J Clin Immunol (2015) 35:604–609
DOI 10.1007/s10875-015-0194-9

ASTUTE CLINICIAN REPORT

Cerebral Vasculitis in X-linked Lymphoproliferative Disease Cured by Matched Unrelated Cord Blood Transplant

Paul E. Gray[1,2] · Tracey A. O'Brien[2,3] · Mayura Wagle[4,5] · Stuart G. Tangye[4,5] · Umaimainthan Palendira[4,5] · Tony Roscioli[6,7] · Sharon Choo[8] · Rosemary Sutton[9] · John B. Ziegler[1,2] · Katie Frith[1,2]

Received: 15 March 2015 / Accepted: 8 September 2015 / Published online: 3 October 2015

Abstract
Vasculitis occurs rarely in association with X-linked lymphoproliferative disease (XLP). There are four published cases of non-EBV XLP-associated cerebral vasculitis reported, none of whom have survived without major cognitive impairment. *Case* A 9-year old boy initially presented aged 5 years with a restrictive joint disease. He subsequently developed dysgammaglobulinemia, episodic severe pneumonitis, aplastic anaemia, gastritis and cerebral vasculitis. A diagnosis of XLP was made, based on flow cytometric analysis and the identification of a novel mutation in SH2D1A, c.96G>C. No peripheral blood lymphocyte clonal proliferation was identified and he was EBV negative, although human herpes virus-7 (HHV7) was detected repeatedly in his cerebrospinal fluid. He underwent a reduced intensity unrelated umbilical cord blood transplant, but failed to engraft. A second 5/6 matched cord gave 100 % donor engraftment. Complications included BK virus-associated haemorrhagic cystitis, a possible NK-cell mediated immune reconstitution syndrome and post-transplant anti-glomerular basement membrane disease, the latter treated with cyclophosphamide and rituximab. At +450 days post-transplant he is in remission from his vasculitis and anti-glomerular basement membrane disease, and HHV-7 has remained undetectable.
Conclusion This is the second published description of joint disease in XLP, and only the fourth case of non-EBV associated cerebral vasculitis in XLP, as well as being the first to be successfully treated for this manifestation. This case raises specific questions about vasculitis in XLP, in particular the potential relevance of HHV-7 to the pathogenesis.

Keywords X-linked lymphoproliferative disease (XLP) · vasculitis · HHV-7 · haematopoietic stem cell transplant

✉ Katie Frith
frith.katie@gmail.com

1 Department of Immunology and Infectious Diseases, Sydney Children's Hospital, Randwick, Australia

2 School of Women's and Children's Health, University of New South Wales, Sydney, Australia

3 Kids Cancer Centre, Sydney Children's Hospital, Randwick, Australia

4 Immunology and Immunodeficiency Group, Immunology Research Program, Garvan Institute of Medical Research, Darlinghurst, Australia

5 St Vincent's Clinical School, University of New South Wales, Sydney, Australia

6 Department of Medical Genetics, Sydney Children's Hospital, Randwick, Australia

7 Kinghorn Centre for Clinical Genomics, Garvan Institute of Medical Research, Darlinghurst, Australia

8 Department of Allergy and Immunology, Royal Children's Hospital, Melbourne, Australia

9 Children's Cancer Institute, Lowy Cancer Research Centre, University of New South Wales, Randwick, Australia

Introduction

X-linked lymphoproliferative-type 1 (XLP1) disease is caused by mutations in *SH2D1A* which encodes SAP (SLAM-associated protein) [1–4]. SAP-deficiency is associated with an inherited predisposition to Epstein Barr Virus (EBV)-driven haemophagocytic lymphohistiocytosis (HLH) and fulminant infectious mononucleosis [4], as well as lymphoma, dysgammaglobulinemia, aplastic anaemia and vasculitis [5, 6]. XLP-associated vasculitis is a rare entity [5, 7, 8], there being only reported 4 cases of non-EBV associated cerebral

