Received: 5 February 2018 | Revised: 13 June 2018 | Accepted: 14 June 2018

DOI: 10.1002/pbc.27344

BRIEF REPORT

WILEY Pediatric Blood & Cancer

SIOP INTERNATIONAL SOCIETY OF PAEDIATRIC ONCOLOGY

aspho The American Society of Pediatric Hematology/Oncology

Is an infectious trigger always required for primary hemophagocytic lymphohistiocytosis? Lessons from in utero and neonatal disease

Maximilian Heeg[1,2,3] | Sandra Ammann[1,4] | Christian Klemann[1,2] | Marcus Panning[5] | Valeria Falcone[5] | Harmut Hengel[5] | Kai Lehmberg[6,7] | Udo zur Stadt[6,7] | Katharina Wustrau[6,7] | Gritta Janka[6] | Stephan Ehl[1,2]

[1]Center for Chronic Immunodeficiency, Medical Center University of Freiburg, Faculty of Medicine, Freiburg, Germany

[2]Center for Pediatrics, Medical Center, University of Freiburg, Faculty of Medicine, Freiburg, Germany

[3]Berta-Ottenstein-Programme, Faculty of Medicine, University of Freiburg, Freiburg, Germany

[4]Cambridge Institute for Medical Research, Biomedical Campus, Cambridge, United Kingdom

[5]Institute of Virology, University Medical Center, Faculty of Medicine, Albert-Ludwigs-University of Freiburg, Freiburg, Germany

[6]Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[7]Divison of Pediatric Stem Cell Transplantation and Immuology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Correspondence
Stephan Ehl, Center for Chronic Immunodeficiency, Breisacher Strasse 115, 79106 Freiburg.
Email: stephan.ehl@uniklinik-freiburg.de

Funding information
Deutsche Kinderkrebsstiftung, Grant/Award Number: DKS 2016.04; Deutsche Forschungsgemeinschaft, Grant/Award Number: SFB1160; Bundesministerium für Bildung und Forschung, Grant/Award Number: 01 EO 0803

M.H. and S.A. contributed equally to this work.

Abstract

In this report, we evaluate the hypothesis that hemophagocytic lymphohistiocytosis in patients with defects of lymphocyte cytotoxicity is usually triggered by infections. We show that in the majority of patients, extensive virus PCR panels performed in addition to routine microbiological investigations remain negative and summarize 25 patients with onset of hemophagocytic lymphohistiocytosis in utero or within the first 10 days of life, in none of which an associated bacterial or viral infection was reported. These observations, even though preliminary, invite to consider a key role of lymphocyte cytotoxicity in controlling T-cell homeostasis also in the absence of apparent infectious stimuli.

KEYWORDS
hemophagocytic lymphohistiocytosis, immunodeficiencies, primary HLH

1 | INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory syndrome caused by uncontrolled immune cell activation.[1] Genetic disorders leading to impaired lymphocyte cytoxicity (familial hemophagocytic lymphohistiocytosis, FHL) are the best characterized risk factor for development of HLH. Infants with cytotoxicity defects are usually asymptomatic at birth. The age at onset of inflammatory symptoms varies, even between affected siblings,[2] indicating that additional factors are required. Many infections can trigger primary and secondary HLH.[3–5] The association of FHL manifestation with infections can be interpreted to indicate that in most cases the triggers of HLH are infections,[6] even if they sometimes escape awareness or detection.

2 | RESULTS

In a recent analysis of our German HLH registry, we noted that in many FHL patients, no infection had been documented.[7] Recommended investigations included blood, urine and stool cultures, PCR for Epstein–Barr virus (EBV), cytomegalovirus (CMV), Adenovirus, Parvovirus, herpes simplex virus and gastrointestinal and/or respiratory virus panels in case of respective clinical symptoms. However,

Abbreviations: CMV, cytomegalovirus; CNS, central nervous system; EBV, Epstein–Barr virus; FHL, familial hemophagocytic lymphohistiocytosis; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GS2, Griscelli syndrome type 2; HLA, human leukocyte antigen; HLH, hemophagocytic lymphohistiocytosis; HHV, human herpesvirus; HSCT, hematopoietic stem cell transplantation; HSV, herpes simplex virus; RSV, respiratory syncytial virus; TTMV, Torque Teno mini virus; TTV, Torque Teno virus

