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CASE REPORT

Dilemmas in the diagnosis and pathogenesis of atypical late-onset familial haemophagocytic lymphohistiocytosis

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Abstract

Objectives. A congenital loss of cytotoxic lymphocyte activity leads to a potentially fatal immune dysregulation, familial haemophagocytic lymphohistiocytosis. Until recently, this disease was uniformly associated with infants or very young children, but it appears now that the onset may be delayed for decades. As a result, some adults are being mis- or under-diagnosed because of their 'atypical' symptoms that are not recognised as immunodeficiency. The clinical picture and histopathology can overlap with those of haematologic malignancy, further complicating the diagnostic thought process. The spectrum of atypical symptoms is poorly defined, and therefore, it is important to describe these cases and the attendant immunological and cellular changes associated with familial haemophagocytic lymphohistiocytosis, in order to improve diagnosis and prevent unintended consequences of symptomatic therapies. **Methods.** A 45-year-old patient presented with suspected T-cell lymphoma and was treated with combination chemotherapy (cyclophosphamide, doxorubicin, vincristine, prednisolone) supplemented with granulocyte-colony stimulating factor (G-CSF). To mobilise stem cells for autologous transplantation, the patient was then treated with high-dose G-CSF and rapidly developed haemophagocytic lymphohistiocytosis. Symptoms resolved temporarily with intensive immunosuppression with alemtuzumab and durably with a subsequent allograft. **Results.** The patient was found to be a carrier of bi-allelic mutations in the STXBP2 protein that is essential for cytotoxic lymphocyte function, and the initial diagnosis has been revised as familial haemophagocytic lymphohistiocytosis. **Conclusion.** This case highlights the difficulty in distinguishing atypical/late-onset familial haemophagocytic lymphohistiocytosis from a malignant process as well as a possible exacerbation of the disease with G-CSF therapy.

Keywords: cytotoxic lymphocyte, G-CSF, natural killer cells, T-cell lymphoma

INTRODUCTION

Familial haemophagocytic lymphohistiocytosis (FHL) is a potentially fatal disorder of immune homeostasis. While usually presenting in children, a proportion of patients develop 'atypical' FHL in adolescence or adulthood.[1] The delayed onset is associated with hypomorphic mutations in FHL-related genes resulting in a partial preservation of cytotoxic lymphocyte activity sufficient to protect carriers from overt haemophagocytic lymphohistiocytosis (HLH) in childhood.[1,2] The spectrum of atypical symptoms in this cohort is broad and presents significant challenges in diagnosis and treatment. We describe an adult patient with initial features suggestive of a T-lineage haematopoietic malignancy who subsequently developed florid HLH associated with the use of granulocyte-colony stimulating factor (G-CSF). We have previously reported the functional and genomic aspects of the case.[3] In this paper, we focus on the clinical and pathological features including the difficulties in diagnosing FHL in the context of suspected malignancy.

CASE REPORT

A previously healthy 45-year-old female of Ashkenazi Jewish descent presented with a 2-month history of mild sensorimotor peripheral neuropathy. Routine workup further demonstrated splenomegaly and mild non-progressive pancytopenia: platelets 72×10^9 L^{-1} (normal range 150–400 $\times 10^9$ L^{-1}), lymphocyte count 1.7×10^9 L^{-1} (normal range 1.0–3.5 $\times 10^9$ L^{-1}), neutrophils 0.6×10^9 L^{-1} (normal range 2.0–8.0 $\times 10^9$ L^{-1}) and haemoglobin (Hb) 98 g L^{-1} (normal range 115–155 g L^{-1}). The ferritin level was 111 μg L^{-1} (normal range 30–150 μg L^{-1}), and liver function was mildly deranged: alkaline phosphatase (ALP) 159 U L^{-1} (normal range 35–105 U L^{-1}) and gamma-glutamyl transferase (GGT) 54 U L^{-1} (normal range < 40 U L^{-1}), but normal alanine aminotransferase (ALT) and bilirubin. She was afebrile.

Bone marrow biopsy demonstrated a moderately hypercellular marrow with reduction in normal trilineage haematopoiesis and multifocal interstitial lymphoid aggregates comprising small, mature $CD3^+$ T cells (Figure 1a) without haemophagocytosis. Immunophenotyping by flow cytometry did not show a clonal B-cell population or an abnormal T-cell population, with normal mature T cells accounting for 93% of lymphocytes with a normal ratio of $CD4^+/CD8^+$ cells of 2.4 and no aberrant loss or gain of surface or cytoplasmic markers detected and negative for CD30 and ALK. However, a subtle monoclonal T-cell receptor (TCR) gene rearrangement peak was observed accounting for a small proportion of T cells (semi-quantitatively assessed as 5% of T cells). Positron emission computed tomography imaging demonstrated diffuse bone marrow uptake and hepatosplenomegaly without nodal enlargement. A presumptive diagnosis of T-cell lymphoma was made.

