

1 SEVERE EBV INFECTION IN PRIMARY IMMUNODEFICIENCY AND THE NORMAL HOST

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16 **Author contribution**

17 Austen Worth; Writing and research selected sections of the manuscript. Final editing of the full
18 manuscript

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1 **Abstract**

2 EBV infection is ubiquitous in humans, but the majority of infections have an asymptomatic or self-
3 limiting clinical course. Rarely, individuals may develop a pathological EBV infection with a variety of
4 life threatening complications (including haemophagocytosis and malignancy) and others develop
5 asymptomatic chronic EBV viraemia. Although an impaired ability to control EBV infection has long
6 been recognised as a hallmark of severe T-cell immunodeficiency, the advent of next generation
7 sequencing has identified a series of Primary Immunodeficiencies in which EBV-related pathology is
8 the dominant feature. Chronic active EBV infection is defined as chronic EBV viraemia associated
9 with systemic lymphoproliferative disease, in the absence of immunodeficiency. Descriptions of
10 larger cohorts of patients with chronic active EBV in recent years have significantly advanced our
11 understanding of this clinical syndrome. In this review we summarise the current understanding of
12 the pathophysiology and natural history of these diseases and clinical syndromes, and discuss
13 approaches to the investigation and treatment of severe or atypical EBV infection.

14

1 **Introduction**

2 Epstein Barr Virus is a γ -herpesvirus characterised by restricted infectious specificity to humans, and
3 latent infection in B lymphocytes. Primary infection with EBV typically occurs in childhood as a
4 symptomless or mild infection, with early infection seen in a higher proportion of the population of
5 low income, compared to high income countries (Cohen 2000; Pariente et al. 2007; Hjalgrim, Friborg,
6 and Melbye 2007)(e.g. 58.9% of Zambian infants aged 12 months are EBV seropositive (Minhas et al.
7 2010) compared to 7.1% of infants in a Swedish infants (Hesla et al. 2013)). By age 30, >95% of
8 adults in Europe and North America are seropositive (Cohen 2000; Pariente et al. 2007; Pembrey et
9 al. 2013). Primary EBV infection in adolescence or adulthood leads to a 25-70% risk of developing a
10 symptomatic EBV infection, known as infectious mononucleosis (IM) (Higgins et al. 2007; McAulay et
11 al. 2007). This is characterised by pharyngitis, benign lymphoproliferation, fever and malaise, and
12 symptoms last up to 6 weeks duration in the majority of patients.

13 Following primary infection, EBV persists within resting memory B-cells (Miyashita et al. 1997) with
14 low immunogenicity (Babcock, Hochberg, and Thorley-Lawson 2000), allowing a life-long infection to
15 be established, which the immune system cannot clear.

16 **EBV Life cycle**

17 EBV has a biphasic life cycle, divided into lytic and latent gene expression programmes (Tsurumi,
18 Fujita, and Kudoh 2005)(figure 1). The lytic gene expression programmes allow EBV to productively
19 infect new cells and new hosts, while establishment of latency is vital to allow life-long persistence
20 of the virus within infected cells, through a highly restricted gene expression profile in order to avoid
21 immune surveillance (Young, Arrand, and Murray 2007).

22 EBV is naturally transmitted through saliva (Gerber et al. 1972), and the first cells thought to be
23 infected by EBV during primary infection are oral epithelial cells and naïve Bcells (Balfour et al.
24 2013). During the lytic cycle, EBV infection is followed by the induction of two immediate early
25 proteins (Rta and EB1) which act as transcriptional activators for a wide range of proteins and viral
26 RNAs involved in viral DNA replication or viral structural proteins. True latency (type 0), where cells
27 express no EBV protein or RNA, but retain senescent episomal EBV DNA, is subsequently established
28 in the resting B memory pool. Although this transition to latency is not fully understood, a model
29 proposing EBV exploitation of the physiological B-cell differentiation pathway is helpful when
30 considering the pathological states following EBV infection (Thorley-Lawson et al. 2013)). In this,
31 infected naïve B-cells enter a proliferative phase during which they express the full range of latent
32 EBV proteins and viral RNAs (latency type 3, equivalent of *in vitro* derived B lymphoblastoid cell
33 lines). A proportion of these cells differentiate further into latency type 2 within the oropharyngeal

1 germinal centres in response to EBV-specific T follicular helper cells, resulting in restriction of EBV
2 protein expression to EBV nuclear antigen (EBNA) 1, latent membrane protein (LMP) 1 and 2
3 proteins only. Further differentiation results in the release of latency type 0 resting B memory cells
4 into the circulation. Sporadically, as these cells recirculate through the oropharynx, a proportion
5 switch from the immunologically silent latent infection, to the productive lytic cycle.

6 The virus is shed at particularly high levels from four-six weeks after initial infection (Dunmire et al.
7 2015), reducing as the infected individual convalesces. Low-level shedding of EBV in saliva continues
8 sporadically for life (Hadinoto et al. 2009), as cycles of lytic reactivation within B-cells and
9 oropharyngeal epithelial cells are interrupted by immunological control, leading to a return to latent
10 infection (Taylor et al. 2015).

11 From epidemiological studies and *in vitro* work, a number of factors have been identified which may
12 drive the change from a latent to a lytic gene expression profile in EBV infected cells (Kenney 2007).
13 In latently infected individuals it has been shown that stress (e.g. sleep deprivation) leads to
14 increased EBV genome production (Uchakin et al. 2011; Mehta et al. 2000), and EBV lytic
15 transcription factor BZLF1 may be induced by glucocorticoids (Yang et al. 2010), demonstrating a link
16 between the host environment and permissivity of viral reactivation. Suppression of T-cell immunity
17 (e.g. after organ transplantation) is also strongly associated with EBV reactivation (Hiwarkar et al.
18 2013), as is concurrent infection with some other pathogens, such as Group A *Streptococcus* (Ueda
19 et al. 2014) and malaria (*Plasmodium falciparum*) (Moormann et al. 2005).

20 **Immune Response to EBV infection**

21 Primary EBV infection in the immunocompetent host leads to distinct antibody responses. The EBV
22 proteins expressed during lytic infection, such as Viral Capsid Antigen (VCA) and Immediate Early
23 Antigen (EA), are often highly immunogenic with antibodies to VCA detectable up to a week before
24 the onset of IM symptoms. Antibody responses to latent proteins are delayed, with anti-EBNA2 IgG
25 appearing at the height of symptoms, and the anti-EBNA1 IgG response developing from 3 months
26 post-infection. The hallmark of primary EBV infection is detectable IgM and rising IgG VCA antibody
27 responses, in the absence of an EBNA1 IgG response (Taylor et al. 2015). In a study of individuals
28 who had recently experienced IM, 88% of individuals were positive for VCA-IgM, 100% were positive
29 for VCA-IgG, and 96% were positive for EBNA1-IgG (Balfour et al. 2013).

30 Studies of individuals in the early stages of EBV seroconversion show an expansion in peripheral
31 Natural Killer (NK) cells at the point of symptom onset, 4-6 weeks after initial infection (Balfour et al.
32 2013). Although NK-cell numbers correlate with virus load in the peripheral blood, they
33 demonstrate little evidence of increased activation, as measured by granzyme B levels. CD8+ T-cell

1 expansion follows the same pattern with a dramatic proliferation of lytic antigen specific CD8+ T-
2 cells during IM, and a much smaller response to latent antigens. Onset of symptoms correlates
3 much more closely with CD8+ cell expansion, than with EBV viral load, supporting a model of IM as
4 an immunopathic disease (Balfour et al. 2013). EBV-specific CD8+ T-cells also develop during
5 asymptomatic EBV infection, however the total CD8+ T-cell counts remain within the normal range,
6 suggesting activated CD8+ T-cell over-proliferation is associated with symptomatic disease
7 (Jayasooriya et al. 2015). CD4+ T-cell expansion also occurs during IM, although this is less dramatic
8 than the CD8+ response. The CD4+ response is predominantly towards latent EBV antigens and
9 demonstrates a wider distribution of epitope responses. A detailed review the immune response to
10 EBV infection can be found elsewhere (Taylor et al. 2015).