TABLE 1 PCR panel

Pt#	HLH diagnosis	Age at onset [y]	TTV	TTMV	Other virus-PCRs and multiplexed nucleic acid tests	GAPDH
Controls 1	SAP-deficiency, EBV triggered HLH	7			EBV	+
Control 2	CMV triggered 2° HLH	0.3			CMV	+
Control 3	2° HLH, no reported trigger	21			CMV	+
Control 4	2° HLH, no reported trigger	0.3			-	+
Control 5	HSV triggered 2° HLH	0			HSV	+
1	FHL2	0.4	–	+	–	+
2	FHL2	0.2	–	+	–	+
3	FHL2	0.1	–	–	–	+
4	FHL2	0.2	–	–	–	+
5	FHL2	0.2	–	–	–	+
6	FHL2	0.2	–	–	–	+
7	FHL2	0.8	+	+	CMV	+
8	FHL3	5.2	–	–	–	+
9	FHL3	0.3	–	–	–	+
10	FHL3	0.3	–	–	–	+
11	FHL3	0.3	–	–	–	+
12	FHL3	0.6	+	–	–	+
13	FHL3	0.1	–	–	–	+
14	FHL3	0.2	–	–	–	+
15	FHL3	1.0	–	+	–	+
16	FHL3	9.0	–	–	–	+
17	FHL3	0.4	–	–	–	+
18	FHL3	0.2	–	+	–	+
19	FHL3	0.1	–	–	CMV	+
20	FHL5	0.2	–	–	–	+
21	FHL5	0.2	–	+	–	+
22	FHL5	0.2	–	+	–	+
23	FHL5	0.8	+	+	–	+
24	FHL5	5.2	–	–	–	+
25	FHL5	0.3	–	–	–	+
26	FHL5	0.3	–	–	–	+

The PCR panel was validated by 5 patients with 2° HLH, where the reported trigger could also be found in the stored biomaterial. All 1° HLH patients were tested for infections in the treating center beforehand. Recommended investigations included blood, urine and stool cultures, PCR for EBV, CMV, adenovirus, parvovirus, herpes simplex virus, and gastrointestinal and/or respiratory virus panels in case of respective clinical symptoms.

The PCR panel included the following viruses: EBV, CMV, HSV1+2, HHV6, RSV, parechoviruses, adenovirus, parvovirus, and BK polyomavirus. Multiplexed nucleid acid tests include: (1) adenovirus 40/41, rotavirus A, *C. difficile*, campylobacter, *E. coli* O157, ETEC LT/ST, salmonella, STEC stx1/stx2, *Shigella, Vibrio cholera*, *Yersinia enterocolitica*, *Cryptosporidium*, *Entamoeba histolytica*, *Giardia* (xTAG Gastrointestinal pathogen panel, Luminex, the Netherlands) (2) Flu A (H1/H3), Flu B, RSV, coronaviruses (229E, OC43, NL63, HKU1), parainfluenza 1–4, hMPV, entero-/rhinovirus, adenovirus, bocavirus (xTAG Respiratory Viral Panel FAST v2, Luminex, the Netherlands).

since patients were not enrolled in a formal protocol, we did not have definite information, whether all microbiological studies had been performed. Assuming that some of the investigations were incomplete, we performed an additional search for viruses using biomaterial stored at the time of HLH diagnosis. The patients are part of a cohort of 87 patients with 1° HLH recruited between 2008 and 2014.[7] Of these, 12 (8% of 61 patients < 2 years and 27% of 26 patients > 2 years) had a documented infection at the time of HLH manifestation, 11 viral infections and one periungual *S. aureus* infection. In 26 of the remaining 75 patients, we had access to biomaterial (7 FHL2, 12 FHL3, and 7 FHL5). Age at onset was < 2 years in 23 patients. Informed consent was obtained (University of Freiburg IRB 143/12; 40/08). We isolated DNA and RNA from the pelleted blood cell fraction remaining after Ficoll separation of peripheral blood mononuclear cells from the initial diagnostic sample. Real-time PCR was performed for EBV, CMV, herpes simplex virus 1+2 (HSV), human herpesvirus 6 (HHV), respiratory syncytial virus (RSV), parechoviruses, adenovirus, parvovirus, and BK polyomavirus using

