X linked lymphoproliferative disease in a United Kingdom family

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Abstract
X linked lymphoproliferative disease (XLP; Duncan's disease) is a rare disorder affecting boys and characterised by a defective immune response to Epstein-Barr virus caused by a mutation in a gene located at chromosome Xq25. Three siblings with XLP in a single UK family are reported and the variation in phenotypic expression of the disease in these siblings described. One of the siblings with life threatening fulminant infectious mononucleosis was successfully treated by chemotherapy, followed by bone marrow transplantation using an unaffected brother as the donor. A healthy baby boy recently born into the family was identified as carrying the defective maternal X chromosome using molecular genetic linkage analysis. This family illustrates the extent of present understanding of this often fatal condition.
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X linked lymphoproliferative disease (XLP; Duncan's disease) is one of six currently recognised, rare (1–2/10⁶), primary X linked immunodeficiency diseases. Up to 1995, 272 males in more than 80 kindreds had been identified worldwide.[1] XLP is caused by a mutation in a gene located at chromosome Xq25, which renders otherwise healthy males unable to mount an effective immune response to Epstein-Barr virus (EBV). The first cases were described over 25 years ago.[2 3] Since then, the phenotypic expression of XLP has been found to be variable and has included fulminant infectious mononucleosis with virus associated haemophagocytic syndrome, acquired dysgammaglobulinaemia, malignant lymphoma, aplastic anaemia, vasculitis, and pulmonary lymphomatoid granulomatosis.[4–6]

The XLP gene has yet to be identified, so definitive diagnosis of XLP requires two or more maternally related boys manifesting an XLP phenotype following EBV infection.[6] In boys without the XLP phenotype but where there is one affected brother, the diagnosis can be confirmed or excluded to a high degree of accuracy using molecular linkage studies and EBV serological analysis.[7] As single affected boys may not be diagnosed, XLP may be more common than generally thought.

After infection with EBV, which is virtually inevitable, XLP carries a poor prognosis. Purtilo *et al* set up the XLP registry at the University of Nebraska Medical Center in 1978 to monitor the outcome of the disease.[8] Of cases recorded by the registry, 75% have died, 70% of whom were under 10 years old. The major cause of death was acute fulminant infectious mononucleosis, which until recently carried a survival rate of only 4%. However, this has been successfully treated with etoposide and cures have been achieved using allogeneic bone marrow transplantation.[9 10]

We present a report of a UK family with XLP, although at least half a dozen other affected boys are known in this country. The family comprises three brothers with XLP, and we describe the successful treatment of two, one of whom presented with fulminant mononucleosis and the other with infectious mononucleosis associated with marrow aplasia and hypoglobulinaemia. We also report the early diagnosis of XLP in the youngest brother at the age of 4 months using molecular linkage analysis.

Methods

XLP FAMILY
Following preliminary indications of XLP in the proband (WW), the family was followed up at the haematology/oncology clinic at Royal Manchester Children's Hospital. Further clinical details of the proband and his siblings are provided in the case reports.

MOLECULAR LINKAGE STUDIES
DNA was extracted using the phenol-chloroform method[11] from EBV immortalised peripheral blood lymphocyte cell lines prepared from each member of the family. Restriction fragment length polymorphism (RFLP) analysis was carried out using the following cDNA probes linked to the XLP gene: p43-15 (DXS42[12]), 36B-2 (DXS10[13]), ST1 (DXS86[14]). Approximately 5 μg of genomic DNA was digested with the appropriate restriction enzyme (DXS42, DXS86: Bgl II; DXS10: Taq I) before electrophoresis through 0.8% agarose gels. Gels were then blotted using positively charged nylon membranes (Appligene-Oncor), which were hybridised to ³²P labelled probes, washed, and visualised by autoradiography. For microsatellite polymorphism analysis, X chromosome microsatellite markers DXS425, DXS1047, DXS737, HPRT, and DXS102—synthesised commercially (VHBio)—were amplified for 25 to 30 cycles using polymerase chain reaction (PCR).