11 **Pathological Responses to EBV infection**

12 Although the majority of individuals infected with EBV have an asymptomatic or self-limiting clinical
13 course, there is a broad range of pathological responses to infection, encompassing prolonged fever
14 and lymphoproliferation (severe IM), haemophagocytic lymphohistiocytosis, autoimmunity and
15 malignancy. These states arise from unregulated cytotoxic and inflammatory responses to EBV
16 infected B-cells, impaired T-cell or NK-cell immune surveillance of EBV infected cells, EBV infection in
17 aberrant (non-B) cells or as yet undefined mechanisms. Each of these pathological mechanisms can
18 be in seen in immunodeficient patients (primary or secondary) or apparently normal hosts.

19 **Haemophagocytic lymphohistiocytosis (HLH)**

20 HLH is a severe, life-threatening immunodysregulatory disorder resulting from the uncontrolled
21 activation and proliferation of T-cells and macrophages, which causes excessive production of
22 cytokines, hyperinflammation and tissue damage. It is classified as either primary HLH, when a
23 family history of the disorder or identified genetic defect is present, or secondary HLH, associated
24 with infection, rheumatological disorders (often termed macrophage activation syndrome) or
25 malignancy. Increasingly, HLH is also recognised as a complication of more “classical” primary
26 immunodeficiencies (PIDs) (Bode et al. 2015). EBV is an important infectious trigger for both
27 primary and secondary HLH. The causes and investigation of primary HLH, and PID-associated HLH
28 are summarised in Table 1.

29 Regardless of the underlying cause, the clinical features of HLH are similar. Clinical manifestations
30 include prolonged fever, hepatosplenomegaly, hepatitis, cytopenias, coagulopathy, rashes and
31 neurological symptoms. The Histiocyte Society has developed diagnostic criteria for HLH, based on
32 both clinical and laboratory findings (Table 2), to help guide the initiation of therapy.

1 In the normal setting, cytotoxic T lymphocytes (CTLs) and NK-cells, once in contact with a virally
2 infected cell, respond by forming an immunological synapse with the target cell, followed by
3 granule-mediated cytotoxicity. This not only clears the virally infected cells but regulates the
4 inflammatory response by removing antigenic stimulus. Familial HLH results from impaired granule-
5 dependent cytotoxicity, resulting in impaired target cell death, continued stimulation of cytotoxic
6 cells and ongoing production of inflammatory cytokines. The clinical features of HLH are a
7 consequence of the resultant uncontrolled macrophage activation and histiocytic transformation
8 (Jordan et al. 2004).

9 Standard of care treatment remains established, highly immunosuppressive, chemotherapeutic
10 protocols (Henter et al. 2007). Alternative and salvage therapies, including the use of anti-
11 thymocyte globulin (ATG) and monoclonal antibodies are reviewed elsewhere (Jordan et al. 2011;
12 Mahlaoui et al. 2007). Supportive care, particularly to prevent and treat infection is critical, and in
13 EBV-HLH, removal of the infectious trigger with multiple courses of Rituximab is essential
14 (Chellapandian et al. 2013).

15 Following induction therapy patients with primary HLH should proceed urgently to Haematopoietic
16 Stem Cell transplant (HSCT). Long term management of secondary EBV-HLH is more difficult;
17 however our practice is to progress to HSCT in patients with relapsing HLH or HLH associated with
18 CAEBV (see below), even in the absence of a defined genetic aetiology. It is critical to achieve
19 remission prior to HSCT as the outcome in patients with active HLH is significantly worse. The
20 introduction of reduced intensity conditioning (RIC) regimes has improved the outlook for patients
21 with primary HLH affording 3-year survival rates of 92% compared to 43% when myeloablative
22 conditioning is used (Marsh, Vaughn, et al. 2010).

23 **Chronic Active EBV (CAEBV)**

24 Chronic Active EBV (CAEBV) was originally used to describe patients with chronic or recurrent
25 infectious mononucleosis (Straus 1988). It is now defined as EBV related illness lasting greater than
26 3 months, associated with systemic EBV positive lymphoproliferative disease (LPD) (with elevated
27 EBV DNA/RNA in affected tissues), and high level EBV viraemia or increased anti-VCA IgG titre, in the
28 absence of defined primary or secondary immunodeficiency (Cohen et al. 2011; Kimura et al. 2012;
29 Okano et al. 2005) (see table 2). CAEBV has been most commonly described in East Asia where the
30 proliferating cells are usually T- or NK-cells (Kimura et al. 2012). This clinically heterozygous
31 condition has overlap with two cutaneous syndromes – Hydroa Vacciniforme-like lymphoma (a
32 recurrent vesiculopapular eruption usually caused by an EBV infected $\gamma\delta$ T-cell infiltration) and
33 mosquito bite sensitivity associated with EBV positive lymphoproliferation (usually NK-cells) (Kimura,

1 Kawada, and Ito 2013) (see figure 2). In Western countries, CAEBV is rarer but usually associated
2 with B-cell proliferation (Cohen et al. 2011). In all of these conditions the lymphocyte, and EBV
3 clonality may be monoclonal, oligoclonal or polyclonal (Kimura et al. 2012).

4 Clinically, CAEBV has an aggressive course, with complications ranging from progression to HLH
5 and/or lymphoma, disseminated intravascular coagulopathy, coronary artery aneurysms, CNS
6 disease, myocarditis, pneumonitis and gastrointestinal perforation (Kimura et al. 2012; Cohen et al.
7 2011). In the largest series of EBV-associated T/NK-cell LPD, 108 Japanese patients demonstrated
8 44% survival at a median follow up of 46 months (range 1 month – 21 years). Patients with isolated
9 cutaneous disease appear to have a better prognosis, and those with CD4+ T-cells as the EBV
10 infected proliferative cell, do worse (Kimura et al. 2012). Complications and prognosis for CAEBV
11 with B-cell lymphoproliferation in the US seems to be similar, although interestingly 42% of these
12 patients developed progressive hypogammaglobulinaemia and B-cell lymphopenia (even in the
13 absences of rituximab therapy) (Cohen et al. 2011).

14 CAEBV patients have significantly higher loads of EBV DNA in mononuclear cells compared to
15 patients with IM (Xing et al. 2013). Characteristically, patients have very high IgG antibody titres to
16 EBV early antigen (EA) and VCA, but lack an IgG response to EBNA1 (Kimura et al. 2003), however,
17 this pattern may be absent in up to 50% of patients and is therefore of limited diagnostic value. The
18 pathophysiology of CAEBV is poorly understood. Despite the above antibody pattern being
19 suggestive of a predominance of EBV lytic cycle infection, T and NK-cells demonstrate EBV infection
20 in type 2 latency (Kimura et al. 2005). CAEBV patients have a hyperinflammatory state with cytokine
21 profiles (raised IL-1 β , IL10, IFN γ) similar to patients with familial HLH (Ohga et al. 2001), suggesting
22 an overlapping pathophysiology, with impaired removal of EBV infected T/NK-cells driving a local
23 inflammatory response in infiltrated tissues, and a resultant pro-oncogenic and haemophagocytic
24 environment (Rickinson 2014).

25 EBV-positive systemic T-cell lymphoproliferative disease (STLPD) is a lymphoma of activated $\alpha\beta$ T-
26 cells. It usually develops following acute EBV infection, but can also arise as a malignant progression
27 from CAEBV (Kimura, Kawada, and Ito 2013). By definition this is a systemic illness caused by clonal
28 proliferation of EBV infected T-cells (Quintanilla-Martinez, Kimura, and Jaffe 2008). It almost
29 invariably is associated with haemophagocytosis, and as the proliferating cells rarely show evidence
30 of atypica, the differentiation from HLH or CAEBV is often difficult. It is perhaps best thought of as
31 the common neoplastic path of lymphoid proliferation in both HLH and CAEBV (Hong et al. 2013)..

32 Treatment of CAEBV and STLPD remains unsatisfactory, with most patients treated according to their
33 predominant clinical syndrome (HLH or lymphoma). Rituximab, antiviral and chemotherapeutic

1 drugs may have a role in stabilising early disease in occasional patients, but any clinical benefit is
2 rarely sustained. Results of early HSCT with RIC are encouraging, suggesting that conservative
3 therapy should be reserved for patients with isolated cutaneous disease and easily controlled
4 inflammation (Kimura et al. 2012; Cohen et al. 2011; Kawa et al. 2011).