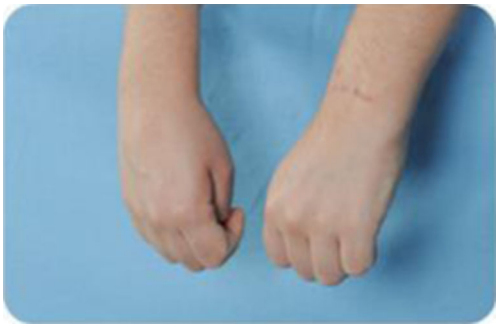


Fig. 1 Symmetrical restrictive arthropathy, persists after HSCT

vasculitis, with only one patient still alive at the time of reporting, that patient being said to be in "a vegetative state" [5, 8, 9].

Case

A 9-year old Australian boy with no relevant family history presented aged 5 years with progressive restriction of movement in the joints of the hands (Fig. 1), wrists, elbows, and lower extremities. The involved joints were tender without erythema or effusions and the skin overlying the involved joints was not thickened or tightened, inflammatory markers were normal and autoimmune serology was negative. MRI scan with contrast of his hands showed mild subcutaneous and peritendinous oedema without evidence of synovial involvement, while biopsy showed a mild lymphocytic synovitis with a mixture of CD4+ and CD8+ T-cells. Overall the presentation was felt to be out of keeping with a diagnosis of Juvenile Idiopathic Arthritis. He was treated with a combination of physiotherapy, hydroxychloroquine and prednisolone with minimal improvement. At this time his serum immunoglobulin levels were normal.

Two years later he suffered two episodes of acute respiratory deterioration separated by several months. These began with low-grade fever and cough, with worsening respiratory distress over the course of a week, alveolitis on chest X-ray (Fig. 2) and requiring mechanical ventilation for more than a week on both occasions. No causative organism was identified despite bronchoalveolar lavage looking for possible viral (multiplex PCR including CMV/EBV), bacterial and fungal causes. He did not develop features of hyperferritnaemia or cytopenias with either episode. He was treated with broad-spectrum anti-microbials and stress doses of hydrocortisone and recovered, however he retained a slowly normalising diffusion defect for some months following the second admission. At this time he was found to have a low IgG = 2.46 g/L (N = 7–16 g/L), but high IgA = 4.19 g/L (N = 0.35–2.33 g/L) and IgM = 4.24 g/L (0.58–2.57 g/L). He had normal T-cell numbers with a normal CD4:CD8 ratio, but an elevated percentage of peripheral blood B-cells (58 %) and almost absent NKT cells. Baseline level of antibody response to vaccines was extremely low for *Tetanus*, *Diphtheria* and 14 serotypes of pneumococcus but with a strong response to *Haemophilus Influenza*.

Three months later he developed transient aplastic anaemia with reduction of all cell lines and a bone marrow aspirate which showed a hypocellular marrow, and markedly depressed erythropoiesis, granulopoiesis and megakaryopoiesis. This episode self-resolved, however he subsequently began to have infrequent episodic left orbital headaches, unilateral blurred vision, and occasional emesis. Brain MRI showed areas of infarction in the left caudate nucleus, basal ganglia, posterior limb of the internal capsule and thalamus, with a normal magnetic resonance angiogram (Fig. 3). Cerebral angiography showed diffuse irregularity of small and medium-sized vessels in all vascular territories in both the supratentorial and infratentorial compartments (Fig. 4), and a fusiform pseudoaneurysm affecting the right posterior inferior cerebellar artery (PICA). CSF analysis demonstrated a mild lymphocytic pleocytosis and an extremely high protein level = 6.75 g/L (N = 0.15–0.45 g/L). EBV PCR from CSF and blood was repeatedly negative, however HHV-7 was repeatedly identified by PCR in CSF over several months. At

