CASE REPORT

Crohn's disease complicated by Epstein-Barr virus-driven haemophagocytic lymphohistiocytosis successfully treated with rituximab

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SUMMARY

Summary We report a case of Epstein-Barr virus (EBV)-driven haemophagocytic lymphohistiocytosis (HLH) in a man with Crohn's disease treated with 6-mercaptopurine and adalimumab therapy who was successfully treated with rituximab therapy alone. This is the first published case in an adult patient with EBV-driven HLH in the setting of thiopurine use and inflammatory bowel disease to be successfully treated with rituximab therapy alone. Here, we will discuss putative immunological mechanisms which may contribute to this potentially life-threatening complication.

BACKGROUND

Haemophagocytic lymphohistiocytosis (HLH), a rare disorder of excessive immunoactivation, can occur in the paediatric and adult populations. HLH is associated with impaired cytotoxic function of natural killer (NK) cells and cytotoxic T cells resulting in excessive macrophage activation and cytokine production.¹⁻⁴ This excessive immune stimulation can result in tissue damage and phagocytosis of haemopoietic cells.

HLH can occur as either a primary or secondary disease. Primary HLH is caused predominantly by mutations in genes responsible for perforin-mediated cytotoxicity and is diagnosed by the combination of a pathogenic mutation together with specific clinical and laboratory parameters (box 1).

Secondary HLH may be seen in association with various conditions which result in immune dysregulation, including malignancy, autoimmune diseases and immunosuppression.¹⁻⁴ In either form, infections are a common trigger with Epstein-Barr virus (EBV) being the most common in children.⁵

The traditional treatment approach includes combination chemotherapy with etoposide, dexamethasone and ciclosporin and, in some cases additional intrathecal methotrexate and haematopoietic stem cell transplantation.¹⁻⁴ There have now been two case reports that demonstrate the efficacy of rituximab therapy alone in EBV-related HLH.^{6, 7} This is an appealing treatment option as it is more targeted and less toxic.

There are a number of reports in the literature describing an association between the development of virally driven HLH and thiopurine use in inflammatory bowel disease (IBD).⁷⁻¹⁴ In a cohort of paediatric patients with HLH and IBD, thiopurine use increased the risk of developing HLH by

100-fold.⁸ We describe the first adult case of EBV-driven HLH in the setting of thiopurine use in IBD that was successfully treated with rituximab therapy alone.

CASE PRESENTATION

A man aged 24 years was diagnosed with ileal Crohn's disease (CD) in 2014 after a colonoscopy to investigate iron deficiency anaemia demonstrated terminal ileitis and ulceration. Subsequent MRI enteroclysis demonstrated ileal inflammation with an entero-enteric fistula. He experienced intermittent lower abdominal pain but no perianal or extra-intestinal manifestations of CD. He was a non-smoker with no other significant medical history. His mother also suffered from CD. At diagnosis, he was started on 40 mg prednisolone daily, 4 g mesalazine daily and 100 mg 6-mercaptopurine (6MP) daily. Prednisolone was weaned over the course of 4 months. Despite this, his disease remained active and adalimumab therapy was started in March 2015.

In June 2015, following a 10-day history of fevers, sore throat and lethargy, he was found to have cervical lymphadenopathy and hepatosplenomegaly, confirmed on CT abdomen. His blood tests revealed a pancytopenia, hepatitis and hyponatraemia. His 6MP was ceased. Thiopurine S-methyltransferase activity was normal (0.83 nmol/gHb/min), while the 6-thioguanine and 6-methylmercaptopurine levels were not outside the therapeutic range (160 pmol/8×10⁸ RBC (235–450 pmol/8×10⁸ RBC) and 2130 pmol/8×10⁸ RBC (<5700 pmol/8×10⁸ RBC), respectively). Further investigations identified a markedly elevated ferritin, soluble CD25, hypertriglyceridaemia and hypofibrinogenaemia (table 1). Although bone marrow biopsy did not demonstrate conspicuous haemophagocytosis, he fulfilled diagnostic criteria for HLH (box 1).¹⁵

Active EBV infection with positive IgM, IgG and heterophile antibodies and a plasma viral load of 3.02×10⁴/L was subsequently noted. Lymphocyte subset analysis revealed a complete absence of NK cells in peripheral blood; therefore, functional studies were not performed acutely (table 1).

