CASE REPORT

EBV-driven diffuse large B-cell lymphoma confined to the liver in a patient with a history of idiopathic CD4 lymphocytopenia

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SUMMARY

Idiopathic CD4 lymphocytopenia (ICL) is a rare immunodeficiency disorder. We describe a 49-year-old woman with a history of ICL who developed hepatic Epstein-Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL). ICL was diagnosed at a time of her presentation with varicella-zoster virus (VZV) meningoencephalitis and chorioretinitis. Her CD4 count subsequently improved but remained at the lower limits of the normal range. Five years later she presented with cough, fever and night-sweat. She was found to have multiple liver nodules on MRI, fluorodeoxyglucose (FDG) avid on the positron emission tomography (PET) CT, histologically defined as DLBCL, EBV positive and of non-germinal centre type. To our knowledge this is the first reported case of EBV-positive DLBCL localised to the liver in the context of ICL. EBV-positive DLBCL typically occurs in immunocompromised individuals. The corticosteroid therapy she received for VZV meningoencephalitis may have contributed to the EBV reactivation with subsequent EBV-driven malignant transformation of B-cells.

BACKGROUND

Although CD4 lymphocytopenia is most commonly associated with HIV infection, it can also be idiopathic CD4 lymphocytopenia (ICL). ICL is poorly understood, with uncertain pathogenesis, prognosis and management. Although, a subset of patients with ICL remains asymptomatic others may present with or develop life-threatening opportunistic infections. A few patients with ICL may develop virally driven (eg, Epstein-Barr virus (EBV), human papillomavirus (HPV)) malignancies. We describe a patient with a history of ICL who developed EBV-driven diffuse large B-cell lymphoma localised to the liver. To our knowledge, this is the first case of hepatic EBV-driven diffuse large B-cell lymphoma (DLBCL) complicating ICL.

CASE PRESENTATION AND INVESTIGATIONS

A previously fit and well 44-year-old Caucasian woman, who had varicella in childhood, was diagnosed with meningoencephalitis and chorioretinitis due to a varicella-zoster virus (VZV) infection. VZV was detected by PCR in the spinal fluid and the anterior chamber of the left eye. At presentation, she had low CD4 count of $0.09 \times 10^9/L$, which was confirmed on subsequent testing. HIV1 and HIV2 IgG test was negative on two occasions. She was

diagnosed with ICL by immunologists elsewhere. She received treatment with valacyclovir and variable doses of corticosteroids (up to 1 mg/kg for a period of 12 months, tapered over the next 6 months and then stopped). She had a residual XII nerve palsy and was blind in the left eye. Her CD4 count improved after 3 years and remained stable at around $0.4 \times 10^9/L$. Within that period, her CD8 count has increased from 0.8×10^9 to $2.0 \times 10^9/L$.

At the age of 48 she returned to the UK and was referred to the immunology clinic because of her medical history. She was clinically very well with no symptoms. Investigations showed normal haemoglobin, mild thrombocytopenia ($88 \times 10^9/L$), leucocytosis $11.1 \times 10^9/L$ with 70% lymphocytosis, normal inflammatory markers, liver and renal function tests, normal serum immunoglobulins and no paraprotein. Lymphocyte subsets showed CD3 $7.49 \times 10^9/L$ ($0.8-2.5 \times 10^9/L$), CD4 $0.4 \times 10^9/L$ ($0.4-1.5 \times 10^9/L$), CD8 $6.73 \times 10^9/L$ ($0.2-1.1 \times 10^9/L$), CD19 (B-cells) $0.12 \times 10^9/L$ ($0.10-0.50 \times 10^9/L$), CD16+CD56 (natural killer cells) $0.19 \times 10^9/L$ ($0.08-0.65 \times 10^9/L$). Despite the absence of HIV risk factors, the HIV1 and HIV2 antibody test was repeated due to persistent CD4 lymphocytopenia and was again negative. TCR $\gamma\beta$ -analysis of peripheral CD3 T cells by immunophenotyping showed no evidence of a clone. Bone marrow biopsy was performed in view of CD8 lymphocytosis and showed a normocellular bone marrow with 5% infiltration by T cells expressing predominantly CD8 but no evidence of lymphoma. Lymphocyte proliferation studies showed normal response to phytohemagglutinin, anti-CD3 and anti-CD3/CD28 antibodies. Autoimmune screen including antinuclear antibody (ANA), extractable nuclear antigen (ENA), double-stranded DNA and anti-neutrophil cytoplasmic antibodies were negative. Abdominal ultrasound showed normal liver, a spleen of 13 cm and no lymphadenomegaly.