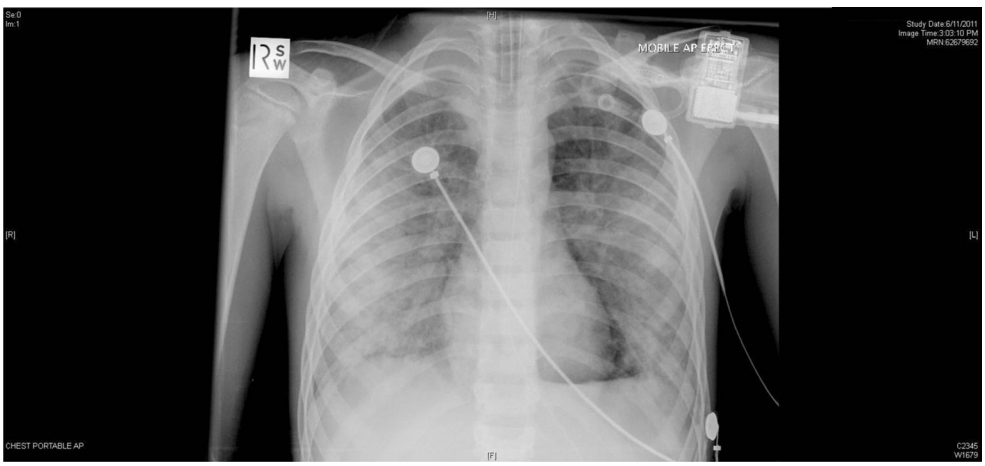
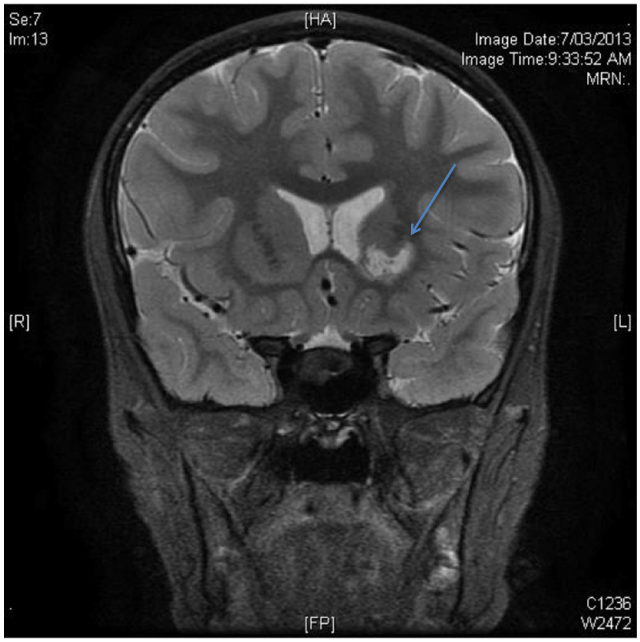
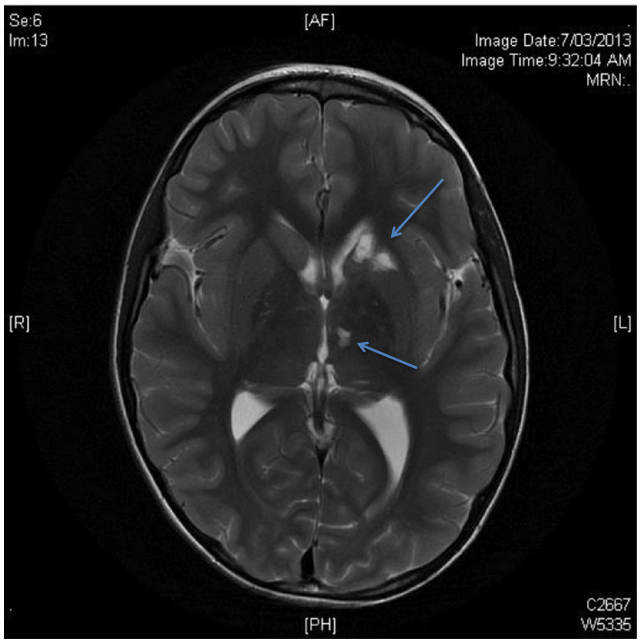


Fig. 2 Extensive bilateral airspace opacity consistent with alveolitis



A



B

Fig. 3 Multiple cerebral infarcts in the caudate nucleus, basal ganglia and thalamus seen on MRI T2 weighted images (stroke protocol) in the **a** transverse and **b** coronal sections

this time he also reported regular symptoms of epigastric discomfort and underwent upper G.I. endoscopy, which demonstrated a chronic active pangastritis, composed predominately of CD8 positive T-cells.