TABLE 2 FHL patients with manifestation of HLH in utero or the first week of life

Patient #	Age at onset	Gestation (wk)	Initial symptoms	HLH criteria		FHL diagnosis	Infection	Ref
				Fulfilled	Tested			
1	24 GW	30	Myocardial hypertrophy, pericardial effusion	5	6	FH	n.r.	[14]
2	24 GW	32	HSM, ascites, hydrocephalus	3	4	FH	n.r.	[9]
3	30 GW	†	Hydrops	2	2	FHL3	no	[15]
4	31 GW	31	Hydrops, fetal distress	5	6	FHL2	no	[16]
5	31 GW	31	Hydrops, fetal distress	4	5	FHL2	no	[16]
6	33 GW	33	Hydrops, ascites, edema	2	2	FHL2	n.r.	[17]
7	34 GW	34	Hydrops	4	4	FHL2	n.r.	[18]
8	34 GW	34	Ascites in utero, petechiae, anemia	6	6	FH	n.r.	reg
9	35 GW	35	Polyhydramnion, HSM, ascites	5	6	FH	no	[19]
10	36 GW	36	Hydrops	4	5	FH	no	[19]
11	36 GW	36	Ascites, fetal distress	6	7	FHL2	no	[20]
12	Birth	32	Ascites, petechiae	5	5	FHL2	no	[21]
13	Birth	33	HSM	6	7	FHL5	no	reg
14	Birth	34	Jaundice, cytopenia, ascites	8	8	FHL3	no	reg
15	Birth	36	HSM, edema, petechiae, resp. distress	4	7	FHL3	no	[22]
16	Birth	36	HSM, petechiae, cytopenia, respiratory distress	7	8	FHL2	no	reg
17	Birth	37	HSM, opisthotonus	5	7	GS2	no	reg
18	Birth	37	Thrombocytopenia	7	8	FHL2	no	reg
19	1 d	39	Fever, petechiae, resp. distress	4	5	FHL2	no	[17]
20	1 d	n.r.	Fever, thrombocytopenia, elevated bilirubin	4	4	FHL2	n.r.	[23]
21	2 d	35	HSM, jaundice, petechiae, cytopenia	7	7	FHL3	no	reg
22	2 d	40	Fever, lethargy	6	6	FHL2	no	[24]
23	5 d	39	Sepsis-like, cytopenia, ileus	1	1	FHL5	n.r.	[25]
24	6 d	39	Hydrops	4	4	FHL2	no	reg
25	8 d	38	HSM	7	7	rec. HLH, consang.	no	reg

Abbreviations: GW, gestational week; i.u., intrauterine; HSM, hepatosplenomegaly; FH, family history; FHL, familial hemophagocytic lymphohistiocytosis; n.r., not reported; Ref, reference; reg, patient information retrieved from German HLH registry; consang, consanguineous.

commercial kits. As quality control, we included PCRs for torque teno virus (TTV) and torque teno mini virus (TTMV), two persisting DNA viruses thought to be prevalent in 20%–40% of children aged 2–4 years[8] and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a positive control. Assuming that local mucosal infections could disseminate in cytotoxicity-deficient patients under HLH conditions, we also performed multiplexed nucleic acid tests for gastrointestinal pathogens and respiratory viruses. We validated the methodology by investigating 3 patients with EBV, CMV, and HSV-1–associated 2° HLH, where the initially detected virus could also be found in the stored biomaterial. Analysis of the 26 FHL samples revealed TTV and TTMV at the expected frequency. Additionally, CMV was identified in 2 patients. Notably, in the 24 remaining FHL patients, all PCR results remained negative (Table 1).

