Progressive reduction of circulating B lymphocytes in patients with X-linked lymphoproliferative disease (XLP)

Inactivating mutations of the *SH2D1A* gene (also termed *SAP*, *DSHP*) determine the occurrence of human X-linked lymphoproliferative disease (XLP) (Nichols *et al*, 1998), a condition associated with inappropriate response to Epstein-Barr virus (EBV) infection. XLP patients who survive fulminant infectious mononucleosis and haemophagocytic syndrome, which cause death in 50% of cases after initial EBV infection, may develop dysgammaglobulinaemia, hypogammaglobulinaemia and/or lymphoproliferative diseases. Inefficient immune response against EBV is unable to control EBV expansion in most cases. Abnormalities in the balance of T helper 1 (Th1)/Th2 responses and accumulation of activated $CD8^+$ T lymphocytes have been described (Czar *et al*, 2001; Belmonte *et al*, 2007). The percentage of memory B cells ($CD27^+$ B lymphocytes) in B lympocytes is reduced in XLP patients surviving primary EBV infection compared to that of normal controls (Malbran *et al*, 2001). However, somatically mutated IgM^+CD27^+, but not Ig-subtype switched B cells could be found in XLP despite the absence of germinal centres in secondary lymphoid organs (Coraglia *et al*, 2010). Likewise, after prolonged culture of peripheral blood mononuclear cells (PBMC), the percentage of residual surviving B lymphocytes having a non-switched memory phenotype ($IgM^+IgD^+CD27^+$) was higher in XLP patients than normal controls (Belmonte *et al*, 2009).

We studied the long-term (>10 years) evolution of the EBV viral load (EBV VL) and B-lymphocyte profile in two surviving hypogammaglobulinaemic siblings of an established XLP family (Malbran *et al*, 2001). One of them (Patient nine) suffered EBV infection as an adult, developed severe EBV-associated disease and was treated with antiviral therapy and rituximab (Milone *et al*, 2005). He recovered from primary EBV infection, became hypogammaglobulinaemic and received substitutive monthly intravenous immunoglobulin (IVIg) infusions until 2013, when he presented a bone marrow aplasia and was successfully allotransplanted. The older sibling (Patient four) developed XLP-related hypogammaglobulinaemia after a tonsilar lymphoma when 4 years old, and remained on gamma globulin treatment for 35 years. During this time, both patients were periodically evaluated for phenotypic and functional changes of both T and B lymphocytes. A predominant terminal effector memory $CD8^+$ T cell phenotype ($CD27^-CD28^-CD8^+$) was observed in Patient nine when compared to his brother (Patient four) (Belmonte *et al*, 2007).

In this communication we report the progressive loss of B lymphocytes observed in both patients during the period of study, with coincidental reduction of the EBV VL. While both patients shared the same genetic defect, their initial encounter with EBV occurred at different ages (childhood in Patient four and adulthood in Patient nine) and they were treated differently. Patient nine was 27 years old and had been asymptomatic before his XLP-marker disease (severe acute EBV infection). He had received preventive IgG replacement since he was genetically diagnosed as XLP. His EBV VL was 18164 EBV copies/10^6 leucocytes during the acute phase of his disease. At this time he received rituximab treatment, with $CD19^+$ lymphocyte reduction to <0·5%, coincident with a reduction of the EBV VL to 278. Two years later, the CD19 values started to increase, reaching the normal range 6 years after rituximab treatment (Fig 1). The EBV VL was still high 2 years after the acute phase of his disease (8620-8750 EBV copies/10^6 leucocytes) and persisted until year five (3969 EBV copies/10^6 leucocytes), becoming undetectable after 6 years (Fig 1). Patient nine has never had any of the clinical symptoms of chronic active EBV disease (Cohen *et al*, 2015). His B lymphocyte values steadily decreased, reaching 3·3–3·4% with negative EBV VL 7 years after his initial EBV disease. This patient's clinical condition required an allogeneic bone marrow transplant that was successfully performed in the USA, and he recovered his good health. We had no access to study the characteristics of his T and B lymphocytes, because he moved out of Argentina.

Patient four, who had his first XLP-related condition at 2 years of age, had low circulating $CD19^+$ cells when we first studied his B lymphocyte profile (2–3%, 33 years after his XLP-marker disease) (Fig 1). These values decreased further, reaching 0·34% after 12 years of follow-up, when he was 45 years of age. During this period he continued receiving substitutive monthly IVIg, and his EBV VL remained below 100 EBV copies/10^6 leucocytes.

As reported before (Malbran *et al*, 2001), memory $CD27^+$ B lymphocytes were lower than normal in XLP. In both patients this value was relatively stable within the B lymphocyte pool, oscillating around 5–14% of the total B lymphocytes (Fig 1), but when the B lymphocyte percentage fell below 3% in Patient 4, the memory B lymphocytes decreased to 0·5%.