A diagnosis of XLP was considered after reduced NK cell lysis of K562 leukaemia cells was identified in peripheral blood, despite normal NK cell degranulation and perforin staining by flow cytometry. Flow cytometric testing showed

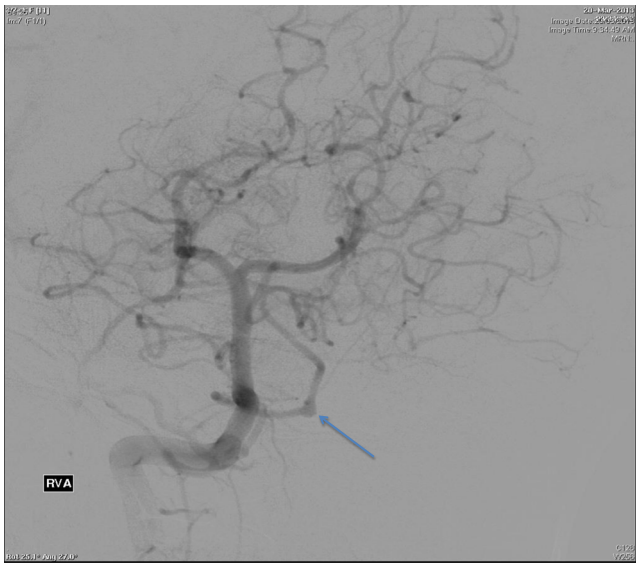
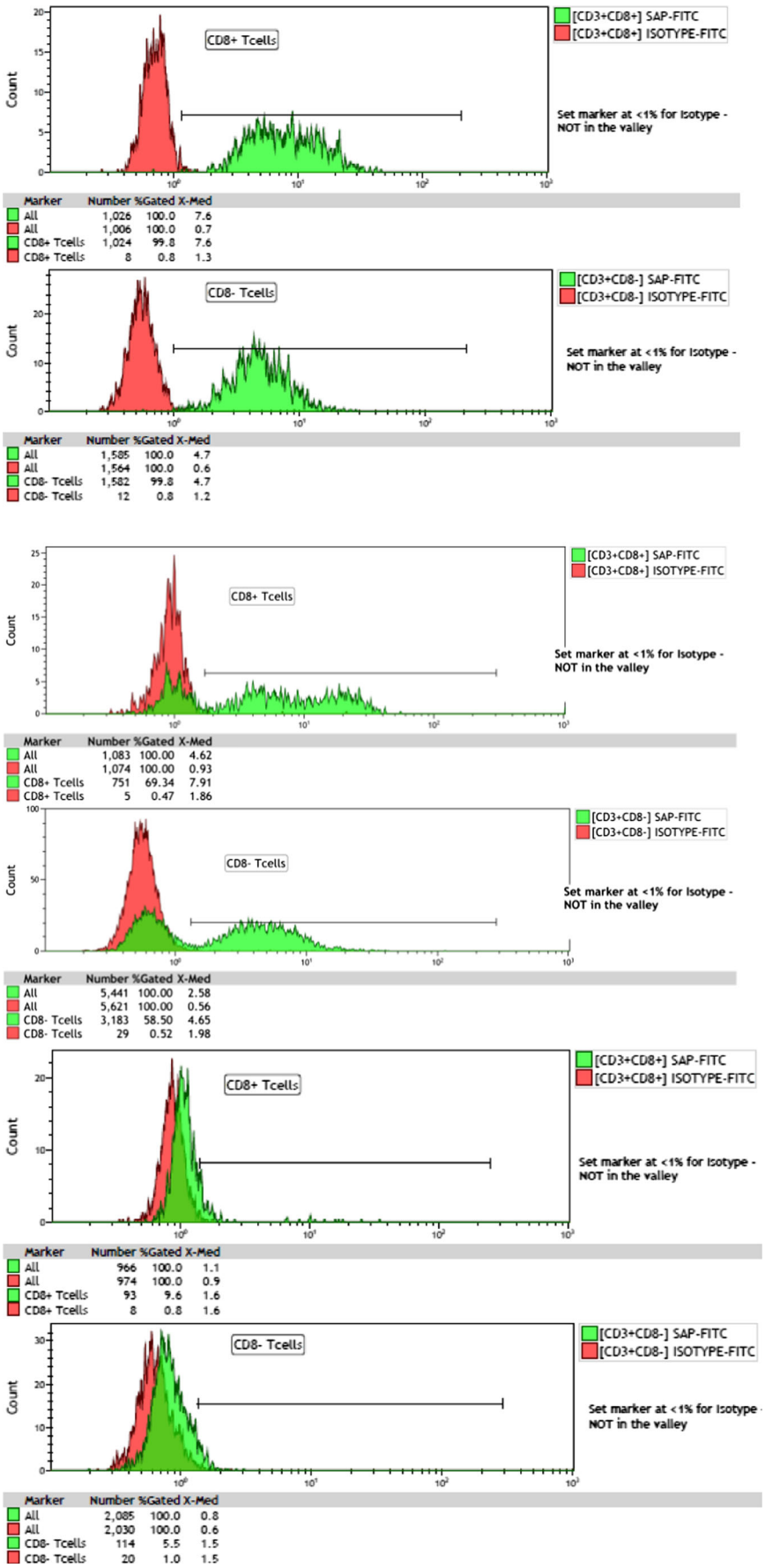