Nine months later she became acutely unwell with dry cough, fever and night sweats. She did not respond to empirical antibiotics and was admitted to her local hospital. Investigations showed raised inflammatory markers and abnormal liver function tests. ANA, ENA, anti-mitochondrial, anti-smooth muscle and anti-liver/kidney/microsome antibodies were negative. An abdominal CT scan showed multiple pathological lesions in the liver and a radiological differential included metastases or lymphoproliferative disease (LPD). She was transferred to our hospital. Investigations showed EBV viraemia of 300 000

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copies/mL. Lymphocyte subsets showed CD4 $0.1 \times 10^9/L$ and CD8 $1.5 \times 10^9/L$. T-cell immunophenotyping showed that 85% of CD4 cells were CD4+CD45RO+ memory cells. CD4 cells had high expression of CD69 activation marker and normal CD38 expression with no evidence of immunosuppression. Of CD8 cells, 85% were activated cytotoxic CD8+CD28+CD27+ cells, 5% were CD8+CD28-CD27+ effector cells and 1% were CD8+CD28-CD27- late effector cells. Of CD8 cells, 60% expressed DR/DQ/DP *ITK* (interleukin-2 inducible T-cell kinase (ITK)) gene (all exons 1–17) sequencing evidenced no mutation. There was no lymphadenopathy on the total body CT scan and no evidence of lymphoma on a repeat bone marrow biopsy which showed only very occasional EBV in situ immunostaining (EBER)-positive cells. Multiple liver lesions were confirmed on MRI. They were fluorodeoxyglucose (FDG) avid on positron emission tomography (PET) CT (figure 1). A liver biopsy showed diffuse proliferation of large, polygonal cells with pleomorphic nuclei and prominent nucleoli and interspersed areas of necrosis (figure 2). Cells were positive for CD20, B-cell lymphoma 6 protein (BCL6), melanoma associated antigen (mutated) 1 (MUM1), EBV, latent membrane protein (LMP)1 and most were CD30 positive; negative for CD3, CD10, anaplastic lymphoma kinase (ALK) and T-cell intracellular antigen 1 (TIA1). The proliferation index was approximately 70%. Monoclonal B-cell proliferation (λ) was confirmed by PCR. The diagnosis was that of DLBCL (EBV positive), non-germinal centre type.

TREATMENT

The patient has completed six cycles of cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone and rituximab (CHOP-R) treatment. She is maintained on prophylactic valgacyclovir.

OUTCOME AND FOLLOW-UP

PET scanning at the end of therapy showed persistent low-grade FDG avidity in the hepatic lesions. Progress imaging 3 months later demonstrated complete resolution of the previous FDG-avid focal liver lesions (figure 1). One year after completing CHOP-R her CD4 count remains low at $0.22 \times 10^9/L$ and CD8 count $1.5 \times 10^9/L$. EBV DNA in peripheral blood was negative. She is well and in clinical remission.

The patient's DNA has been stored and is awaiting whole exome sequencing to identify potential immune gene defects accounting for her ICL.