In both patients the absolute B and $CD4^+$ T lymphocyte values were lower (0·021–0·095 $\times$ 10^9 B lymphocytes/l; 0·0016–0·0113 $\times$ 10^9 memory B lymphocytes/l; 0·32–

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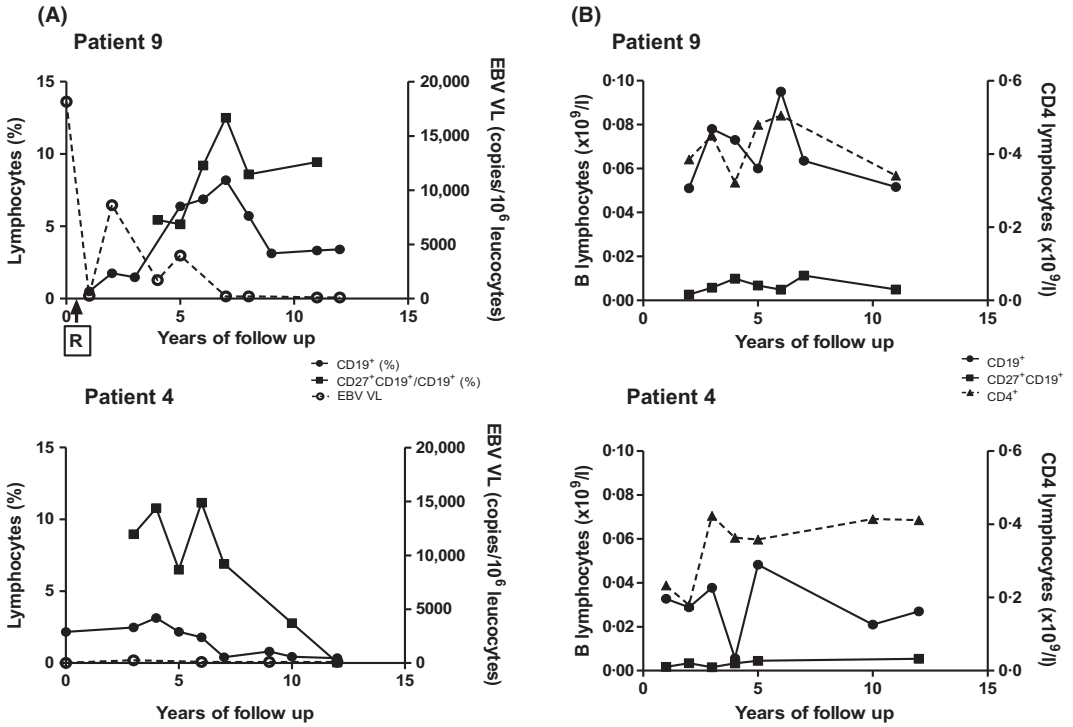

Fig 1. (A) B lymphocyte and EBV viral load in XLP patients during 12 years of follow up. Upper panel: Patient nine; lower panel: Patient four. Patient nine received rituximab (R) treatment during the acute phase of his EBV-related disease (Milone *et al*, 2005). Y axis: left side, percentage (%) of $CD19^+$ B lymphocytes (●-●) and % of memory B lymphocytes within the B cell region, as determined by flow cytometry, ($CD27^+CD19^+/CD19+$) (■-■). Y axis: right side, EBV viral load (EBV VL) expressed as EBV copies/10^6 leucocytes (**o**–**o**). X axis: years of follow-up. Laboratory determinations were performed in samples drawn before the monthly IgG replacement therapy in both patients over the 12-year period. For Patient nine (year 1) the initial date of study was immediately after recovery of the acute phase of the EBV disease, while for Patient four the initial sample was drawn after 33 years of his XLP-marker disease. (B) Absolute values of B lymphocytes ($CD19^+$ and $CD27^+CD19^+$) and CD4 T lymphocytes. Upper panel: Patient nine; lower panel: Patient four. Y axis: left side, absolute values of $CD19^+$ B lymphocytes (●-●) and memory B lymphocytes ($CD27^+CD19^+$) (■-■) within the B cell region, as determined by flow cytometry. Y axis: right side, $CD4^+$T cell counts (▲-▲).

$0{\cdot}5 \times 10^9$ $CD4^+$ T lymphocytes/l) compared to those of normal controls ($0{\cdot}072–0{\cdot}2 \times 10^9$ B lymphocytes/l; $0{\cdot}028–0{\cdot}043 \times 10^9$ memory B lymphocytes/l; $0{\cdot}6–0{\cdot}9 \times 10^9$ $CD4^+$ T lymphocytes/l), while $CD8^+$ T lymphocytes were increased ($0{\cdot}7–3{\cdot}0 \times 10^9$ $CD8^+$ T lymphocytes/l in XLP and $0{\cdot}32–0{\cdot}7 \times 10^9$ $CD8^+$ T lymphocytes/l in normal controls).

It is difficult to explain why B lymphocytes were progressively reduced in these XLP patients. Several factors must be taken into account. It is known that germinal centre persistence and formation are affected in XLP patients probably because T_{FH}-B contacts are transient in the absence of SH2D1A (SAP) (Qi *et al*, 2008). This may lead to impaired B cell memory formation (Malbran *et al*, 2001; Coraglia *et al*, 2010). The long period of continuous IgG replacement may alter the balance of circulating B cells and their function. Inhibition of BCR-dependent and BCR-independent antigen presentation by B lymphocytes after IVIg infusion was demonstrated (Paquin Proulx *et al*, 2010). Also, $CD8^+$ T lymphocytes reacting against EBV-infected B lymphocytes could contribute to reduce the B cell count over a long period.

Replacement treatment with IgG will continue to be mandatory for the protection of these patients if they do not undergo allogeneic transplantation and recover the balance of their immune system. In any event, the progressive loss of B lymphocytes after many years of XLP evolution must be taken into account as an additional complication of this genetic defect.

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Author contributions

PB and CP performed the experiments, analysed data and wrote the manuscript. AM was the physician in charge of the XLP patients. AM and MMEB designed the study, analysed data and wrote the manuscript.

Conflict of interest

The authors declare no competing financial interests.

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