Fig. 4 Widespread small vessel irregularity on cerebral angiography with a pseudoaneurysm of the right posterior inferior cerebral artery (PICA)

a lack of SLAM-associated protein (SAP) expression in his lymphocytes, while sequencing of genomic DNA using a previously described technique [10], detected a novel missense mutation in SH2D1A, NM_001114937.2: c.96G>C; g.X, 123480588,G>C, resulting in a substitution of an Arginine to a Serine at amino acid 32 in this transcript. This mutation was predicted to be pathogenic (Provean score = −5.78 deleterious http://provean.jcvi.org/index.php, SIFT damaging) and different amino acid substitutions at the same position (R32Q [11] and R32T [12]) have previously been reported as associated with HLH. Further genetic studies confirmed his mother to be a carrier of the implicated allele, while flow cytometric analysis showed that approximately 50 % of her cells failed to express the mutated protein, confirming her carrier status (Fig. 5).

Steroid therapy and aspirin thromboprophylaxis were commenced prior to haematopoietic stem cell transplant. During the 3 months from diagnosis to transplant, intermittent transient (c. 20–30 min) hemi-sensory events occurred which were thought to be the result of arterial spasm. Although a cerebral MRI demonstrated new small infarcts in the splenium and corpus collosum, these were not associated with persisting functional deficits. Because of previous reports of a γδT-cell clone underlying XLP-cerebral vasculitis, we assessed peripheral blood using both flow cytometry and a PCR-based system for detection of clonal rearrangements of T-cell receptor delta, gamma and beta genes in purified mononuclear cell DNA (comprehensive Biomed 2 system) [13], and no such clone was identified.