DIFFERENTIAL DIAGNOSIS AND TREATMENT

Treatment consisted of two cycles of combination chemotherapy (cyclophosphamide, doxorubicin, vincristine, prednisolone, CHOP), with a mixed response comprising reduction in the bone marrow infiltrate but increasing splenomegaly. Etoposide was added, and four further cycles were administered with the addition of 300 μg G-CSF on days 6, 8, 10 and 12 of each cycle. A repeat bone marrow biopsy at the end of treatment demonstrated persistent but reduced marrow T-cell burden (Figure 1b), and the decision was made to consolidate with an autologous stem cell transplant.

Five days after the final course of chemotherapy, stem cells were mobilised using 300 μg G-CSF daily for 10 days. Fourteen days after completion of G-CSF, there was worsening of pancytopenia, and at 34 days, the patient was readmitted to hospital with high-grade fevers and increasing splenomegaly.

Investigations showed a marked pancytopenia (Hb 91 g L^{-1}, WCC 0.9×10^9 L^{-1}, platelets 8×10^9 L^{-1}) and worsening liver derangement (ALT 735 U L^{-1}, bilirubin 198 U L^{-1}, GGT 685 U L^{-1}, ALP 785 U L^{-1}). Ferritin (16 634 μg L^{-1}) and soluble CD25 (> 60 000 pg mL^{-1}, normal range < 2678 pg mL^{-1}) were both markedly elevated, and fibrinogen was reduced (0.9 g L^{-1}, normal range 2.0–5.0 g L^{-1}). Triglyceride levels were within normal range. Serologic and PCR-based testing for a range of infectious aetiologies including viral hepatitis A/B/C/E, CMV, EBV, HIV, HSV, adenovirus, HTLV1/2 and cultures for bacterial and fungal organisms were all negative. Bone marrow biopsy demonstrated marked