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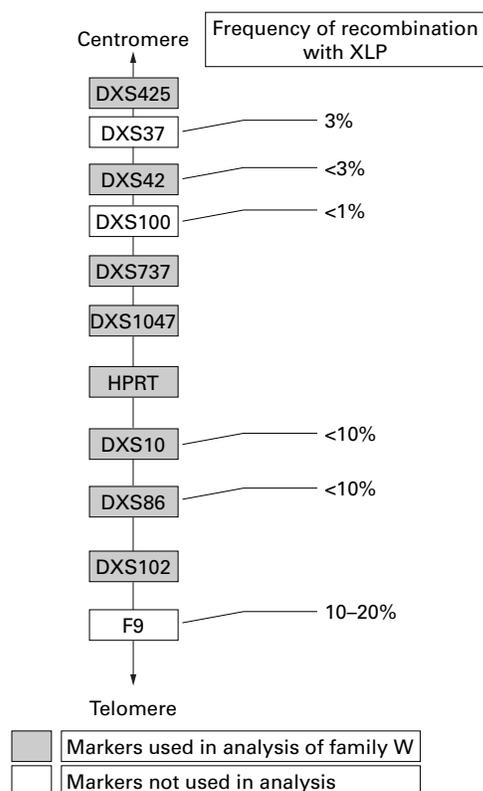email: gmtaylor@man.ac.uk

Figure 1 Order and recombination frequencies of markers flanking the XLP locus. (Recombination frequencies provided by T Gross, Department of Pathology, University of Nebraska Medical Center, USA).

Analysis of PCR products was carried out using 8% non-denaturing polyacrylamide gels and visualised by silver staining. The order of microsatellite markers shown in fig 1 was based on a two dimensional crossover map of the human X chromosome (University of Wash-

ington Genlink Server: www.genlink.wust.edu/mmp). The suspected proximity of these markers to the proposed XLP gene is also shown.

Results

The pedigree of family W is shown in fig 2. The family consists of mother and father who are both alive and well, and four boys aged 8, 6, and 4 years, and 8 months. The three affected boys are described below.

PROBAND

WW is a 6 year old male born at 33 weeks' gestation. His parents are unrelated, clinically well, and there is no family history of primary immunodeficiency. The pregnancy was complicated by varicella zoster infection in the third trimester and WW developed chickenpox neonatally, complicated by osteomyelitis of the right tibia. He recovered and remained well to the age of 18 months. He had no adverse reactions to his immunisations.

