

Contribution of the Epstein–Barr virus to the molecular pathogenesis of Hodgkin lymphoma

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Although the morphology of the pathognomonic Reed–Sternberg cells of Hodgkin lymphoma (HL) was described over a century ago, it was not until recently that their origin from B lymphocytes was recognised. The demonstration that a proportion of cases of HL harbour the Epstein–Barr virus (EBV) and that its genome is monoclonal in these tumours suggests that the virus contributes to the development of HL in some cases. This review summarises current knowledge of the pathogenesis of HL with particular emphasis on the association with EBV.

variable (*V*) region, indicating that they originate from germinal centre or post-germinal centre B cells. However, only L&H cells of NLPHL demonstrate intraclonal *V* gene diversity due to ongoing mutation, suggesting that they originate from differentiating germinal centre B cells. In contrast, in 25% of classical HL, HRS cells carry non-functional “crippling” mutations in the *IGVH* gene rearrangements, suggesting that HRS cells originate from pre-apoptotic germinal centre B cells that were rescued from apoptosis by transforming events⁷ (fig 1).

The lack of expression of functional surface immunoglobulin (B-cell receptor; BCR) is the hallmark of classical HL, and while in some cases this is certainly due to non-functional or destructive rearrangements, other mechanisms can also account for the loss of functional BCR. For example, the loss of immunoglobulin-specific transcription factors, BOB-1, OCT2 and PU.1 in HRS cells has been reported.^{8–11} It has also been shown that, in a minority of cases, HRS cells carry mutations in the octamer region of the immunoglobulin gene promoter, which can prevent binding of Ig-specific transcription factors.^{12–13} Epigenetic silencing can also suppress Ig transcription in HRS cells.¹⁴

Microarray profiling has revealed the down-regulation in classical HL of B-cell lineage gene expression, including many components of BCR signalling.¹⁵ *ID2*, which causes a global down-regulation of B-cell genes by directly interacting and negatively regulating B-cell specific transcription factors, such as E2A and PAX5, is strongly and uniformly expressed in HRS cells. Amplification or genomic gain of the *ID2* locus has been reported in 50% of patients with HL and may account for its over-expression in some cases.^{16–17} Activation of *NOTCH1*, which is expressed by HRS cells, can also suppress the B-cell phenotype¹⁸ and could contribute to the characteristic loss of B-cell identity. It has been suggested that the down-regulation of B-cell identity might allow HRS cells or their progenitors to escape the apoptosis that should occur in the absence of functional BCR, though there is no direct experimental evidence in support of this at present.¹⁹

Occasionally, HL tumours express T-cell antigens, including granzyme B and T-cell intracellular antigen (TIA)-1. In some cases this has been shown to represent aberrant expression of T-cell antigens by HRS cells that show evidence of *IGH* gene rearrangement and are thus assumed to be B cell in origin.²⁰ However, in the same study a single HL case showed expression of T-cell markers and

Hodgkin lymphoma (HL) is characterised by the disruption of normal lymph node architecture and the presence of a minority of malignant Hodgkin/Reed–Sternberg (HRS) cells amid a background of non-neoplastic cell populations comprising T- and B-lymphocytes and other cell types.¹ HRS cells and their reactive neighbouring cells cross-talk via a complex of cytokine and cell contact dependent interactions; these probably include proliferative and anti-apoptotic signals favouring tumour cell survival and expansion.²

The Revised European American Lymphoma (REAL)/World Health Organization (WHO) lymphoma classification³ divides HL into two major types: classical and nodular lymphocyte predominant HL (NLPHL) (table 1). Classical HL is further separated into four subtypes: nodular sclerosis (NS), mixed cellularity (MC), lymphocyte depletion (LD) and a newly defined entity known as “lymphocyte rich classical” (LRC) HL. NLP and classical HL are separated on the basis of morphological, immunophenotypic and clinical differences.⁴ For example, HRS cells of NLPHL have different morphology, being referred to as lymphocytic and histiocytic (L&H) cells; they rarely express the classical HL markers CD15 or CD30, but regularly express B-cell antigens such as CD20 and CD19, which are usually absent in HRS cells of classical HL.⁵

ORIGIN OF HRS CELLS

Early phenotypic studies suggested that HRS cells might be derived from macrophages, dendritic reticulum cells or granulocytes. However, the identification of clonally rearranged immunoglobulin (Ig) genes by PCR analysis of single HRS cells micromanipulated from HL tissues provided evidence not only of their malignant character but also of their origin from B cells.⁶ Tumour cells of both HL types carry somatic *IGH* mutations in the

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Table 1 Comparison of classical and nodular lymphocyte predominant Hodgkin lymphoma

REAL/WHO classification	Morphology/immunophenotype of HRS cells	EBV association	Ig status
Classical Hodgkin lymphoma Nodular sclerosis Mixed cellularity Lymphocyte depletion Lymphocyte-rich	Typical HRS cells which are CD15+, CD20-, CD30+, CD45-	Positive or negative	Lack BCR expression Destructive or non-functional <i>IGH</i> rearrangements or loss of Ig-specific transcription factors
Nodular lymphocyte-predominant Hodgkin lymphoma	Atypical "popcorn" cells which are CD15-, CD20+, CD30-, CD45+,	Negative	Express BCR Functional rearrangements of Ig genes Evidence of intraclonal diversity indicating ongoing somatic hypermutation

REAL/WHO, Revised European American Lymphoma (REAL)/World Health Organization; HRS, Hodgkin/Reed-Sternberg; EBV, Epstein-Barr virus; BCR, B-cell receptor.

also TCR gene rearrangements, indicating that at least a minority of HL tumours are genuinely of T-cell origin.