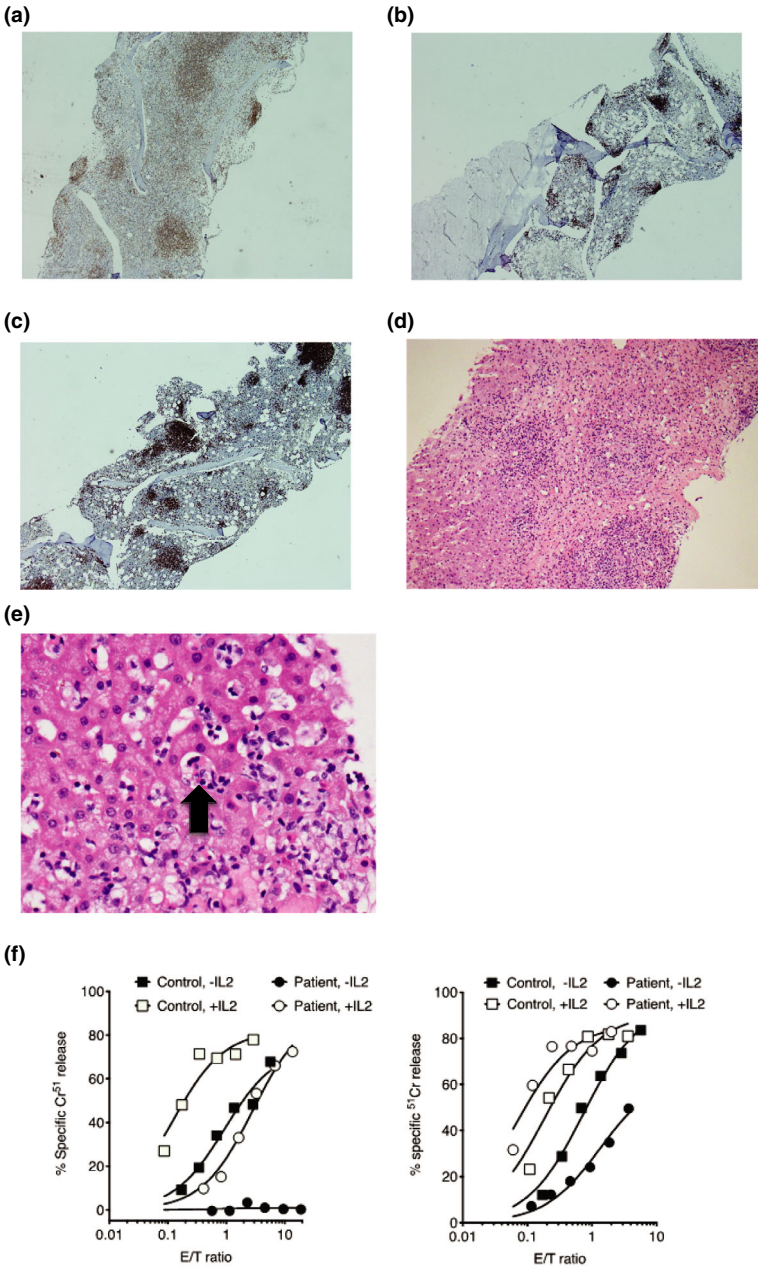

Figure 1. Tissue biopsies and NK function testing. **(a)** Diagnostic bone marrow sample demonstrating hypercellular marrow with fibrosis and 50–60% T-cell burden (anti-CD3 immunostain, ×40 magnification). **(b)** Post-CHEOP trephine demonstrating normalisation of cellularity and reduction in the T-cell infiltrate (anti-CD3 immunostain, ×40 magnification). **(c)** Post-G-CSF trephine with an increase in T-cell infiltration (anti-CD3 immunostain, ×40 magnification). **(d)** Liver biopsy specimen showing polymorphous lymphocytic infiltration and areas of necrosis (haematoxylin and eosin stain, ×60 magnification). **(e)** Liver biopsy specimen after diagnosis of HLH demonstrating increased macrophage activity and haemophagocytosis (arrow) (haematoxylin and eosin stain, ×100 magnification). **(f)** Natural killer cell cytotoxicity following heterologous bone marrow transplantation was assessed using ^{51}Cr release assay. Thus, peripheral blood mononuclear cells were isolated from the whole blood using Ficoll, incubated overnight in a complete cell culture media in the absence or in the presence of 100 U mL^{-1} IL2. The cells were then mixed with $Na_2{}^{[51]}CrO_4$-labelled MHC class I-deficient K562 target cells at the indicated Effector:Target (E/T) cell ratios normalised for % natural killer cells ($CD16^{+}/CD56^{dim+}/CD3^{-}$). After 4 h of co-incubation at 37°C, the cells were centrifuged at 500 *g* for 4 min, and the released ^{51}Cr was measured in the supernatant using gamma counter. Spontaneous ^{51}Cr release from K562 cells was assessed in the absence of PBMC, and the total (100%) ^{51}Cr content was estimated using Triton X-100 lysed cells. The percentage-specific ^{51}Cr release was calculated using the following formula: ([Experimental Release − Spontaneous Release]/[Total Release − Spontaneous Release]) × 100. Left: natural killer cell cytotoxicity at the time of diagnosis. Right: natural killer cell activity 18 months later, after heterologous bone marrow transplantation.

increase in the T-cell lymphoid infiltration (Figure 1c) without overt haemophagocytosis. Percutaneous liver core biopsy demonstrated a dense portal polymorphous lymphoid infiltrate and hepatocellular necrosis (Figure 1d). Immunohistochemistry of the liver biopsy revealed a mixed, activated, T-cell predominant lymphoid population without evidence of an abnormal phenotype. A targeted lymphoid gene panel using next-generation sequencing (NGS) on involved liver tissue did not demonstrate any hotspot mutations associated with T-cell malignancy. Repeat TCR gene rearrangement on bone marrow and liver tissue at this point demonstrated a polyclonal pattern.

HLH was considered (seven of eight HLH-2004 criteria fulfilled[4]), and intravenous methylprednisolone was commenced with an immediate response including resolution of fevers and marked improvement in liver function. Further questioning regarding family history revealed the death of a male sibling at age 30 from an HLH-like syndrome. Additional investigations in our patient at this time included a repeat liver biopsy, which demonstrated a persistent but reduced hepatic portal T-cell infiltrate with active haemophagocytosis (Figure 1e); further review attributed these changes to hepatic involvement by HLH. Genetic testing and functional natural killer cell assays demonstrated bi-allelic mutations in the STXBP2 gene (c.1001C>T (p.P334L) and c.474_483del_insGA (p.C158Wfs*78) resulting in protein degradation,[3] and abrogated natural killer cell function (cytotoxicity and degranulation; Figure 1f, on the left)[3] – all diagnostic of FHL5. Importantly, natural killer cell cytotoxicity was absent in unstimulated cells (Figure 1f, on the left -IL2), but recovered following IL-2 stimulation, consistent with previous reports of patients with STX11/STXBP2 mutations in contrast to other forms of FHL.[5–7] Interestingly, the same combination of mutations and associated HLH was previously reported in a young child of Ashkenazi Jewish descent, whose twin brother remained healthy at the time.[8]

High-dose glucocorticoid therapy was tapered and T cell-depleting HLH directed therapy with the anti-CD52 antibody alemtuzumab was commenced, given every 2–4 weeks based on peripheral blood T-cell recovery to $> 0.4 \times 10^9$ L^{-1} detected by flow cytometry. This continued for 9 months as a bridge to transplant during which liver enzymes and ferritin levels remained mildly deranged, but there were no other clinical features of HLH. The patient subsequently underwent a matched unrelated-donor allogeneic stem cell transplant, with no further symptoms of HLH and restoration of natural killer cell activity (Figure 1f, on the right) in the context of full donor chimerism.