5 **EBV driven malignancy and autoimmunity**

6 EBV-driven malignancy is seen in both immunocompetent and immunodeficient patients, with
7 malignant transformation of lymphoid cells (T-, B- and NK-cells), and non-haematopoietic cells
8 (Figure 2). Pathophysiology is multifactorial with the following being key pathogenic processes; (1)
9 loss of immune surveillance / EBV-mediated immune evasion, (2) EBV infection induced growth factor
10 and cytokine production, (3) EBV oncogene expression (particularly LMP1 and LMP2A) and (4)
11 genetic / epigenetic alteration of the host genome. The relative importance of each of these
12 mechanisms varies for each individual malignancy (Murata, Sato, and Kimura 2014; Rickinson 2014;
13 Taylor et al. 2015).

14 EBV has also been implicated in the pathophysiology of autoimmunity, best characterised by
15 associations with multiple sclerosis and systemic lupus erythematosus (Taylor et al. 2015; Thacker,
16 Mirzaei, and Ascherio 2006)

17 **Common human genetic variation in EBV infection and immunity**

18 Susceptibility to EBV infection and disease may be described as a spectrum. At one end lie rare
19 monogenic mutations with large effects (Houldcroft and Kellam 2015), and at the other end are
20 common polymorphisms with small effects, leading to subtle changes in risk of, and response to,
21 infection. In the middle is a less well defined category of low-frequency variants which are
22 associated with disease phenotypes, but are also found in apparently healthy individuals (Manso et
23 al. 2014), with no clear impact on gene function.

24 Genome-wide association studies (GWAS) have identified a number of common genetic variants
25 underlying population response to EBV infection. Individuals vary in their antibody titres to EBNA1
26 in a heritable manner. A series of GWAS have identified single nucleotide polymorphisms (SNPs) within
27 HLA class II genes as important for antibody response to EBV, specifically IgG antibodies to EBNA1
28 (Hammer et al. 2015; Pederagnana et al. 2014; Rubicz et al. 2013). Pederagnana and Rubicz identified a
29 cluster of SNPs near HLA-DRA, (although it was unclear which SNP drove the relationship with EBNA1
30 IgG titres), and Hammer et al identified HLA-DRB1*07:01 as associated with lower anti-EBNA-1 IgG
31 titres (beta: -0.17). Candidate gene studies have also associated anti-VCA IgA and IgG titres to
32 variants in a number of genes (Houldcroft and Kellam, 2015), but these variants have yet to be
33 validated by the more agnostic GWAS approach

1 This approach has also suggested variants within the HLA class 1 system as being important for
2 susceptibility to EBV infection (Durovic et al. 2013), and risk of symptomatic infection (IM versus
3 silent seroconversion) (McAulay et al. 2007). Evidence from epidemiological studies further
4 supports a common genetic basis for infectious mononucleosis susceptibility, as studies of IM risk in
5 twins (Hwang et al. 2012) demonstrate that monozygotic twins have twice the relative risk of
6 concordance for symptomatic IM compared to dizygotic twins. Similarly, first degree relatives
7 (Rostgaard, Wohlfahrt, and Hjalgrim 2014) show a heritable component of IM risk, based on studies
8 of individuals hospitalised with severe IM, with rate ratios of IM increasing as genetic relatedness
9 increased.

10 **EBV genome variation and its role in disease**

11 In addition to genetic susceptibility of the host, it is possible that genetic variability in the EBV
12 genome may play a role in the pathogenesis of severe EBV infection.

13 The genome of EBV is approximately 184kb long, formed of linear double-stranded DNA. Following
14 primary infection, EBV persists in B-cells as an episome and does not normally integrate into the
15 human genome, although aberrant EBV integration events are seen in some EBV-positive cancers
16 Raab-Traub 2007). Genetically and phenotypically, there are two types of EBV (1 and 2) and these
17 types have different geographic distributions. EBV type 1 is most prevalent and occurs worldwide,
18 while EBV type 2 seropositivity shows widespread geographic variation and is reported in 20-25% of
19 EBV seropositive individuals in parts of Africa and Melanesia (Young et al. 1987), and is found at
20 lower frequencies outside these regions. Co-infection with EBV type 1 and EBV type 2 is infrequently
21 detected (Chang et al. 2009). Recombinant strains between EBV type 1 and EBV type 2 have been
22 also reported (Burrows et al. 1996).

23 EBV type 2 transforms B-cells more poorly than EBV type 1, and there is some *in vivo* evidence that
24 type 2 EBV transforms T-cells more successfully than B-cells (Coleman et al. 2015). There are other
25 patterns of diversity across individual EBV genes, but they do not lead to such clear patterns of
26 genome differentiation as the type 1/type 2 distinction (Palser et al. 2015).

27 The number of EBV genome sequences from healthy individuals is very small and there are few EBV
28 genome sequences from patients with EBV-specific susceptibilities. Identifying disease-associated
29 viral variants will require more EBV sequences from pathogenic infections, with age and location
30 matched controls.

1 **Primary Immune Deficiencies associated with severe EBV disease**

2 Herpesvirus infections are particularly problematic for patients with monogenic defects of the
3 immune system, and severe or persistent EBV infection is a hallmark combined or innate primary
4 immunodeficiency. Among these diverse disorders there is a subset of conditions which appear to
5 have a particular susceptibility to developing pathogenic consequences of EBV infection, and these
6 are summarised below. Interestingly these disorders share a number of functional immune defects
7 including cytotoxicity, T-cell receptor signalling, effective antibody production, cell migration and
8 regulation of apoptosis (table 3). Additionally several patients with combined immunodeficiency
9 have chronic asymptomatic EBV viraemia. Optimal management of these patients is unknown,
10 however careful long term monitoring is essential.

11 Investigation of patients presenting with severe or atypical EBV infection should focus on early
12 identification of a possible PID or characterisation of an HLH or CAEBV clinical state. Detailed
13 phenotyping of T- and B-cell differentiation, and functional assays including cytotoxic granule
14 release and cytotoxic cell killing assays, provide a useful screen for PID and primary HLH, allowing
15 targeted protein expression assay and genetic sequencing, and guide the bioinformatics of next
16 generation sequencing data. A multidisciplinary approach including malignant Haematologists,
17 Rheumatologists, Infectious Diseases physicians and Clinical Geneticist is recommended. A proposed
18 investigation algorithm is shown in figure 3.

19 **X-linked Lymphoproliferative Disease (XLP) / SAP deficiency**

20 XLP-1 is a primary immunodeficiency whose clinical features include HLH, lymphoma and
21 dysgammaglobulinaemia. (Seemayer et al. 1995; Sumegi et al. 2000; Booth et al. 2011). Overall
22 mortality has reduced over time but HLH still remains fatal in the majority of patients with this
23 manifestation (Booth et al. 2011). XLP results from mutations in the *SH2D1A* gene which encodes
24 the SLAM-associated protein (SAP). SAP is an intracellular adaptor molecule expressed in T-, NK- and
25 NKT-cells, and is a key regulator of normal immune function. Immune defects described in XLP
26 patients include reduced or absent NKT-cells (Nunez-Cruz et al. 2008), abnormal NK- and CD8+ T-cell
27 cytotoxicity (Parolini et al. 2000; Tangye et al. 2000; Dupre et al. 2005) and compromised
28 reactivation induced cell death, all of which could explain the abnormal response to viral infection
29 (Snow et al. 2009). Defective CD4+ T follicular helper cell function leads to impaired antibody
30 function, and lack of memory B-cells and long lived plasma cells (Veillette et al. 2008; Crotty et al.
31 2003; Qi et al. 2008).

32 Although XLP is associated with an increased susceptibility to severe EBV disease, the finding that up
33 to 35% of patients are EBV negative at diagnosis supports our understanding of XLP as a disorder of

1 severe immune dysregulation, with HLH, lymphoma and humoral abnormalities described in EBV
2 negative patients (Booth et al. 2011). No significant difference in mortality was seen between EBV
3 positive and EBV negative patients.