He was treated initially with intravenous tazocin 4.5 g four times daily. Once HLH was diagnosed, he was started on dexamethasone 20 mg daily. An initial improvement in the cytopenias and hepatitis occurred; however, after 10 days of dexamethasone, he was still spiking high temperatures and had ongoing pancytopenia (table 1). Rituximab

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Box 1 Diagnostic criteria for haemophagocytic lymphohistiocytosis

Molecular identification of an HLH-associated gene mutation (ie, *PRF1*, *UNC13D*, *STX11*, *STXBP2*, *Rab27A*, *SH2D1A*, or *BIRC4*)

OR

Five of the following eight findings

- ▶ Fever $\geq 38.5^{\circ}\text{C}$
- ▶ Splenomegaly
- ▶ Peripheral blood cytopenia, with at least two of the following:
 - Haemoglobin $< 9\text{ g/dL}$
 - Platelets $< 100\,000/\mu\text{L}$
 - Absolute neutrophil count $< 1000/\mu\text{L}$
- ▶ Hypertriglyceridaemia (fasting triglycerides $> 265\text{ mg/dL}$) and/or hypofibrinogenaemia (fibrinogen $< 150\text{ mg/dL}$)
- ▶ Haemophagocytosis in the bone marrow, spleen, lymph node or liver
- ▶ Low or absent NK cell activity
- ▶ Ferritin $> 500\text{ ng/mL}$
- ▶ Elevated soluble CD25 (soluble IL-2 receptor α) $> 2400\text{ U/mL}$

800 mg (375 mg/m^2) was then given, which resulted in a rapid improvement clinically and in his laboratory parameters. Within 48 hours, he became afebrile and there was a clear improvement in his cytopenias, hepatitis and ferritin levels (table 1). Prior to his second dose of rituximab, the EBV viral load had dropped and he was discharged from hospital (table 1). His dexamethasone was weaned and he was given one further dose of rituximab 1 week later after which his EBV viral load became undetectable (table 1).

Genetic testing did not reveal an underlying cause for his HLH and NK cell deficiency. Specifically, we did not find any mutations in genes associated with familial HLH including *PRF1*, *UNC13D* or *Syntaxin 11*. Testing for X linked lymphoproliferative syndrome type 1 and 2 did not find mutations in *SH2* domain containing either protein 1A (*SH2D1A*) or *BIRC4* and immunophenotyping revealed normal intracellular SLAM-associated protein (SAP) and X linked inhibitor of apoptosis protein (XIAP) expression.

Serial testing of NK cell number and function, via a flowcytometric cytotoxic killing assay, showed improvement and eventual normalisation following treatment. Three months later, adalimumab was restarted and has remained well. He has been advised to avoid thiopurine therapy indefinitely, given the recognised association between the development of virally induced HLH and thiopurine therapy.⁸

Table 1 Patient's results

Laboratory parameter	At presentation	Postdexamethasone	Post first dose of rituximab	Post second dose of rituximab
Haemoglobin (135–180 g/L)	82	90	108	143
White cell count ($4\text{--}11 \times 10^9/\text{L}$)	1.06	2.92	4.69	7.14
Neutrophil ($2\text{--}7.5 \times 10^9/\text{L}$)	0.39	1.02	1.96	3.09
Lymphocytes ($1.2\text{--}4 \times 10^9/\text{L}$)	0.27	0.46	0.94	2.11
Platelets ($150\text{--}400 \times 10^9/\text{L}$)	101	154	235	257
Bilirubin ($< 20\text{ }\mu\text{mol/L}$)	33	21	18	6
Alkaline phosphatase (30–110 U/L)	394	378	228	51
ALT ($< 40\text{ U/L}$)	288	393	267	17
GGT ($< 60\text{ U/L}$)	1030	966	659	37
Albumin (35–50 g/L)	31	27	34	47
Ferritin (30–620 $\mu\text{g/L}$)	11 300	8140	3000	81
EBV viral load (copies/mL)	30 200	1870	4180	0
Soluble CD25 (186–2678 pg/mL)	22 718	—	4214	—
Triglycerides ($< 1.7\text{ mmol/L}$)	2.8	2.2	1	1.4
NK cell number ($93\text{--}575 \times 10^6/\text{L}$)	0	24	—	208
NK cell function ($> 17\%$ target cell killing)	—	15	—	69
Fibrinogen (2–4 g/L)	1.8	2.2	2	3.1
Maximum temperature ($^{\circ}\text{C}$)	39.1	39.4	37.6	36.7

DISCUSSION

Thiopurine treatment in IBD is associated with an increased risk for developing virally driven HLH.^{7–14} This may be attributable either to viral reactivation or an inability to control primary viral infection. The underlying mechanisms are unclear but may involve dysfunction of NK and cytotoxic T cells, given their importance in clearing virally infected cells.