Subsequent detailed investigation of the family history revealed that the sibling initially presented with a seizure and was found to have a lymphohistiocytic infiltrate on brain and bone marrow biopsy without evidence of malignancy. After initially responding to HLH-directed therapy, the patient relapsed within 2 years, and without an identified allogeneic stem cell transplant donor succumbed to progressive HLH after brief responses to CHOP and alemtuzumab. He reportedly had normal NK function testing at diagnosis, although it should be noted that the precise method of NK function testing could not be established and may have occurred in the presence of IL-2 stimulation, which is now known to restore the activity of NK cells in patients with STXBP2 mutations.

DISCUSSION

Familial haemophagocytic lymphohistiocytosis typically has a fulminant onset in infants or young children, but in adolescents and adults the initial presentation of the disease can be atypical without an apparent inflammatory component. The spectrum of presentations is wide and includes hepatic failure, neurologic symptoms, fever of unknown origin and even growth arrest. The HLH-2004 criteria were developed for paediatric populations, and alternative criteria have been proposed that recognise the heterogeneous presentation in adults.[9] Familial forms in adults can also differ in initial laboratory findings, with the typical ferritin level being considerably lower.[10] In our case, the diagnosis of familial HLH was delayed as the presentation was insidious: the ferritin level was normal at the outset, haemophagocytosis was not a prominent morphological feature, and none of the previously reported atypical features of STXBP2-associated FHL – gastrointestinal and bleeding problems, hearing deficit or hypogammaglobulinemia[11] – were observed in this patient. Furthermore, the family history was under-appreciated until much later in the clinical course.

In addition, the presence of an apparent clonal T-cell infiltrate complicated the clinical picture. A number of adolescent/adult patients with hypomorphic mutations in perforin or other related genes have been diagnosed with haematological malignancies prior to the development of HLH symptoms.[12,13] Indeed, defects in cytotoxic functions may themselves predispose to malignant transformation.[2] Confirming the diagnosis of a T-cell NHL, however, can be difficult and often depends upon a combination of histologic features, demonstration of immunophenotypic or molecular aberrancy and the presence of a compatible clinical picture, including the exclusion of reactive causes. Furthermore, florid T-cell infiltrates can themselves be a pathologic feature of HLH[14] and T-cell clonality has been observed in FHL without an underlying malignant process.[15,16] Mechanisms behind such clonality are not fully established, but reactions against a viral or auto-antigen are possible and may have occurred in this case. The initial presumptive diagnosis of a T-cell malignancy was considered because of monoclonal TCR gene rearrangement, but the low specificity of this test, discordant results between the amount of infiltrate and degree of monoclonality, inability to reproduce the findings at other time points, the subsequent lack of demonstrable changes by immunophenotyping or NGS, and the comparable illness of her brother strongly support the possibility that the liver and marrow infiltrates were attributable to FHL alone.

The subsequent onset of florid HLH was temporally related to G-CSF, raising the possibility that cytokine stimulation was an exacerbating event. Several case reports suggest that G-CSF can trigger florid HLH in patients with haematological malignancies, but unlike the current study none of the reported cases were analysed for FHL-related genes.[17–20] The mechanism by which this occurs is unknown; however, it is plausible that G-CSF further stimulated activated macrophages through the G-CSF receptor, leading to hyperactivation and hypersecretion and a self-perpetuating cycle of inflammation which persisted in the absence of supraphysiological stimulus. Interestingly, while G-CSF was administered between chemotherapy cycles, it showed no adverse effect, perhaps because it was counteracted by concurrent therapy (i.e. glucocorticoids and etoposide) that is commonly used to treat familial and secondary HLH.

This case highlights the difficulty in distinguishing atypical/late-onset FHL from a malignant process as well as a possible exacerbation with G-CSF therapy. It is often underappreciated that FHL may present in adolescence and adulthood as a primary neurological, inflammatory or lymphoproliferative cancer-like condition,[1,3,21] and it is important to consider this diagnosis in circumstances when the immunopathology is not definitive for a haematologic malignancy.[22] In such cases, it may be prudent to take a comprehensive family history and consider excluding reduced natural killer function, particularly if G-CSF therapy is being considered.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

AM wrote the manuscript. AG and IV conceived the paper and reviewed/edited the manuscript.

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