DISCUSSION

ICL is a rare and heterogeneous immunodeficiency of unknown aetiology.¹ It was defined by the CDC in 1992 as 'an absolute CD4-lymphocyte count of less than $0.3 \times 10^9/L$ or of less than 20% of total T cells, documented on more than one occasion, with negative HIV testing, and the absence of any immunodeficiency or therapy causing CD4 lymphopenia.'^{2–4} ICL can be associated with life-threatening opportunistic infections (most commonly cryptococcal and non-tuberculous mycobacterial infections and progressive multifocal leucoencephalopathy). Malignancies can occur and are typically related to opportunistic pathogens or reactivated latent viral infections.¹ A subset of patients remains asymptomatic. Differential diagnosis includes infections (HIV1, HIV2, human T-lymphotropic virus (HTLV-1), HTLV-2, mycobacterial infections, EBV, cytomegalovirus (CMV)), hematological conditions (aplastic anaemia, myelodysplasias, lymphomas), autoimmune diseases (SLE, Sjogren's syndrome), medications (corticosteroids, chemotherapy, immunosuppressants) genetic syndromes (X-linked immunodeficiency with magnesium defect and EBV infection and neoplasia (XMEN), Unc119) and

Figure 1 Transverse slice of fluorodeoxyglucose positron emission tomography (FDG PET) pretreatment demonstrates increased uptake in liver lesions (upper left). Postchemotherapy FDG PET demonstrates complete metabolic resolution of abnormal findings (lower left). Pretreatment maximum intensity projection (MIP) images demonstrate multiple areas of increased uptake in the liver and a focus of uptake in the mediastinum. Postchemotherapy (far right) demonstrates complete metabolic response.

Figure 2 H&E liver biopsy at $\times 200$ magnification (left) showing atypical large lymphoid cells in a background of necrosis. The CD20 immunostaining (centre) confirms the B-cell nature of the proliferation and the EBER immunostaining (right) demonstrates that this is an EBV-driven B-cell lymphoma.

others (eg, combined immunodeficiencies).¹ Mechanisms leading to low CD4 count include decreased production, increased destruction or tissue sequestration of CD4 cells. T-cell defects described in ICL include reduced T-cell proliferation, impaired TCR signalling, impaired cytokine production, low numbers of circulating naïve T cells, impaired chemotaxis and augmented apoptosis.¹ Recently, genetic defects in magnesium transporter 1 (*MAGT1*) gene,⁵ recombination activating gene 1 (*RAG1*)⁶ and uncoordinated 119 (*UNC119*) gene⁷ have been described in a subset of ICL patients.

ICL is usually detected after the occurrence of an opportunistic infection in a person without known immunodeficiency or immunosuppression. This occurs in approximately 80% of patients or is otherwise an incidental finding on blood tests.¹ Our patient presented with reactivation of latent VZV infection. VZV reactivation typically manifests as zoster in immunocompetent individuals and is more common with advancing age. In immunocompromised patients, VZV is more likely to spread to the central nervous system causing meningoencephalitis,⁸ as in this case.

The distinction between cause and effect in a patient with an infection and a low CD4 count is difficult as CD4 lymphopenia can occur during infections. Also, transient ICL has been reported by Zonios *et al*¹ in a prospective natural history cohort study of 39 individuals with ICL and a median follow-up of 49.5 months, where CD4 count normalised in 19.4% of those patients after an average of 31 months. Although our patient's CD4 counts have improved 3 years after she first presented, they remained at the lower limits of the range (around $0.35\text{--}0.4 \times 10^9/\text{L}$) and were less than 20% of total T cells. Currently, 12 months after chemotherapy, our patient's CD4 count remains low at $0.22 \times 10^9/\text{L}$ which remains consistent with ICL.² In the cohort described by Zonios *et al*,¹ only one case of EBV-positive LPD was observed, although there are some reports of EBV-negative lymphomas in patients with ICL.¹

DLBCL is a neoplasm of large B lymphoid cells. Morphological, biological and clinical studies have subdivided DLBCL into subgroups and distinct entities; DLBCL may present with nodal or extranodal disease.⁹ EBV-positive DLBCL is an EBV-driven clonal B-cell lymphoid proliferation.¹⁰ It can occur in patients receiving immunosuppressive treatment, in those with immunodeficiency or sometimes in the elderly individuals, presumably due to accelerated immunological senescence.