He then developed infectious mononucleosis, complicated by hepatitis and aplastic anaemia. EBV infection was confirmed by the identification of the virus in B lymphocytes grown in vitro from his peripheral blood mononuclear cells cultured in the presence of cyclosporin A. Scanty EBV genome was also seen in the lymphoid infiltrate from liver biopsy using immunofluorescence, but EBNA IgG was negative. Cytotoxic T lymphocytes, which are thought to cause some of the tissue injury in this disease, were increased in number and activated: $CD8^+$ lymphocytes were increased (7.6×10^9/l), with a reversed CD4/CD8 ratio of 1:2. These T cells had an unusual phenotype, with high levels of MHC class II expression (47% $CD3^+DR^+$) suggesting activation, but little IL-2 receptor
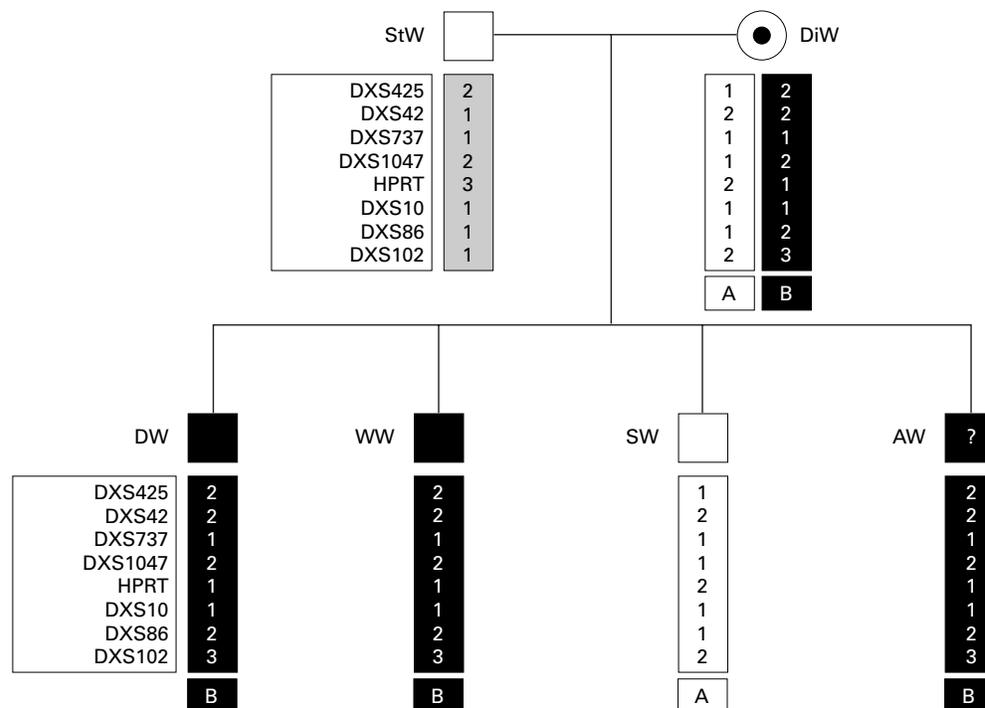

Figure 2 Analysis of RFLP and microsatellite markers flanking the XLP locus in family W.

expression (3% CD25). There was a normal proliferative response to T cell mitogens (PHA, ConA, PPD, candida). His aplastic anaemia responded to a combination of oral prednisolone, broad spectrum antibiotics, and aciclovir, but hepatosplenomegaly and raised transaminase levels persisted. Although his immunoglobulins were within the normal range at presentation, he developed hypoglobulinaemia three months later (IgG 2.4 g/l, IgA 0.08 g/l, IgM 0.27 g/l), and has immunoglobulin infusions every three weeks. He remains clinically well on these infusions.

CASE 2

DW, the oldest of the four brothers, presented in March 1997 at the age of 7 years with a brief history of fever and lethargy. He had not previously experienced any major medical problems. He rapidly developed a generalised itchy rash and hepatitis. A monospot was positive. Treatment with intravenous aciclovir and immunoglobulin infusion was ineffective and he developed bone marrow failure. Hyponatraemia occurred secondary to inappropriate antidiuretic hormone secretion. He became confused and had a single brief generalised seizure. Electroencephalography showed non-specific high voltage slow wave activity and magnetic resonance imaging revealed areas of high signal in both basal ganglia. Lumbar puncture confirmed the presence of atypical lymphoid cells and a raised protein concentration (5.2 g/dl). Macrophages showing haemophagocytosis could not be found in either bone marrow or cerebrospinal fluid samples, but because there were clinical features consistent with the diagnosis of virus associated haemophagocytic lymphohistiocytosis (HLH), he was started on treatment with the HLH 94 protocol.[15] He improved clinically and there was rapid clearance of the abnormal cells from his cerebrospinal fluid. He then underwent an allogeneic bone marrow transplantation from his fully HLA matched unaffected sibling (SW). After the transplant, he developed graft versus host disease of both bowel and liver, needing high dose steroid treatment, but has made otherwise excellent progress.