4 Management relies on appropriate treatment of HLH and lymphoproliferation, with most patients
5 requiring immunoglobulin replacement therapy. Rituximab is routinely used to reduce EBV viral load
6 and manage EBV related complications. Survival following HSCT is 81% but mortality increases to
7 50% in patients with HLH. Survival for un-transplanted patients is reported as 63% but again
8 outcome is extremely poor in the context of HLH with survival plummeting to 19% (Booth et al.
9 2011). A murine model of XLP has been corrected using HSC gene therapy and this approach may
10 offer, in the future, an alternative treatment strategy for patients lacking a suitable donor for HSCT
11 (Rivat et al. 2013).

12 **XIAP deficiency**

13 X-linked inhibitor of apoptosis (XIAP) deficiency is caused by mutations in the *BIRC4* gene and
14 although initially described as XLP-2 due to similarities in clinical presentation to boys with SAP
15 deficiency (Rigaud et al. 2006), it is now recognised as a more complex disorder of immune
16 dysregulation, with a wide spectrum of clinical manifestations. XIAP is ubiquitously expressed and
17 appears to have several roles in immune cells including in NOD-1 and NOD-2 signalling, and
18 detection of bacterial infection, alongside its anti-apoptotic role (Aguilar and Latour 2015). Patients
19 with XIAP deficiency have reduced NKT-cell numbers and lymphocytes demonstrate increased
20 activation induced cell death (AICD). NK-cell cytotoxicity is normal (Marsh, Madden, et al. 2010).
21 Diagnosis can be made through flow cytometric analysis of protein expression and genetic analysis.
22 A functional assay demonstrating impaired TNF α production in response to NOD2 pathway
23 stimulation in monocytes has also been described (Ammann et al. 2014).

24 A number of case series have now been published which confirm the main clinical features as HLH
25 (frequently recurrent and of a more indolent course than seen in other primary HLH diseases),
26 splenomegaly, colitis and periodic fevers (Pachlopnik Schmid et al. 2011; Yang et al. 2012;
27 Speckmann et al. 2013; Aguilar and Latour 2015). In contrast to XLP patients, lymphoma has not
28 been reported in patients with XIAP deficiency. Hypogammaglobulinaemia is also less common (67%
29 vs 33%) (Pachlopnik Schmid et al. 2011) and has been described subsequent to EBV infection.
30 Interestingly female carriers may also exhibit symptoms including erythema nodosum and
31 inflammatory bowel disease (Dziadzio et al. 2015). The outcome for XIAP patients receiving HSCT
32 following myeloablative conditioning is poor with historical data reporting a survival of 14% (Marsh
33 et al. 2013). In most cases mortality was due to transplant related toxicity, highlighting the

1 sensitivity of these patients to drug side effects, likely related to the loss of XIAP's anti-apoptotic
2 function. Results are more favourable with RIC; survival increases to 55% overall, and 86% if
3 patients are in remission from HLH at the time of transplant. Minimal intensity conditioning using
4 anti-CD45 monoclonal antibodies has also been successfully employed (Worth et al. 2013).

5 **ITK deficiency**

6 Interleukin-2 inducible T-cell kinase (ITK) is another recently described autosomal recessive PID
7 associated with EBV-driven LPD, Hodgkin lymphoma and, more unusually, non-Hodgkin lymphoma
8 (Huck et al. 2009; Serwas et al. 2014; Mansouri et al. 2012; Stepensky et al. 2011; Linka et al. 2012).
9 ITK is a member of the TEC kinase family (which includes BTK) and is required for normal
10 development and signalling in lymphoid cells. Progressive reduction in CD4+ T-cells, naïve CD4+ T-
11 cells and NKT-cell numbers is a common feature along with hypogammaglobulinaemia. Clinical
12 features are primarily related to EBV associated lymphoproliferation but opportunistic infections
13 associated with T-cell deficiencies have also been reported. 9 patients have been described to date,
14 8 presenting with LPD between the ages of 3 and 13 years (Ghosh et al. 2014). 6 patients have died
15 with 5 succumbing within 2 years of presentation despite treatment, demonstrating the devastating
16 course of this condition. Fever and lymphadenopathy were found in all patients, with
17 hepatosplenomegaly and lung involvement in 5/8 symptomatic individuals. The presence of
18 autoimmunity in 3, and HLH in 2 suggests an underlying immune dysregulatory component to this
19 disease but all patients so far have been EBV+ at diagnosis making it difficult to dissect out the role
20 of EBV. ITK deficiency can be diagnosed by immunoblot to detect protein expression, with
21 confirmatory sequence analysis. The response to chemotherapy treatment of malignancy is
22 variable. Some benefit has been shown for rituximab and aciclovir therapy, but steroids do not
23 appear to ameliorate the clinical features (Ghosh et al. 2014; Cipe et al. 2015). Two patients have
24 received HSCT (1 MSD, 1 haploidentical donor) with one patient surviving (Ghosh et al. 2014). The
25 optimal management strategy for patients with ITK deficiency is yet to be determined but close
26 monitoring is essential.

27 **CD27 deficiency**

28 CD27 deficiency is a diagnosis to consider in patients with severe EBV disease,
29 hypogammaglobulinaemia and recurrent infection. It is inherited in an autosomal recessive fashion.
30 As an increasing number of cases are reported, our understanding of the clinical spectrum of this
31 disease is improving. CD27 is a co-stimulatory molecule important for the development T, B and NK-
32 cells, in particular memory B-cells. As CD27 is a widely used marker in the analysis of B- and T-cell
33 differentiation, flow cytometry offers a simple and reliable screen for this disorder, through

1 assessing CD27 expression across B-, T- and NK-cells. All proven CD27 deficient patients have either
2 absent CD27 expression (9/11 tested) or severely reduced expression (2/11) (Alkhairy et al. 2015).
3 Reduced numbers of NKT-cells have been reported in severely affected individuals but impaired NK-
4 cell function may be a more consistent immunological finding (Alkhairy et al. 2015). Impaired T-cell
5 dependent B-cell responses due to defective CD4+ T-cell help lead to compromised cellular and
6 humoral immunity, and patients may be misdiagnosed with CVID (van Montfrans et al. 2012). To
7 date 17 patients have been described and it is apparent that the clinical phenotype is variable,
8 ranging from asymptomatic absence of memory B-cells and hypogammaglobulinaemia, to EBV driven
9 HLH and LPD, with no genotype-phenotype correlation (van Montfrans et al. 2012; Salzer et al. 2013;
10 Alkhairy et al. 2015). Median age at presentation of symptomatic patients was 6 years in this cohort
11 (range 1-22 years) with a reported mortality of 29% (Alkhairy et al. 2015). Many patients received
12 immunoglobulin replacement therapy, rituximab and appropriate lymphoma treatment. Three
13 patients underwent RIC mismatched unrelated cord blood transplant and are alive with the longest
14 follow up of 4.5 years. Close monitoring of asymptomatic patients is crucial to allow early
15 intervention in EBV driven disease.

16 **XMEN (X-linked, magnesium defect, EBV, neoplasia)**

17 XMEN is a recently described serious PID caused by mutations in the *MAGT1* gene, encoding the
18 magnesium transporter 1 protein (Li et al. 2011; Li et al. 2014). It is characterised by chronic EBV
19 infection with high viral loads, and increased susceptibility to lymphoma and LPD. To date 8 patients
20 have been described with an age at diagnosis of 3-58 years (Ravell, Chaigne-Delalande, and Lenardo
21 2014; Dhalla et al. 2015). None have developed HLH or other overt features of immune
22 dysregulation, unlike the other X-linked lymphoproliferative disorders. A decreased CD4:CD8 ratio is
23 a consistent finding with abnormal TCR signalling, but significant humoral defects have not been
24 described (Li et al. 2014). Due to abnormal magnesium flux in NK and T-cells, viral specific cytotoxic
25 cells fail to sufficiently control EBV infection. Patients also develop other viral infections such as
26 Molluscum, HSV and VZV, and recurrent sinopulmonary infections (Ravell, Chaigne-Delalande, and
27 Lenardo 2014). Haematological malignancy is reported in all post-pubertal patients described, with
28 many experiencing LPD early in life. Recurrent malignancy is described in 2 patients.