We then sought further evidence that FHL can manifest in the absence of infection. For this, we followed the sporadic observation that FHL patients can already present with signs of HLH in utero or within the first 10 postnatal days, when exposure to known and unusual pathogens is limited.[9] We performed a retrospective survey in addition to a systematic literature search. The following criteria had to be fulfilled for patient inclusion: (i) a genetic diagnosis of FHL OR a positive family history of HLH OR recurrent HLH AND (ii) fulfillment of ≥5/8 HLH criteria if all were tested OR no more than 2 normal HLH criteria if less than 8 were tested AND (iii) onset of HLH-related symptoms in utero or within the first 10 postnatal days.

Twenty-five patients were included (Table 2; references in Supplementary Information References S1). The most prominent clinical features were fetal hydrops, most likely as a result of anemia, and splenomegaly (Table 2). The most frequent HLH criteria were splenomegaly in 24 and bicytopenia in 20 infants (Supplementary Information Table S1). A genetic diagnosis was established in 19 patients (12 FHL2, 4 FHL3, 2 FHL5, and 1 Griscelli syndrome). Of the 25 infants, 21 died of HLH or of complications of therapy, 3 patients are alive after hematopoietic stem cell transplantation (HSCT), but neurologically impaired and only one is well after transplantation. Interestingly, in 18 of 25 cases no infectious trigger was documented despite extensive investigations for intrauterine or neonatal infections, and in the remaining 7 reports no microbiological investigations were mentioned.

3 | DISCUSSION

This report focuses on the question of whether HLH manifestation in FHL patients always requires an infectious trigger. We report on two patient cohorts with primary HLH with a low incidence of infections. Obviously, some limitations require discussion. In the first cohort, the analysis was restricted to viral DNA or RNA isolated from a blood fraction that is not standard for virus detection. Although we could detect EBV, CMV, HSV, TTV, and TTMV in high copy numbers in this material, we could not validate all of the viruses tested in our study, in particular viruses with replication restricted to mucosal sites. Also, viruses may have initiated the immune reaction, but already been eliminated at the time of blood sampling. In addition, unknown viruses or organisms other than viruses causing localized or systemic infections were not covered in our panel. On the other hand, the infection rate of 16% (14/87) was similar to the 19% (32/168) observed in a recent report.[10] Higher rates ranging from 28% (5/18)[4] to 42% (25/60)[11] up to 81% (13/16)[5] had been reported earlier, but in these studies, 1° and 2° HLH could not be readily differentiated. It is also notable that in our cohort the immunosuppressive HLH therapy did not lead to secondary emergence of a pathogen in the FHL infants presenting without apparent trigger. In the second cohort, although an infection was the key initial differential diagnosis and extensive investigations for neonatal infections were therefore performed, the lack of standardized testing represents a limitation. Furthermore commensal bacteria or viruses that escaped awareness of the physician may become pathogenic in immunodeficient patients. Also, exposure to bacteria or transplacental transmission of pathogens could have triggered HLH in these infants without causing infectious illness. However, the fact that not a single case was reported with an associated infection challenges the concept that HLH manifestations in FHL patients may always require an overt infection.

Therefore, we propose that besides infections other triggers should also be considered in primary HLH. This is further supported by the observation of isolated central nervous system (CNS) HLH without detectable trigger in FHL patients (M. Heeg, unpublished), where HLH appears to be initiated in a primarily sterile compartment. We speculate that noninfectious triggers provoking tissue damage, the associated danger signals or even developmental processes in the fetus that require expansion of cytotoxic lymphocytes could be sufficient to initiate the immune activation. The occasional association of disease onset with vaccination supports these possibilities. However, it is difficult to envisage how such moderate localized and transient stimuli can result in systemic polyclonal activation of up to 80% of all T cells.[12] In perforin-deficient mice, at least the large majority of hyperactivated T cells is specific for the triggering virus.[13] The specificity of these T cells in human patients remains an enigma. Understanding their specificity might help to further elucidate the pathogenesis of HLH. The unique amplification of cytotoxic (HLA-DR$^+$ CD127$^-$PD1$^+$ CD57$^+$ perforin$^+$) CD4$^+$ T cells observed in 1°, but not in virus-induced 2° HLH may provide additional clues.[12]

From a biological viewpoint, our findings and considerations suggest that in humans lymphocyte cytotoxicity is key to control T-cell activation, also to minor noninfectious stimuli, in a way that prevents an extensive amplification of a dramatic, potentially lethal immune response. This puts lymphocyte cytotoxicity to the center of immune regulation and maintenance of T-cell quiescence, far beyond its activity in virus control. A better understanding of the initiation of immune reactions in HLH and the role of perforin and the secretory pathway in controlling them will therefore be highly relevant for understanding the regulation of human immune responses. Ultimately, it will also provide insights relevant for prophylaxis and intervention in this life-threatening disease.