EBV is typically associated with LPD and lymphomas due to its B-cell lymphotropism and oncoviral properties. EBV-associated B-cell lymphoma occurring as a localised mass in the subcutaneous tissue was described in a 49-year-old woman previously healthy and with no apparent immunodeficiency.¹¹ In the setting of immunodeficiency, EBV is associated with LPD in patients with

primary or secondary immunodeficiency (including HIV) and in recipients of solid organ or bone marrow transplants on immunosuppressive therapy.¹⁰ EBV-positive LPD can be extranodal in post-transplant patients: a case of hepatic DLBCL was recently described in a kidney transplant recipient.¹² The precise mechanism of EBV-driven malignant B-cell transformation is unclear. Genetically determined susceptibility and certain HLA types may result in an abnormal response to primary EBV infection in certain parts of Asia, where EBV-positive DLBCL 'of the elderly' is more common.¹³ T cells (in particular cytotoxic CD8 cells) play a crucial role in antiviral immunity and in suppressing EBV-associated oncogenesis. Therefore, impaired T-cell immunity may lead to abnormal control of the proliferation of EBV-infected B cells. However, our patient had high numbers of activated early effector CD8 cells suggestive of strong antiviral response with good lymphocyte proliferative responses. ITK deficiency, an autosomal recessive disorder characterised by lymphoproliferation and immune dysregulation following EBV infection, was not detected on genetic testing in this case. Treatment with steroids can cause chronic immunosuppression and favour EBV reactivation. Our patient had previously received prolonged periods of high dose corticosteroid therapy for VZV meningitis and developed lymphoma 4 years after stopping steroids. Nevertheless, the immunosuppressive effect of high-dose steroids may have contributed to the EBV reactivation due to reduced immune surveillance and resulted in malignant transformation of EBV-infected B cells.

CD8 lymphocytosis was another immunological feature in this case. It was first noted shortly after the first presentation with VZV meningoencephalitis, and the CD8 count has gradually increased over time. Although, we are not able to look for EBV-specific T-cell clones in our diagnostic lab, we believe that CD8 lymphocytosis reflected the attempts of the immune system to control EBV reactivation. We found no evidence of monoclonal expansion of peripheral CD8 cells on our TCR $\nu\beta$ panel. Also, there was no evidence of cytotoxic immune exhaustion, as the percentage of late effector CD8+CD28–CD27– cells was very low (1%) and the majority of CD8 cells were activated early effector cells (CD8+CD28+CD27+) expressing activation markers CD69, CD38 and major histocompatibility complex class II antigens DR/DQ/DP.

In summary, we described a middle-aged woman with a history of ICL who initially presented with VZV-meningoencephalitis and retinitis and who subsequently developed hepatic EBV-positive DLBCL. She has responded to chemotherapy. Steroids may have played a role in the pathogenesis of her lymphoma. In addition, she most likely has an underlying undefined functional T-cell defect which resulted in insufficient immunological surveillance, EBV reactivation and malignant transformation of B cells. Recently, a variant of ICL named

XMEN has been described in three male patients with a mutation in *MAGT1* gene; one of them died of chronic EBV-associated lymphoma at the age of 45.⁵ Whole or partial genome sequencing with next generation sequencing technologies is becoming a tool for diagnosing the genetic defects in rare diseases. Our patient will undergo whole exome sequencing as part of the National Institute of Health Research (NIHR) whole exome sequencing study of patients with primary immunodeficiencies. In the near future, this approach may enable us to detect molecular defects in complex cases with suspected immunodeficiency such as the patient we described.

Learning points

- ▶ Idiopathic CD4 lymphocytopenia (ICL) is a rare heterogeneous immunodeficiency disorder of uncertain pathogenesis, prognosis and management.
- ▶ Patients with ICL may develop severe opportunistic infections(s) and/or malignancies, although a subset of patients remain asymptomatic long term.
- ▶ Malignancies in ICL may be related to opportunistic pathogens or to the reactivation of latent viruses with oncogenic properties such as Epstein-Barr virus or human papillomavirus.
- ▶ EBV-related B-cell lymphoproliferative disease with extranodal involvement can complicate ICL.
- ▶ New generation technologies such as whole-genome sequencing will enable to identify potential molecular defects underlying rare primary immunodeficiencies such as ICL.

