperferritinemic syndromes are in great prominence in the face of the COVID-19 pandemic by the SARS-CoV-2, a virus associated with concepts such as “cytokine storm”, hyperinflammatory state, and HLH itself like the SARS-CoV, MERS-CoV (Riley *et al.*, 2020; Copaescu *et al.*, 2020; Soy *et al.*, 2020)

However, despite the clear evidence of hyperinflammation, some do not meet diagnostic criteria for sHLH indicating that in those cases a still not well-defined hyperinflammatory syndrome may be the cause. Authors believe it could be an associated innate neutrophilic hyperinflammatory response or virus-associated-cytokine release syndrome, a condition referred to only as COVID-Cytokine Storm Syndrome or other

possible differential diagnoses (see Figs. 1 and 2).

In 2014, it was validated a score of 9 variables, the HScore, to estimate the risk of an individual having HLH. In 2020, given the COVID-19 pandemic and the identification of a subgroup of patients with sHLH, a condition with high mortality, a Lancet recommendation proposed screen patients with severe COVID-19, using the HScore system to guide the immunomodulating/ immunosuppressive therapies (Wood *et al.*, 2020). However, the use of this system has been the subject of controversy regarding its sensitivity, given the clinical and laboratory peculiarities of these patients (Wood *et al.*, 2020).

The current view of HLH is an inability to adequately restrict the stimulating effects of various immunological triggers, presenting as primary or familial HLH (pHLH) and reactive or secondary (HLH). It is a common terminal pathway, but with different “roots” (La Rosée, 2015).

Defects in granule-mediated cytotoxicity in the innate (NK cells) and adaptive (TCD8 cells) immune system, whether in signaling, traffic, exocytosis, or perforin formation (Arceci, 2008) appear to be the common underlying mechanism. Inherited variations in genes already

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associated with pHLH may play a still unclear role in HLH in adults (Sepulveda and de Saint Basile, 2017; Miao et al., 2017). pHLH is traditionally considered a childhood disease and is even called pediatric HLH. However, cases of late-onset pHLH (adults) started to be published, probably due to hypomorphic or low-penetrance mutations. In the past years, several articles have pointed out the frequent presence of genetic mutations responsible for pHLH in adults, such as *PRF1*, *UNC13D*, *STXBP2*, *LYST*, and others (Table 1), intending to evaluate the possible predisposition to develop HLH. However, it is noteworthy that no clinical implications have been established yet, and further studies are needed (Miao et al., 2017).

In other words, the hypothesis is that changes in the cytotoxic function due to hypomorphic missense and splice-site mutations decrease the “immune threshold” so that HLH can be triggered by infections or other immune stimuli. This suggests that the adult form may be a truly polygenic condition.

The Fas-dependent apoptosis mechanisms are also implied in the pathophysiology. After stimulation by specific T cells, dendritic cells (DC) are susceptible to death by the activated T cells themselves, by mechanisms dependent on perforin and Fas. It has been shown to limit the accumulation of this cell type, to prevent autoimmunity and

systemic inflammation (Chen et al., 2012).

In experimental studies, the perforin deficiency, together with the deletion of Fas-dependent apoptosis mechanisms in dendritic cells (DC), results in a cell accumulation, uncontrolled T cell activation, and production of $\text{INF-}\gamma$, especially by TCD8. Thus, the absence of synergism between perforin and the Fas pathway in maintaining DC homeostasis and prevent the onset of a disordered inflammatory cascade would be an important mechanism in the pathophysiology of HLH (Chen et al., 2012).

In HLH, the increased antigen presentation, their persistence - due to the deficiency in mediated death by cytolytic granules - and repeated $\text{INF-}\gamma$ stimulation “feed” the activation of antigen-presenting cells and T cells. It produces an exaggerated inflammatory response leading to hypersecretion of pro-inflammatory cytokines, such as $\text{INF-}\gamma$, $\text{TNF-}\alpha$, IL-1, IL-4, IL-6, IL-8, IL-10, IL-18, and IL-33 (La Rosée, 2015). Moreover, there is also a possible failure in the feedback of inhibitory cytokines, such as IL-10, TGF-beta, and Fas-ligand (Arceci, 2008).