29 Two patients received HSCT at the ages of 23 and 45 years but both died in the early post-transplant
30 period from transplant related complications (Li et al. 2014). There is a suggestion from *in vitro* data,
31 and use in 2 patients, that oral magnesium supplementation can increase NK-cell cytolytic activity
32 and EBV control. Although highly experimental, it appears safe and well tolerated (Chaigne-
33 Delalande et al. 2013).

1 **STK4 Deficiency**

2 Serine threonine kinase 4 (STK4) (also known as MST1) deficiency is an autosomal recessive
3 combined immunodeficiency, characterised by progressive CD4+ lymphopenia. STK4 is a
4 ubiquitously expressed constituent of the HIPPO signalling pathway, which regulates cell
5 proliferation, migration and apoptosis (Zhao, Tumaneng, and Guan 2011). Specifically, in human
6 immune cells STK4 plays a critical role in preventing lymphocyte apoptosis (Abdollahpour et al. 2012;
7 Nehme et al. 2012), thymic egression (Tang et al. 2015) and leucocyte migration (Dang et al. 2016).

8 13 patients with STK4 deficiency are reported in the published literature (Nehme et al. 2012; Halacli
9 et al. 2015; Crequer et al. 2012; Abdollahpour et al. 2012; Dang et al. 2016). Combining these
10 patients with our experience of 3 unpublished cases (16 in total), 13 patients have been exposed to
11 EBV, and 11 have developed chronic viraemia. 5 patients have developed EBV driven
12 lymphoproliferation or malignancy at a median follow up of 11 years of age. Additionally, these
13 patients have recurrent invasive bacterial infections, severe cutaneous viral infections,
14 mucocutaneous candidiasis and autoimmune cytopenias. In some kindred, intermittent neutropenia
15 and congenital cardiac defects have been described. 7 patients have been treated by HSCT. 4
16 patients died of a combination of infectious, toxicity related and GVHD complications, the remaining
17 3 patients are alive and well, and apparently cured of their immunodeficiency (Nehme et al. 2012;
18 Dang et al. 2016).

19 **CTP synthetase 1 (CTPS1) Deficiency**

20 CTPS1 deficiency has been recently described in 8 patients, as an autosomal recessive combined
21 immunodeficiency, caused by a defect in lymphocyte proliferation following antigen receptor
22 stimulation (Martin et al. 2014). CTP synthesis contributes to the free cellular CTP pool, essential for
23 efficient cell division. CTPS1 activity is induced following TCR activation, and deficiency results in a T-
24 cell proliferative defect despite normal TCR activation signalling. Clinically, these patients are
25 susceptible to severe viral infections, and capsulated bacterial infection, suggesting both a functional
26 defect of T-cell cytotoxicity and T-independent B-cell immunity. The clinical penetrance of
27 immunodeficiency appears high, with the majority of patients presenting within the first 2 years of
28 life. All patients developed chronic EBV viraemia, with 4/8 patients developing severe IM and 3/8
29 developing CNS LPD. 6 patients received an HSCT and 4 remain alive, well, and free of symptoms.

30 **Coronin 1A Deficiency**

31 Coronin 1A deficiency was originally described as a thymic egress defect causing T- B+ NK+ severe
32 combined immunodeficiency (SCID)(Shiow et al. 2008). Like other immunodeficiencies caused by
33 actin cytoskeletal defects, Coronin 1A deficiency impacts on a wide range of lymphocyte processes,

1 including development, survival, TCR signalling, immune synapse formation and migration (Foger et
2 al. 2006; Punwani et al. 2015; Mace and Orange 2014; Mugnier et al. 2008). Impaired calcium flux
3 and f-actin accumulation at the immune synapse, result in increased T-cell apoptosis, compounding
4 the CD4+ lymphopenia. A total of 9 patients have been described with Coronin 1A deficiency (Shiow
5 et al. 2008; Moshous et al. 2013; Mace and Orange 2014; Stray-Pedersen et al. 2014; Punwani et al.
6 2015; Yee et al. 2016), the majority with a typical SCID clinical presentation. Patients have an
7 immunophenotype of absent or low naïve T-cells, severely impaired T proliferative responses,
8 normal levels of total immunoglobulins, and impaired (but not absent) vaccine responses. Unlike
9 other forms of SCID, Coronin 1A deficient patient have normal volume thymic tissue. 5/9 patients
10 have developed EBV driven B-cell lymphomas, and generally these have been at an earlier age than
11 in other susceptible PIDs, with 4/5 patients developing EBV-driven LPD prior to 15 months of age. 2
12 patients died of their lymphomas prior to HSCT. No patients have developed HLH or severe IM. 3
13 patients to date have been treated with HSCT. 1 is alive and well, but 2 died following HSCT (GVHD,
14 relapsed diffuse large B-cell lymphoma).

15 **Activated Phosphatidylinositide 3-Kinase delta syndrome (APDS)**

16 Gain of function mutations in the phosphatidylinositide 3-kinase delta (PI3K δ) subunit p110 δ cause a
17 combined immunodeficiency of variable clinical severity, characterised by recurrent sino-pulmonary
18 infections, increased susceptibility to viral infections, lymphoproliferation, bronchiectasis and an
19 autosomal dominance inheritance pattern (Angulo et al. 2013; Lucas et al. 2014). PI3K δ is involved
20 in downstream signalling from T- and B-cell antigen receptors, costimulatory receptors, cytokine
21 receptors and some Toll-like receptors (Okkenhaug 2013). Unregulated activity results in
22 hyperactivation of the Akt-mTOR pathway, inducing excessive terminal differentiation of effector
23 lymphocytes, increased activation induced cell death in T-cells, impaired cytokine production and
24 impaired immunoglobulin class switching in B-cells (Angulo et al. 2013; Lucas et al. 2014). Although
25 neither haemophagocytic syndrome nor severe IM have been described in APDS, a high incidence of
26 chronic EBV viraemia has been described (Kannan et al. 2015; Lucas et al. 2014). Of 43 patients with
27 APDS described in the literature, 9 (21%) have developed haematological malignancy or LPD, of
28 which 3 were EBV positive, and two were undefined (Crank et al. 2014; Angulo et al. 2013; Kannan et
29 al. 2015; Lucas et al. 2014; Kracker et al. 2014; Hartman et al. 2015). There is one published patient
30 who has been successfully treated by HSCT and we have transplanted a second patient without
31 complication (unpublished). Inhibition of mTOR activity with Rapamycin has been used to
32 successfully ameliorate the disease and has improved the immunophenotype in patients (Lucas et al.
33 2014). With the availability of selective PI3K δ inhibitors, pharmacological blockade offers an
34 attractive line of treatment for these patients.

1 **Radiosensitive SCID**

2 Defects of the non-homologous DNA end joining mechanism result in T- B- NK+ SCID, but clinical
3 severity of defects in this pathway are heterogeneous, with several patients described with a
4 hypomorphic phenotype. Hypomorphic DNA ligase IV and Artemis gene mutations demonstrate
5 susceptibility for EBV driven-LPD or diffuse large B-cell lymphoma, however HLH has not been seen
6 (Woodbine, Gennery, and Jeggo 2014; Moshous et al. 2003; Toita et al. 2007; Enders et al. 2006).
7 Although numbers are small for each of these conditions, the incidence of EBV LPD seems to be
8 between 20-50% of described patients.

9 **Ataxia Telangectasia (AT)**

10 AT is an autosomal recessively inherited syndrome characterised by progressive cerebellar ataxia,
11 oculomotor dyspraxia, oculocutaneous telangiectasia, immunodeficiency and susceptibility to
12 malignancy. It is caused by mutations in the protein ATM, which plays an integral role in DNA repair
13 and cell cycle checkpoint control. A recently published French registry study demonstrated a 19.1%
14 incidence of lymphoma in patients with AT by 20 years of age. Approximately 1/3rd of these
15 lymphomas were Hodgkin Diseases (all tested were EBV related) and 2/3^{rds} were NHL (50% EBV
16 positive) (Suarez et al. 2015). HLH, SIM or chronic EBV viraemia has not been described in AT.