Contributors This case was complex and required multidisciplinary management. The teams involved were immunology, haematology, nuclear medicine and histopathology. MDz has been involved in the patient's care. She has written the case report and reviewed the literature. RC has supervised the immunological laboratory tests and their interpretation. He supervised MDz in describing and discussing the immunological laboratory findings in this case. He contributed to the part of discussion covering the role of T-cell immunity in virally-driven oncogenesis. CM was responsible for the patient's care after she was diagnosed with lymphoma. He supervised MDz in describing the haematological findings, and in discussing the

role of EBV in haematological malignancies within the text. He contributed to the part of discussion regarding lymphomas and immune senescence. TW was involved in the diagnostic imaging of the patient with PET-CT. He provided images for figure 1 and provided figure legend. MDe was involved in the formulating the histological diagnosis of lymphoma. She has described the histological findings and provided images and legend for figure 2. She also critically reviewed the manuscript before its submission. SLS was involved in the patient management throughout her illness. He suggested writing this case report and reviewed the manuscript. He contributed the part of discussion regarding genetic defects recently described in ICL.

Competing interests None.

Patient consent Obtained.

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REFERENCES

- 1 Zonios D, Falloon J, Bennett J, *et al.* Idiopathic CD4+ lymphocytopenia: natural history and prognostic factors. *Blood* 2008;112:287–94.
- 2 Anonymous. Unexplained CD4-T-lymphocyte depletion in persons without evident HIV infection—United States. *MMWR Morb Mortal Wkly Rep* 1992;41:541–5.
- 3 Smith DK, Neal JJ, Holmberg SD. Unexplained opportunistic infections and CD4 T-lymphocytopenia without HIV infection: an investigation of cases in the United States. The Centers for Disease Control Idiopathic CD4-T-lymphocytopenia Task Force. *N Engl J Med* 1993;328:373–9.
- 4 Zonios D, Sheikh V, Sereti I. Idiopathic CD4 lymphocytopenia: a case of missing, wandering or ineffective T cells. *Arthritis Res Ther* 2012;14:222.
- 5 Li F, Chaigne-Delalande B, Kanellopoulou C, *et al.* Signaling role for Mg2+ revealed by immunodeficiency due to loss of MagT1. *Nature* 2011;475:471–6.
- 6 Kuijpers T, Ijspeert H, van Leeuwen E, *et al.* Idiopathic CD4+ T lymphopenia without autoimmunity or granulomatous disease in the slipstream of RAG mutations. *Blood* 2011;117:5892–6.
- 7 Gorska MM, Alam R. A mutation in the human Uncoordinated 119 gene impairs TCR signalling and is associated with CD4 lymphopenia. *Blood* 2012;119:1399–406.
- 8 Kleinschmidt-DeMasters BK, Gilden D. Varicella-zoster virus infections of the nervous system. Clinical and pathologic correlates. *Arch Pathol Lab Med* 2001;125:770–80.
- 9 Jaffe E, Pittaluga S. Aggressive B-cell lymphomas: a review of new and old entities in the WHO classification. *Hematol Am Soc Hematol Educ Program* 2011;2011:506–14.
- 10 Carbone A, Ghoghini A, Dott G. EBV-associated lymphoproliferative disorders: classification and treatment. *Oncologist* 2008;13:577–85.
- 11 Pozdnyakova O, Spieler P, Abraham J, *et al.* Epstein-Barr virus—associated diffuse large B-cell lymphoma in an immunocompetent woman. *J Clin Oncol* 2010;28:e75–8.
- 12 Makhdooni K, Moghaddam AG, Oskue AA, *et al.* Extranodal diffuse large B cell lymphoma confined to liver in a kidney transplant recipient. *Iran J Kidney Dis* 2010;4:336–9.
- 13 Castillo J, Beltran B, Miranda R, *et al.* Epstein-Barr virus—positive diffuse large B-cell lymphoma of the elderly: what we know so far. *Oncologist* 2011;16:87–96.

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