The pathophysiology of HLH may also be related to the activation of Toll-like receptors (TLR), in the context of viral infections, TLR9 may be particularly important. This endosomal receptor can recognize viral or bacterial dsDNA, preferably unmethylated cytidine phosphate

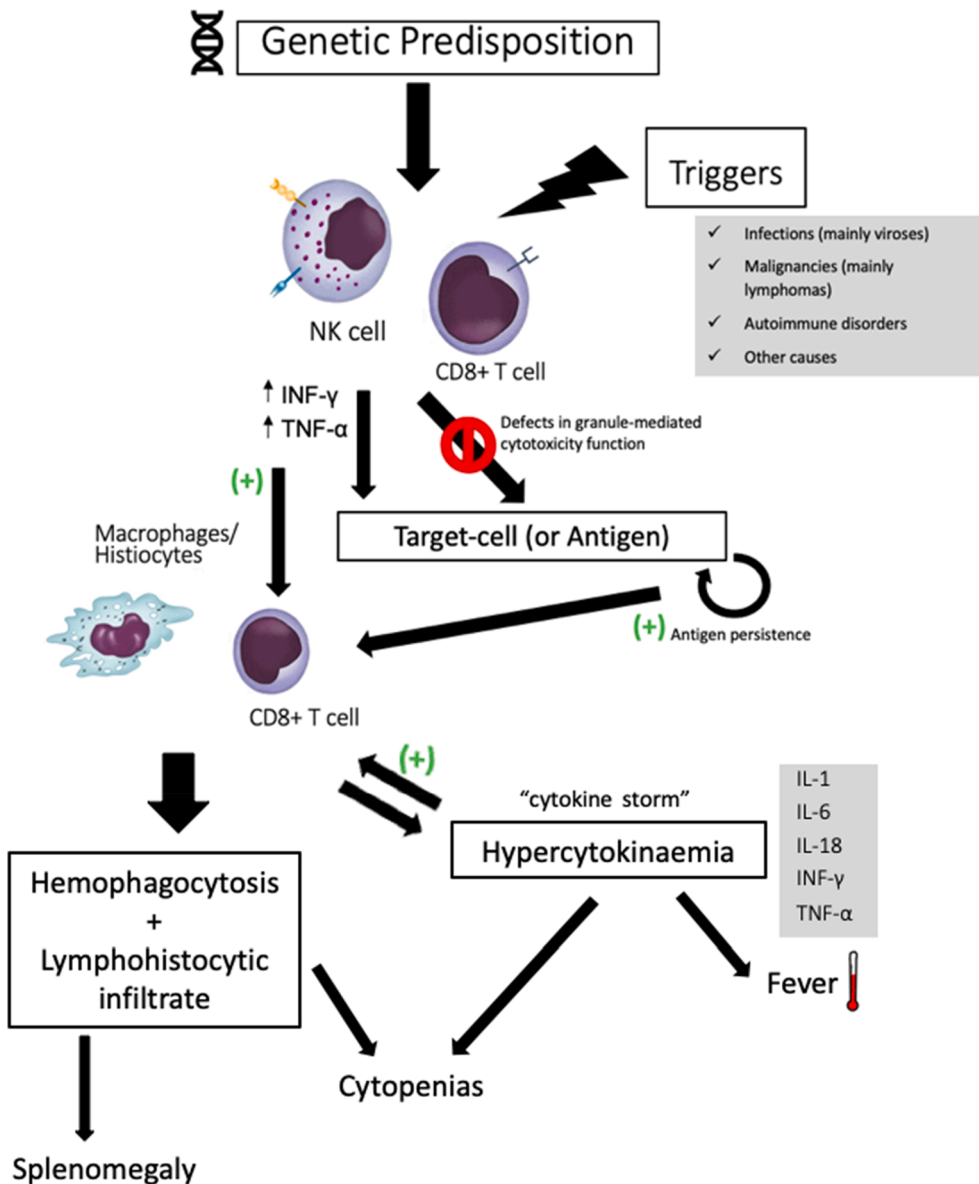


Fig. 1. Pathophysiology of HLH. Certain triggers (infections, autoimmune diseases, malignancies, among others), induce an inflammatory cascade, leading to the stimulation of NK cells and TCD8 lymphocytes, whose impaired function, whether by monogenic (hereditary) defects already well characterized in pHLH, or by heterozygous mutations in loci that control granule-mediated cytotoxicity, still little explored especially in sHLH difficult the elimination of the antigen or target-cell. The persistence of the antigen prolongs the already dysfunctional inflammatory response and the sustained action of macrophages and lymphocytes. Thus, an environment of hyperkitokinaemia and hyperactivity is created. Hypercytokinaemia is mainly responsible for fever and cytopenias, while the lymphohistiocytic infiltrate is responsible for splenoomegaly and of course has a certain contribution to cytopenias.