17 **CD16 deficiency and other NK defects**

18 Although only 3 patients have been described with homozygous mutation in the gene coding for
19 CD16, two have developed EBV related severe complications (prolonged IM (de Vries et al. 1996),
20 EBV-associated B-LPD (Grier et al. 2012)). Patients have normal numbers of NK-cells, but impaired
21 NK-cell cytotoxicity, and affected patients suffered from severe viral infections (particularly VZV and
22 HPV in addition to EBV). Other monogenic disorders of NK cells (GATA2 and MCM4 deficiencies) also
23 demonstrate specific EBV susceptibility, and are summarised in table 3 (Spinner et al. 2014; Gineau
24 et al. 2012; Hughes et al. 2012).

25 **Other Primary Immunodeficiencies with EBV susceptibility**

26 Although all diseases with impaired T-cell function or number will struggle to respond appropriately
27 to EBV infection, there are several other PIDs which, whilst not having the high penetrance of EBV-
28 associated disease of the above conditions, still frequently develop significant EBV pathology.
29 Patients with Wiskott Aldrich Syndrome are at high risk of developing malignancy, particularly EBV-
30 driven B-cell lymphoma. Historical data suggests that without HSCT, over 10% of patients will
31 develop malignancy with a median age of onset of 9.5 years (Sullivan et al. 1994). There are also
32 occasional cases of EBV-driven HLH in Wiskott Aldrich Syndrome (Pasic, Micic, and Kuzmanovic 2003;
33 Bode et al. 2015). Autoimmune lymphoproliferative syndrome (ALPS) is a disorder of impaired

1 lymphocyte apoptosis. There is a linear risk of developing lymphoma with age, and at 30 years 15%
2 of patients have developed lymphoma, with almost all being EBV positive (Price et al. 2014). Two
3 patients have also developed HLH (Bode et al. 2015). WHIM (Warts, Hypogammaglobulinaemia,
4 Immunodeficiency and Myelocathexis syndrome) is characterised by a susceptibility to severe
5 papilloma virus and herpesvirus infections. Two cases of EBV-associated LPD (fatal in one case) have
6 been described (Chae, Ertle, and Tharp 2001; Imashuku et al. 2002).

7 **Conclusions and Future Directions**

8 Significant advances in recent years have increased our understanding of the host-virus interaction
9 in EBV infection and better characterised the pathophysiology of severe and aberrant EBV infection.
10 We remain, however, far from truly appreciating the complexities of genetically determined
11 susceptibility to pathological EBV-associated states. Treatment of these rare disorders, however,
12 remains inconsistent and optimal therapeutic approaches are largely unknown. Ongoing
13 identification of new monogenic PIDs with specific EBV-susceptibility, and further characterisation of
14 the phenotype and natural history of already described conditions, will aid management strategies
15 for these patients. For patients without a discernible immunodeficiency, biomarkers to predict
16 severe disease progression and those patients who would benefit from early HSCT are urgently
17 needed. With improving understanding of the pathophysiology of these conditions, identification of
18 targeted biologic, cellular or small molecule therapies offers the best hope of managing these
19 patients effectively and safely in the future.

20

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28

29

1 **Figure Legends**

2 **Figure 1: The Epstein-Barr virus life cycle**

3 Following initial infection (typically through saliva) EBV establish a biphasic life cycle within the host,
4 allowing non-productive genome maintenance in situations of immune surveillance, termed the
5 latent cycle, and reactivating to the productive, infectious lytic cycle when in situations of primary
6 infection or immune suppression. EBV induces different stages of latency (in B cells of different
7 differentiation states, with progressively less viral protein and RNA production as B cells become
8 more differentiated / less activated. Individuals with chronic active EBV or other EBV genetic
9 susceptibilities may be unable to mount an effective CTL or antibody response to lytically replicating
10 EBV-positive cells, leading to high virus loads and constitutive immune activation. Abbreviations:
11 VCA – Viral capsid antigen, EA- Early antigens, EBER – EBV encoded small RNAs, EBNA – EBV nuclear
12 antigen, LMP – Latent membrane protein, T_{fh} – T follicular helper cell.

13

14 **Figure 2: Clinical manifestations of EBV infection**

15 Although the vast majority of EBV infected individuals have an asymptomatic or self limiting primary
16 infection (infectious mononucleosis), rare individuals develop more severe clinical syndrome as a
17 consequence of an impaired ability to control lytic or latent EBV infection, or a the establishment of
18 latent EBV infection in aberrant (non-B-cell) types. These complications can be immune
19 dysregulatory, leading to lymphproliferation and a local or systemic hyperinflammatory state, or
20 they can be malignant. Increasingly it appears that these pathologies are closely linked, with
21 dysregulated inflammatory responses driving EBV-induced malignant proliferation. As a consequent
22 there is considerable overlap between many of the malignant and inflammatory EBV clinical
23 syndromes. The relationship between these main syndromes is shown in the figure. Arrows
24 represent clinical overlap or progression between individual clinical syndromes.

25

26 **Figure 3: Clinical investigation algorithm for chronic or pathological EBV infection**

27 We advocate an aggressive approach to the investigation of chronic EBV viraemia and pathological
28 consequences of EBV infection. Establishing the detailed immune state of the host, and the
29 pathophysiology of infection, facilitates close monitoring of patients, assists targeting therapies, and
30 helps identify which patients may benefit from early stem cell transplant. The above algorithm
31 provides an exhaustive list of investigation for patients presenting with EBV – associated
32 haemophagocytic syndromes, CAEBV, chronic EBV viraemia, SIM, and EBV-driven malignancy. It

1 provides a framework for selecting investigations according to an individual patient's clinical
2 presentation and disease progress. We do not propose that every patient should undergo every
3 investigation listed above. Management and assessment of these patients benefits from a
4 multidisciplinary approach involving haematologists, immunologists, rheumatologist, Infectious
5 diseases specialists, and specialist immunodeficiency and molecular genetics laboratory services.
6 *Abbreviations: TRECs – T-cell recombination excision circles, IgGAME – IgG, IgA, IgM, IgE, DHR -*
7 *dihydrorhodamine assay, NBT - nitroblue tetrazolium assay, EM – electron microscope, FBC – full*
8 *blood count, LDH – Lactose dehydrogenase, sCD25 – soluble CD25, PID – Primary Immunodeficiency*
9 *, fHLH – familial HLH, WES – whole exon sequencing, WGS – whole genome sequencing, ANA –*
10 *antinuclear antigen autoantibody, dsDNA – double stranded DNA autoantibody, ENA – Extractable*
11 *nuclear antigen autoantibodies, Urinary alb:creat – urinary albumin : creatinine ratio, CMV –*
12 *cytomegalovirus, HSV – Herpes simplex virus 1 and 2, VZV – varicella zoster virus, HHV6/7 – Human*
13 *herpes virus 6 and 7, Hiv – human immunodeficiency virus, BM – bone marrow, VCA – Viral caspid*
14 *antigen, EA- Early antigens, EBER – EBV encoded small RNAs, EBNA – EBV nuclear antigen*