Blood samples from DW were tested for EBV DNA by PCR and remained negative throughout the illness. EBV VCA IgM was positive from the onset, and two weeks into the illness EBV VCA IgG became positive (1/640), probably secondary to passive acquisition of antibody with the immunoglobulin infusions, as his EBNA has remained low. His immunoglobulins have so far remained within the normal range.

CASE 3

SW, the 4 year old brother of WW and DW, remains well, with a positive EBNA IgG consistent with normal seroconversion and a healthy phenotype.

CASE 4

Just after DW's nearly fatal presentation, which resulted in the diagnosis of XLP being confirmed and discussed with the family, a healthy term baby boy, AW, was born. Molecular studies using microsatellite and RFLP analysis at 4 months of age showed that AW carries the defective maternal X chromosome (fig 2). WW, DW, and AW have a normal karyotype with no large structural abnormality apparent. AW has been started on prophylactic three weekly immunoglobulin infusions. Suitable unrelated bone marrow transplant donors are presently being sought for AW and WW.

As DiW—the mother of the brothers—carries the defective gene, her EBV serology was also tested. She showed definite seroconversion, with a high level of anti-VCA IgG of < 1/640, which is often seen in mothers of XLP children, and an anti-EA IgG of < 1/40.

Discussion

As far as we are aware, this is one of the first XLP families from the UK to be reported (see addendum). It illustrates various aspects of diagnosis and management of this rare and serious condition. The phenotypic variation is apparent, with one brother suffering from fulminant infectious mononucleosis/viral associated HLH, and the other from less severe infectious mononucleosis associated with hypoglobulinaemia and transient aplastic anaemia. DW, despite being comatose with encephalitis, hepatitis, and bone marrow failure, responded well to treatment, and survived bone marrow transplantation. Genetic linkage analysis has confirmed that AW has inherited the defective maternal X chromosome.

Until recently, treatment of XLP boys has been largely unsuccessful. However, Seemayer *et al*[1] reported that of 157 boys with fulminant infectious mononucleosis/virus associated HLH (usual survival 4%), five have recently been successfully treated with chemotherapy and four have received etoposide (VP16) to quell macrophage activation, resulting in quiescent disease and survival. Currently, the only curative treatment is allogeneic bone marrow transplantation. Age at time of transplant appears to be critical: all four boys less than 15 are alive and well two years or more after transplant, whereas all four who were over 15 died within 90 days from complications of transplantation.[1] We used a similar treatment regimen for DW, with excellent results. The HLH-94 protocol was applied, based on his clinical features and the experience of others in treating this condition. As described above, no cytological evidence of haemophagocytosis was found in his bone marrow or cerebrospinal fluid. We recommend that boys with this life threatening clinical XLP phenotype should receive chemotherapy with etoposide/HLH-94 protocol followed if possible by bone marrow transplantation.

Despite the suggestion by Lamartine *et al* that the gene for XLP has been localised to a small 130 kb segment of the X chromosome,[16] its precise identification remains elusive. Characterisation of the gene will allow antenatal screening of at risk families. As in this case, the definitive diagnosis of XLP can have major implications for other members of the family. Early determination of the health of other

family members using genetic tests can do much to allay fears, while parenteral immunoglobulin containing neutralising antibodies against EBV can be offered as a possible prophylactic measure.[17][18]

The immune defect in these patients remains unknown. Cytotoxic T lymphocytes are normally thought to limit the expanding pool of infected B cells and thus confer immunity to EBV.[18] As we showed in the case of WW, children with XLP seem to have large numbers of activated cytotoxic T lymphocytes, but not only are these T cells ineffective at controlling the virus infection, but their excessive unfocused activity results in a large amount of host tissue damage. This is likely to be at the root of many of the XLP phenotypes, with the exception of lymphomas, which are caused by excessive B lymphocyte proliferation secondary to EBV immortalisation. Characterisation of the XLP gene will also shed some light on specific immune regulatory mechanisms controlling infections.

Addendum

Note added in proof: since submitting this paper, details of another UK XLP family have been drawn to our attention.[19]

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