Disease	Gene	Protein	Defect
FHL1	<i>Unknown</i>	Unknown	Unknown
FHL2	<i>PRF1</i>	Perforin	Pore formation (ultimately results in apoptosis)
FHL3	<i>UNC13D</i>	Munc13.4	Priming of cytolytic granules, defective degranulation
FHL4	<i>STX11</i>	Syntaxin11	Vesicle docking and fusion
FHL5	<i>STXBP2</i>	Munc18.2	Vesicle docking and fusion
Chédiak Higashi	<i>LYST</i>	LYST	Vesicle trafficking
GrisCELLI 2	<i>RAB27A</i>	RAB27A	Vesicle fusion
Hermansky-Pudlak 2	<i>AP3B1</i>	AP3	Vesicle trafficking
XLP1	<i>SH2D1A</i>	SAP	Multiple, including CD8+ T/NK cell cytotoxicity
XLP2	<i>XIAP</i>	XIAP	Multiple signalling pathways

FHL: Familial HLH

Fig. 2. Cytolytic pathway genes associated with familial or primary HLH. These mutations also represent the molecular diagnostic criteria for HLH-2004.

Table 1

Genetic classification of familial haemophagocytic lymphohistiocytosis, and the respective proteins and associated defects.

Disease	Gene	Protein	Defect
FHL1	<i>Unknown</i>	Unknown	Unknown
FHL2	<i>PRF1</i>	Perforin	Pore formation (ultimately results in apoptosis)
FHL3	<i>UNC13D</i>	Munc13.4	Priming of cytolytic granules, defective degranulation
FHL4	<i>STX11</i>	Syntaxin11	Vesicle docking and fusion
FHL5	<i>STXBP2</i>	Munc18.2	Vesicle docking and fusion
Chédiak Higashi	<i>LYST</i>	LYST	Vesicle trafficking
GrisCELLI 2	<i>RAB27A</i>	RAB27A	Vesicle fusion
Hermansky-Pudlak 2	<i>AP3B1</i>	AP3	Vesicle trafficking
XLP1	<i>SH2D1A</i>	SAP	Multiple, including CD8 + T/NK cell cytotoxicity
XLP2	<i>XIAP</i>	XIAP	Multiple signalling pathways

FHL: Familial HLH

guanosine oligonucleotides (CpG), preponderantly expressed by antigen-presenting cells, especially dendritic cells, B cells, and T cells. Murine models with repeated stimulation of TLR9 by CpG, without the addition of exogenous antigen, result in an HLH/MAS-like phenotype in immunocompetent mice (Wood et al., 2020). Unlike what has been seen so far, in this model, despite the pathophysiology being dependent on IFN- γ , only a small population of CD8 + T cells are activated, and the development of the disease is independent of B or T cells, bringing the idea that only activation of innate immune cells via TLR9 is enough to cause HLH/MAS. Likewise, NK cells would also be expendable for the pathophysiology of HLH, however, it is suggested that both cell populations are important for maximal development of the disease. Behrens et al. further suggest that TLR or IFN- γ blockade are important pathways with therapeutic relevance in HLH (Behrens et al., 2011).

Independent of the pathway, the result is the accumulation of lymphohistiocytic infiltrates in multiple organs and systems. Macrophages

non-selectively phagocytize hematopoietic elements, leading to one of the main microscopic findings, hemophagocytosis, not only in the bone marrow and peripheral blood. Fever is caused by endogenous pyrogens, such as IL-1, IL 6, and TNF- α . Fatigue and, above all, cytopenias, reflect the suppressive activity in hematopoiesis by TNF- α , INF- γ , and the heavy subunit of ferritin, as well as hemophagocytosis itself. Still, the presence of these two cytokines can lead to inhibition of lipoprotein lipase or stimulate the synthesis of triglycerides, leading to hyperlipidemia. The hypofibrinogenemia and increased fibrin degradation products may be a result of an increase in plasminogen activator, expressed by macrophages. Hyperferritinemia can result from exaggerated secretion by activated macrophages. Thus, the so-called “cytokine storm” is related to the development of the main clinical and laboratory characteristics of HLH (Arceci, 2008; Chen et al., 2012).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics approval

Not Applicable.

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