ACKNOWLEDGMENTS

This work was supported by the SFB1160: IMPATH (TP1), the BMBF (01 EO 0803), and Deutsche Kinderkrebsstiftung DKS 2016.04. Maximilian Heeg is a Bertha-Ottenstein Scholar of the Medical Faculty of the University of Freiburg.

AUTHOR CONTRIBUTIONS

MH, SA, and SE analyzed data and wrote the manuscript. MP, VF, and HH performed virological analysis. US performed genetic investigations. MH, SA, CK, KL, KW, GJ, and SE provided clinical information. SE supervised the project. All authors commented on the manuscript.

CONFLICT Of INTEREST

SE has a scientific collaboration with UCB, which is *not* related to this study.

ORCID

Maximilian Heeg http://orcid.org/0000-0002-9594-1377

REFERENCES

1. Sepulveda FE, de Saint Basile G. Hemophagocytic syndrome: primary forms and predisposing conditions. *Curr Opin Immunol*. 2017;49:20–26.
2. Allen M, De Fusco C, Legrand F, Clementi R, et al. Familial hemophagocytic lymphohistiocytosis: how late can the onset be? *Haematologica* 2001;86:499–503.
3. Ansuini V, Rigante D, Esposito S. Debate around infection-dependent hemophagocytic syndrome in paediatrics. *BMC Infect Dis*. 2013;13:15.
4. Sung L, Weitzman SS, Petric M, King SM. the role of infections in primary hemophagocytic lymphohistiocytosis: a case series and review of the literature. *Clin Infect Dis*. 2001;33:1644–1648.
5. Henter J-I, Ehrnst A, Andersson J, Elinder G. Familial hemophagocytic lymphohistiocytosis and viral infections. *Acta Paediatr*. 1993;82:369–372.
6. Jordan MB. An animal model of hemophagocytic lymphohistiocytosis (HLH): CD8$^+$ T cells and interferon gamma are essential for the disorder. *Blood*. 2004;104:735–743.
7. Ammann S, Lehmberg K, zur Stadt U, et al. Effective immunological guidance of genetic analyses including exome sequencing in patients evaluated for hemophagocytic lymphohistiocytosis. *J Clin Immunol*. 2017;37:770–780.

8. Spandole S, Cimponeriu D, Berca LM, Mihăescu G. Human anelloviruses: an update of molecular, epidemiological and clinical aspects. *Arch Virol*. 2015;160:893–908.
9. Shah AJ, Kapoor N, Cooper RM, et al. Pre- and post-natal treatment of hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2009;52:139–142.
10. Bergsten E, Horne A, Aricó M, et al. Confirmed efficacy of etoposide and dexamethasone in HLH treatment: long-term results of the cooperative HLH-2004 study. *Blood*. 2017;130:2728–2738.
11. Aricò M, Janka G, Fischer A, et al. Hemophagocytic lymphohistiocytosis. *Leukemia*. 1996;10:197–203.
12. Ammann S, Lehmberg K, zur Stadt U, et al. Primary and secondary hemophagocytic lymphohistiocytosis have different patterns of T-cell activation, differentiation and repertoire. *Eur J Immunol*. 2017;47:364–373.
13. Murali-Krishna K, Altman JD, Suresh M, et al. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. *Immunity*. 1998;8:177–187.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Heeg M, Ammann S, Klemann C, et al. Is an infectious trigger always required for primary hemophagocytic lymphohistiocytosis? Lessons from in utero and neonatal disease. *Pediatr Blood Cancer*. 2018;65:e27344. https://doi.org/10.1002/pbc.27344