15

Table 1: Genetic disorders associated with primary HLH

Condition	Gene	Protein	Normal function	Diagnosis
Familial HLH				
FHL1	Unknown	Unknown	Unknown	Abnormal degranulation assay
FHL2	<i>PRF1</i>	Perforin	Pore formation	Abnormal perforin expression, gene sequencing
FHL3	<i>UNC13D</i>	Munc 13-4	Vesicle priming	Abnormal degranulation assay, gene sequencing
FHL4	<i>STX11</i>	Syntaxin 11	Vesicle fusion	Abnormal degranulation assay, gene sequencing
FHL5	<i>STXBP2</i>	Munc 18-3	Vesicle fusion	Abnormal degranulation assay, gene sequencing
X-linked lymphoproliferative disorders				
XLP	<i>SH2D1A</i>	SAP	Signalling molecule	Abnormal SAP expression, gene sequencing
XIAP deficiency	<i>BIRC4</i>	XIAP	Signalling molecule	Abnormal XIAP expression, gene sequencing
HLH with pigmentary dilution				
Chediak Higashi	<i>LYST</i>	LYST	Vesicle trafficking	Hair shaft microscopy, abnormal degranulation assay, giant granules in several cell types, gene sequencing
Griscelli Type 2	<i>RAB27A</i>	RAB27A	Vesicle docking	Hair shaft microscopy, abnormal degranulation assay, gene sequencing
Hermansky-Pudlak type 2	<i>AP3B1</i>	AP3B1	Vesicle trafficking	Abnormal degranulation assay, gene sequencing
Other causes of HLH with primary immunodeficiency				
ITK deficiency	<i>ITK</i>	ITK	Signalling molecule	Abnormal ITK expression, gene sequencing
CD27 deficiency	<i>CD27</i>	CD27	Co-stimulatory molecule	Abnormal CD27 expression, gene sequencing
APDS	<i>PIK3CD</i>	PI(3)K p100δ	Signalling molecule	Gene sequencing
SCID	<i>ILR2G, RAG-1</i>		T cell development	Abnormal protein expression, gene sequencing
DiGeorge Syndrome	<i>Chromosome 22q11 deletion</i>		T cell development	CGH microarray
Wiskott Aldrich Syndrome	<i>WASP</i>	WASp	Various, including Vesicle trafficking	Abnormal protein expression, gene sequencing
Chronic Granulomatous Disease	<i>CYBB, NCF1, NCF2</i>	p91-phox, p47-phox, p67-phox	Oxidative burst	Abnormal DHR/NBT assay, abnormal protein expression, gene sequencing

Table 1: **Causes of primary HLH and other Primary Immunodeficiencies with HLH as a clinical feature.** FHL - Familial haemophagocytic Lymphohistiocytosis syndrome, XLP - X-linked lymphoproliferative disease, SAP - SLAM associated protein, XIAP - X-linked inhibitor of apoptosis, ITK - IL-2 inducible T-cell kinase deficiency, XMEN - X-linked immunodeficiency with magnesium defect, APDS - activated PI3kinase delta syndrome, SCID - severe combined immunodeficiency, DHR - dihydrorhodamine assay, NBT - nitroblue tetrazolium assay.

Table 2: Comparison of diagnostic criteria for HLH and CAEBV

Haemphagocytic Histiocytosis	Chronic Active Epstein Barr Infection
<i>Clinical definition requires ≥ 5/8 of criteria below;</i>	<i>Persistent illness lasting > 6 months either;</i>
Fever	→ Following primary EBV infection or → Associated with significantly increased EBV viraemia demonstrated by <ul style="list-style-type: none"> • marked elevation of blood EBV DNA by PCR • Significant rise in IgG antibody titres to; VCA (≥ 1:5120) or EA (≥ 1:640)
Splenomegaly	
Cytopenias (affecting ≥2 of 3 lineages in peripheral blood) <ul style="list-style-type: none"> → Haemoglobin <9g/dL (<10g/dl if under 4 weeks of age) → Platelets <100 x10⁹/L → Neutrophils <1.0 x10⁹/l 	Some or all of the following clinical features (present in ≥ 10% patients); <ul style="list-style-type: none"> → Fever → Hepatomegaly and / or splenomegaly → Excessive lymphadenopathy → Hepatitis → Cytopenia → Skin rash → Hypersensitivity to mosquito bites → Hydroa vacciniforme
Hypertriglyceridaemia and/or hypofibrinogenaemia <ul style="list-style-type: none"> → Fasting triglycerides ≥3.0mmol/L → Fibrinogen ≤1.5g/L 	
Haemophagocytosis in bone marrow or spleen or lymph node with no evidence of malignancy	Tissue infiltration with lymphocytes (e.g. lymph nodes, bone marrow, liver, skin, lung, eye, CNS)
Low or absent NK cell activity	Increased EBV in infiltrated tissues demonstrated by;
Ferritin >500µg/L	→ In situ hybridisation for EBER
Soluble CD25 (IL-2 receptor) >2400 U/ml	→ EBV DNA positivity by PCR
	→ EBV protein positivity by immunohistochemistry
<i>If a molecular diagnosis consistent with HLH is confirmed (see table 1) then full clinical criteria do not need to be fulfilled</i>	<i>Absence of any primary or secondary immunodeficiency, or other pathological cause of symptoms</i>

Table 2: Diagnostic criteria for Haemophagocytic Lymphohistiocytosis (HLH) (Henter et al. 2007) and Chronic active EBV infection (CAEBV) (Kimura et al. 2001; Kimura et al. 2003; Cohen et al. 2011). Definitions for CAEBV vary slightly between different studies (duration of symptoms 3-6 months, necessary criteria for EBV viraemia and tissue based disease). EBER – Epstein Barr Virus-encoded RNA, VCA – viral capsid antigen, EA – Early antigen.

Table 3 Clinical and Immunopathology of Primary Immunodeficiencies associated with Complex EBV infection

	Clinical Manifestation						Immune Function			Pathophysiology			
	HLH	Severe IM	Chronic EBV viraemia	Malignant susceptibility	Autoimmunity	EBV as disease trigger	Naive T cell number	T cell proliferation	Antibody production	Cytotoxicity	TCR signalling	Cell migration / thymic egress	Dysregulation of apoptosis
XLP	Dark Red	Red	Red	Dark Red	Red	Dark Red			Dark Blue	Dark Green	Dark Green		
XIAP def	Dark Red	Red	Light Red		Red	Light Red			Light Blue				Dark Green
ITK def	Light Red		Dark Red	Dark Red	Red	Dark Red	Dark Blue	Light Blue		Dark Green	Dark Green		
CD27 def	Dark Red		Red	Dark Red		Dark Red			Dark Blue	Dark Green	Dark Green		
XMEN			Dark Red	Dark Red	Light Red	Dark Red	Light Blue	Light Blue		Dark Green	Dark Green		
STK4 def			Dark Red	Dark Red	Light Red	Dark Red	Dark Blue					Dark Green	Dark Green
CTPS1 def		Red	Dark Red	Dark Red		Dark Red	Light Blue	Dark Blue	Light Blue				
Coronin1A				Dark Red		Dark Red	Light Blue	Dark Blue	Light Blue	Dark Green	Dark Green	Dark Green	Dark Green
APDS			Red	Light Red	Red	Light Red	Dark Blue	Dark Blue	Dark Blue		Dark Green		Dark Green
RS-SCID			Light Red	Dark Red	Red	Dark Red	Dark Blue	Dark Blue	Dark Blue		Dark Green		
AT				Light Red		Dark Red	Light Blue		Light Blue			Light Green	Dark Green
CD16 def		Red	Dark Red	Dark Red		Dark Red				Dark Green			
GATA2 def			Light Red	Light Red		Light Red	Light Blue			Dark Green			
MCM4 def				Light Red		Light Red				Dark Green			Light Green
WAS	Light Red	Light Red	Light Red	Dark Red	Red	Dark Red		Light Blue	Light Blue	Dark Green	Dark Green	Dark Green	
ALPS	Light Red			Dark Red	Red	Dark Red							Dark Green
WHIM				Light Red		Light Red	Light Blue	Light Blue	Light Blue			Dark Green	Light Green

Clinical Manifestation

Frequency

Dark Red	>75% patients
Red	25 – 75% patients
Light Red	<25% patients
Very Light Red	Sporadic cases
White	Not described

Immune Function

Severity

Dark Blue	Full defect
Blue	Severe defect
Light Blue	Partial defect
Very Light Blue	Mild defect
White	No defect

Pathophysiology

Severity

Dark Green	Confirmed defect in humans
Light Green	Predicted / animal model suggestive
White	No defect / Unknown

Table 3 Clinical and Immunopathology of Primary Immunodeficiencies associated with Complex EBV infection: HLH - Haemophagocytic lymphohistiocytosis, IM – infectious mononucleosis, TCR – T-cell receptor, XLP – X-linked lymphoproliferative syndrome, XIAP – X-linked inactivator of apoptosis, ITK - IL-2 inducible T-cell kinase deficiency, XMEN - X-linked immunodeficiency with magnesium defect, STK4 – Serine threonine kinase 4, CTPS1 – Cytosine triphosphate synthetase 4, APDS - activated PI3kinase delta syndrome, RS-SCID – Radiosensitive severe combined immunodeficiency, AT – Ataxia telangectasia, WAS – Wiskott Aldrich Syndromw, ALPS – Autoimmune lymphoproliferative syndromw, WHIM – Warts, hypogammaglobulinaemia, immunodeficiency and myelocathexis syndrome. % frequencies of clinical manifestations, and severity of immune function defect are based on the author’s review of all published case reports of each disease, experience of cases at the authors’ host department, and discussions with other expert centres.