The patient initially underwent a 6/6 cord blood transplant with conditioning including alemtuzemab, treosulphan, fludarabine and cyclophosphamide, but failed to engraft and



Control

Patient

Mother

◀ **Fig. 5** Absent SLAM associated protein (SAP) expression on patient's CD8+ T cells and CD8- T cells compared with normal expression in control and two populations in carrier mother

underwent autologous recovery. A second transplant using a 5/6 cord blood and conditioned with reduced dose busulphan and fludarabine engrafted successfully with 100 % ongoing donor chimerism. The post-transplant course was complicated by severe haemorrhagic cystitis secondary to BK virus infection, resulting in bladder perforation. He also had an episode of fever, hyperferritinemia (1892 μg/L), bilateral pulmonary infiltrates and acute confusional state, at the same time as demonstrating accelerated engraftment of NK cell activity and function (NK cells = 97 % of 2.2 × 10^9/L lymphocytes with exceptionally high in vitro lysis of K562 leukaemia cells (x 3–100 vs. control)). At day +169 post-transplant he developed haematuria and was diagnosed with anti-glomerular basement membrane (anti-GBM) disease. This was treated with corticosteroids, cyclophosphamide and rituximab with normalisation of anti-GBM antibody levels and ongoing stable moderate renal impairment.

With regard to his primary disease, prior to treatment for anti-GBM disease he had normal serum immunoglobulin levels (IgG 7.04 g/L, IgA 0.99 g/L and IgM 0.45 g/L) and is in clinical remission from his vasculitis, with his most recent CSF protein reducing in a linear manner over time (most recently 0.9 g/L at day 415 post-HSCT). Cerebral MRI demonstrates stable lesions with small new foci of white matter involvement in the frontal lobes bilaterally, a probable reflection of peri-transplant changes. Repeat cerebral angiography was not performed because of the risk of contrast to his renal function. Notably, his CSF HHV-7 was negative on post-transplant testing.

Discussion

Non-HLH manifestations of XLP include dysgammaglobulinaemia, lymphoma, aplastic anaemia [5, 7], and T-cell pulmonary lymphomatoid granulomatosis [14]. We only identified two cases of XLP with arthritis in the literature, one of which had no clinical description given [3], the other which very much mirrored the joint restriction seen in the current case [15]. In that case there was painful restriction of a similar array of joints without swelling, however the symptoms appeared to progress to muscle weakness with vasculitis, which was not found on biopsy of connective tissues in our patient.

Vasculitis is a rare but well described complication of XLP, with associated vascular aneurysms reported in a number of patients [14]. To our knowledge there are only 4 published cases of non-EBV -associated cerebral vasculitis in XLP [5, 8, 9], two of which are well described. One of those patients had multiple aneurysms, and died from a subarachnoid haemorrhage [8], another failed to be controlled by a wide variety of immunosuppression and died [8], while a third presented with seizures widespread white matter changes, and at the time of reporting was said to be in a vegetative state [9]. Two patients were adult at presentation and had the same C163T mutation introducing a stop codon at arginine 55, and no viral cause was identified, while both had post mortem evidence of a CD8 positive T-cell vasculitis and clonal expansion of γδ T-cells [8].

The current case is unique for a number of reasons. Firstly, the novel c.96G>C mutation may have played a role in his unusual presentation. While we were unable to detect a T-cell clonal proliferation as a potential driver of inflammation, HHV-7 was identified repeatedly in CSF but disappeared post-transplant. This virus has previously been identified as a potential driver of XLP-associated HLH [16], while human herpes viruses have been associated with gamma/delta T-cell clones in other circumstances [17]. We would hypothesise that failure to clear local infection with HHV7 may have driven lymphocyte proliferation and been responsible for the cerebral vasculitis. Failure to clear a persistent virus is consistent with our observations during transplant when the patient developed symptoms localised to sites of previous disease (lung and brain) concurrent with massive NK cell engraftment. Our interpretation is that pre-transplant NK-cell deficiency undermined his ability to clear HHV-7, as related viruses are controlled by NK cells [18], and that he underwent an NK-cell mediated immune reconstitution syndrome against such an organism. Finally and most importantly, this is the first patient reported with XLP-associated cerebral vasculitis to successfully undergo haematopoietic stem cell transplant.

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