A recent review identified 22 patients who developed HLH in the setting of IBD.⁹ Within this cohort, CD was more common than ulcerative colitis and patients on thiopurine therapy were most commonly affected (70%). TNF inhibitors were used in five patients, four of which were in combination with a thiopurine as in our case. The vast majority of patients had an acute infection with cytomegalovirus (CMV), or EBV, and despite treatment, five patients died (table 2). Haemophagocytosis was observed in only half of the patients and NK cell function was not reported.¹⁰

Thiopurines prevent lymphocyte proliferation and increase apoptosis of activated lymphocytes which are essential in the immunological defence against viruses such as CMV and EBV.¹⁶ Our patient had undetectable NK cells, which returned to normal following cessation of thiopurine therapy and resolution of his HLH, potentially highlighting an effect of thiopurines on NK cells. In systemic lupus erythematosus and patients who had undergone renal transplant, there is a significant reduction in NK cell activity in those treated with the thiopurine azathioprine (AZA), a finding that has not been reported with other immunosuppressants.^{17–18} Interestingly, our patient only developed HLH 4 months following the addition of adalimumab therapy. The contribution of other immunosuppressants such as TNF inhibitors either alone or in combination with thiopurines to the risk of HLH in IBD is unclear.

The only other published case of HLH in the setting of CD and AZA therapy was in a girl aged 10 years who also had an absence of NK cells. She also had reduced perforin expression at presentation of her HLH.⁷ She was found to have a heterozygous nucleotide substitution in PRF1 gene. Repeat testing following HLH resolution revealed normal perforin expression and NK cell numbers. This mutation has previously been

thought to be non-pathogenic but may have increased her susceptibility to HLH.⁷

It is tempting to consider the presence of a common underlying molecular defect, which predisposes patients to EBV-driven HLH and IBD. As only a very small subset of patients with IBD develops this complication, possibilities include a rare genetic polymorphism and/or an as-yet undetermined environmental insult. The role of common perforin polymorphisms such as Ala91Val is controversial, but some heterozygous individuals have mildly reduced perforin expression. It is, therefore, possible that these polymorphisms, although not sufficient on their own, may increase the risk of developing HLH.⁷ To date, there has been limited investigation into underlying genetic causes in the cases published.

Chronic haemorrhagic inflammatory colitis is a well-recognised feature of patients with X linked lymphoproliferative disease type 2 due to mutations in XIAP.¹⁹ Recent reports have shown that XIAP is involved in activation of the nucleotide-binding oligomerisation domain containing 2 (NOD2) receptor.²⁰ NOD2/Card15 variants are also well known to be associated with an increased risk of CD.²¹ Given the importance of a Th1-driven cytokine response in CD and HLH, there may be underlying disease-specific factors which contribute to HLH in a subset of patients with CD.^{22–23}

This case reinforces the efficacy of rituximab therapy in EBV-driven HLH. Rituximab works by removing the EBV-infected B-cells, which are thought to drive the excessive immune response seen in HLH.^{6–7} This therapeutic approach is much more targeted, specific and less toxic when compared with traditional chemotherapy in patients with EBV-driven HLH. Rituximab has been used in other cases of EBV-driven HLH in adults with IBD on thiopurines but usually in combination with other agents, including etoposide.^{8–14} Our patient was treated with rituximab prior to the development of any end-organ complications of HLH which may have contributed to the success of rituximab therapy in our case. There is only one similar case reported in a child who had rituximab in the absence of chemotherapy.⁷ This is the first report of an adult with EBV-driven HLH in the setting of thiopurine therapy for IBD to be successfully treated with single-agent rituximab therapy.

Although the association between EBV-driven HLH and IBD is being increasingly recognised, HLH remains a rare condition and there are still many unanswered questions. Further research and extensive genetic testing of index cases (which can be provided by next generation sequencing) may prove useful in elucidating possible links between IBD and the development of HLH, particularly in the setting of thiopurine therapy. Increasing awareness of this association among physicians is also paramount to ensure early recognition and prompt management of this life-threatening condition.

Table 2 Characteristics of inflammatory bowel disease patients who developed haemophagocytic lymphohistiocytosis⁹

Inflammatory bowel disease	Number of patients (%)
CD	18 (81.8)
UC	4 (18.1)
Immunosuppressive therapy	
Any thiopurine therapy	17 (77.2)
Thiopurine alone	9 (40.9)
Thiopurine in combination with steroids	4 (18.1)
Thiopurine in combination with TNF inhibitor	4 (18.1)
Corticosteroids alone	3 (13.6)
TNF and corticosteroids	1 (4.5)
Trigger	
CMV	12 (54.5)
EBV	8 (36.4)
Other	2 (9.1)
Mortality	5 (22.7)
EBV	3 (13.6)
CMV	2 (9.1)

Learning points

- ▶ Rituximab is an effective, safe and more specific therapy for Epstein-Barr virus (EBV)-driven haemophagocytic lymphohistiocytosis (HLH).
- ▶ There is an association between the development of EBV-driven HLH in the setting of thiopurine therapy for treatment of inflammatory bowel disease.
- ▶ Awareness of this association is important to ensure early recognition and treatment, given the high mortality.

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