Figure 1

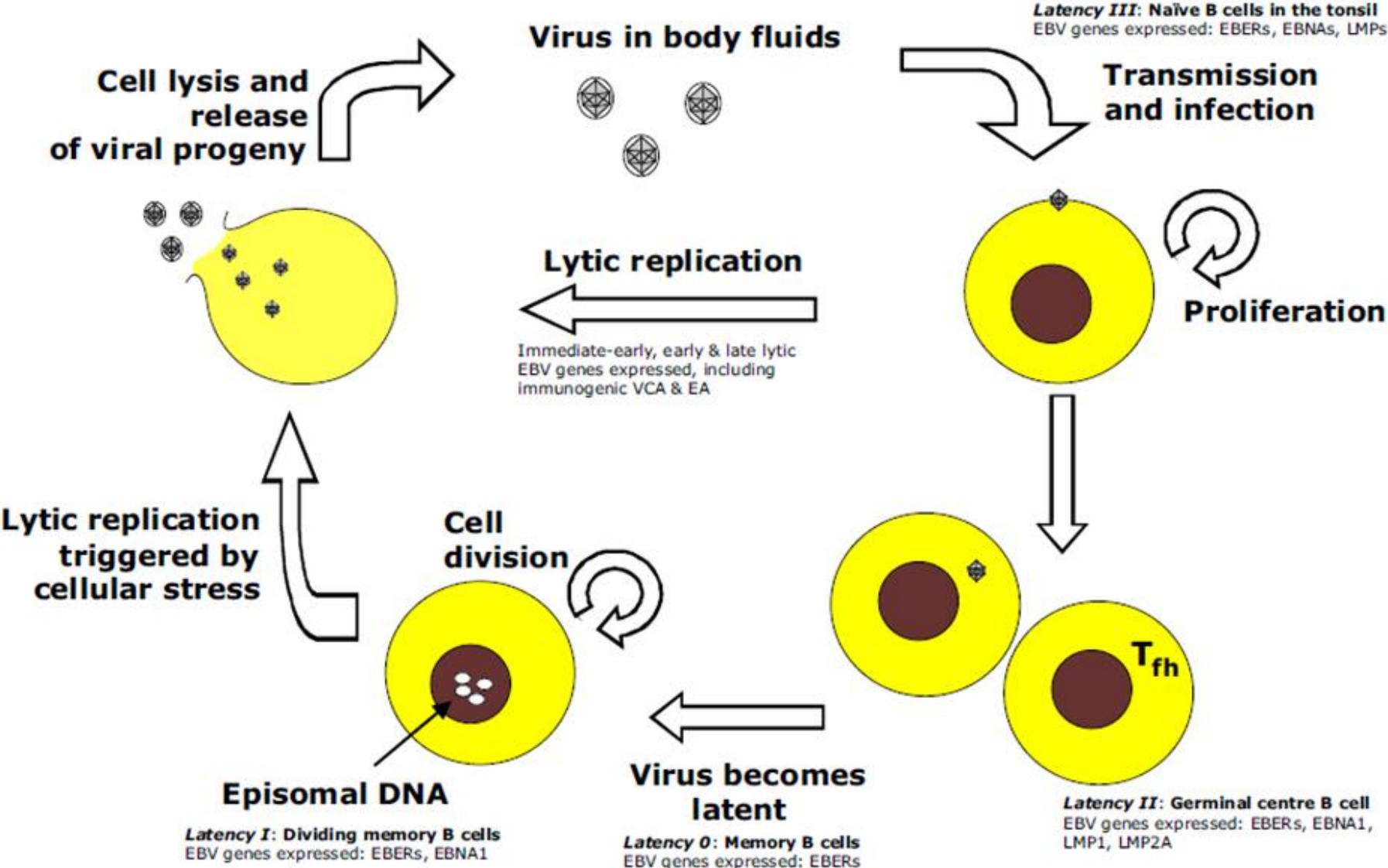


Figure 2
Asymptomatic

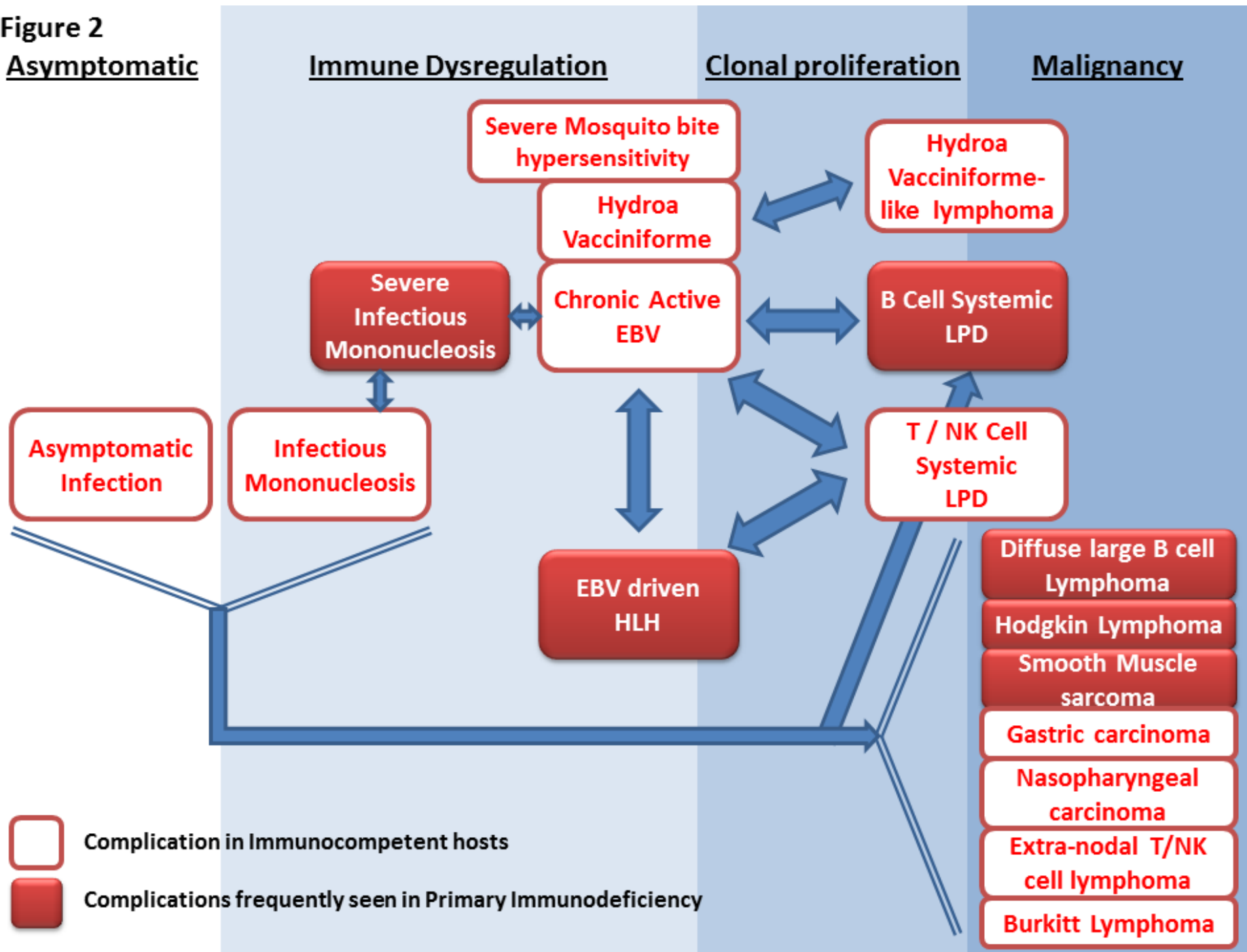


Figure 3