EBV AND HODGKIN LYMPHOMA

EBV is a ubiquitous human herpesvirus that infects over 90% of humans and persists for the lifetime of the person.²¹ First evidence that EBV might be involved in the pathogenesis of HL was provided by the detection of raised antibody titres to EBV antigens in HL patients when compared with other lymphoma patients,²² and furthermore, that these raised levels preceded the development of HL by several years.²³ With the advent of cloned viral probes and Southern blot hybridisation methods, EBV DNA was initially detected in 20–25% of HL.²⁴ In situ hybridisation provided the first demonstration of the existence of viral DNA in the HRS cells.^{25–26} Subsequently, the demonstration of the abundant EBV-encoded RNAs (EBER1 and EBER2) in HRS cells provided a sensitive method for detecting latent infection in situ. This technique is now generally accepted as the "gold standard" for the detection of latent EBV infection in clinical samples²⁷ (fig 2).

In EBV-associated HL, viral genomes are found in monoclonal form, indicating that infection of the tumour cells occurred prior to their clonal expansion.²⁵ In the majority of cases, EBV persists throughout the course of HL and is also found in multiple sites of HL.²⁸

Although most EBV infections in children are asymptomatic or cause non-specific symptoms, when primary infection is postponed until adolescence it can result in infectious mononucleosis (IM) in 50% of patients.²¹ The relative risk of developing HL in individuals with a history of IM, relative to those with no prior history, was shown to range between 2.0 and 5.0.²⁹ It has recently been shown that the risk of EBV-positive HL is increased four-fold after IM, whereas the risk of EBV-negative HL is not increased.^{30–31}

The possibility that EBV may contribute to the pathogenesis of HL early in the transformation of the progenitor cells but is subsequently lost ("hit and run"), prompted the search for evidence of defective rearranged EBV DNA in tumours that by conventional testing (eg by detection of the EBERs) are virus negative. Such defective rearranged viral DNA has been previously detected in some cases of virus-negative sporadic Burkitt lymphoma,³² and could result in partial elimination of EBV episomes from infected cells through the expression of BZLF1. In support of this possibility, Gan *et al* amplified sequences that span abnormally juxtaposed BamHIW and Z fragments (which characterise defective heterogeneous EBV DNA) from 2/24 EBER-negative HL tumours in which the standard viral genome could not be detected.³³ However, others using fluorescence in situ hybridisation (FISH) found no evidence of integrated EBV genomes in EBV-negative HL.³⁴ Furthermore, using quantitative PCR assays that spanned the

whole genome, no evidence of deletion of EBV genomes in EBV-positive HL, or retention of EBV genomes in EBV-negative HL tissues, was found.³⁵ Therefore, it seems unlikely that EBV contributes to the development of EBV-negative HL.

The rate of detection of EBV in HL depends on factors such as country of residence, histological subtype, sex, ethnicity and age. EBV-positive HL is less common in developed populations, with percentages of 20–50% for North American and European cases,^{27–38} 57% for HL in China,³⁹ but much higher rates in underdeveloped countries.^{40–41} The increased incidence of EBV-positive HL in underdeveloped countries could be due to the existence of an underlying immunosuppression similar to that observed for African Burkitt lymphoma in a malaria-infected population. This is supported by higher EBV-positive rates in HL from HIV-infected patients.⁴² Alternatively, the timing of EBV infection (which is likely to occur earlier in developing countries) might also be important.

EBV is more commonly associated with the MC subtype and less frequently with the other subtypes.^{43–44} Furthermore, it is now generally accepted that NPLHL is an EBV-negative disease.⁴⁵ HL in the older age groups and in children, especially boys under 10 years, has been shown to be more likely to be EBV-associated than HL in young adults.^{46–48} This has led to the suggestion that HL consists of three disease entities: HL of childhood (EBV-positive, MC type); HL of young adults (EBV-negative, NS type); and HL of older adults (EBV-positive, MC type).⁴⁸ The higher proportion of EBV associated HL cases in older adults, is probably due to reduced immunosurveillance and increased viral reactivation occurring as a consequence of advancing years.⁴⁹ The infrequent association of EBV with HL in young adulthood prompted the suggestion that a second virus might be involved, although there is little direct evidence to support this at present.^{50–52} EBV-positive rates are generally higher in males than in females. EBV-positive HL also affects more Asians and Hispanics than whites or blacks,⁴⁶ and in the UK is more common in South Asian children compared with non-South Asian children.⁵³

TRANSFORMING EVENTS IMPLICATED IN HRS PATHOGENESIS

A number of cell signalling pathways are known to be aberrantly activated in HL. The PI3K/Akt pathway was recently shown to be constitutively activated in HL-derived cell lines and in HRS cells from primary tumour tissues, where it was shown to contribute to the survival of these cells.⁵⁴ The active phosphorylated form of ERK is also aberrantly expressed in cultured and primary HL cells; inhibition of the upstream MEK kinase inhibited the phosphorylation of ERK and the proliferation of HL cell lines.⁵⁵ Kube *et al* have demonstrated the constitutive activation of STAT3 in HRS cells.⁵⁶ STAT6 and STAT5a constitutive activation has also been reported.^{57–58}

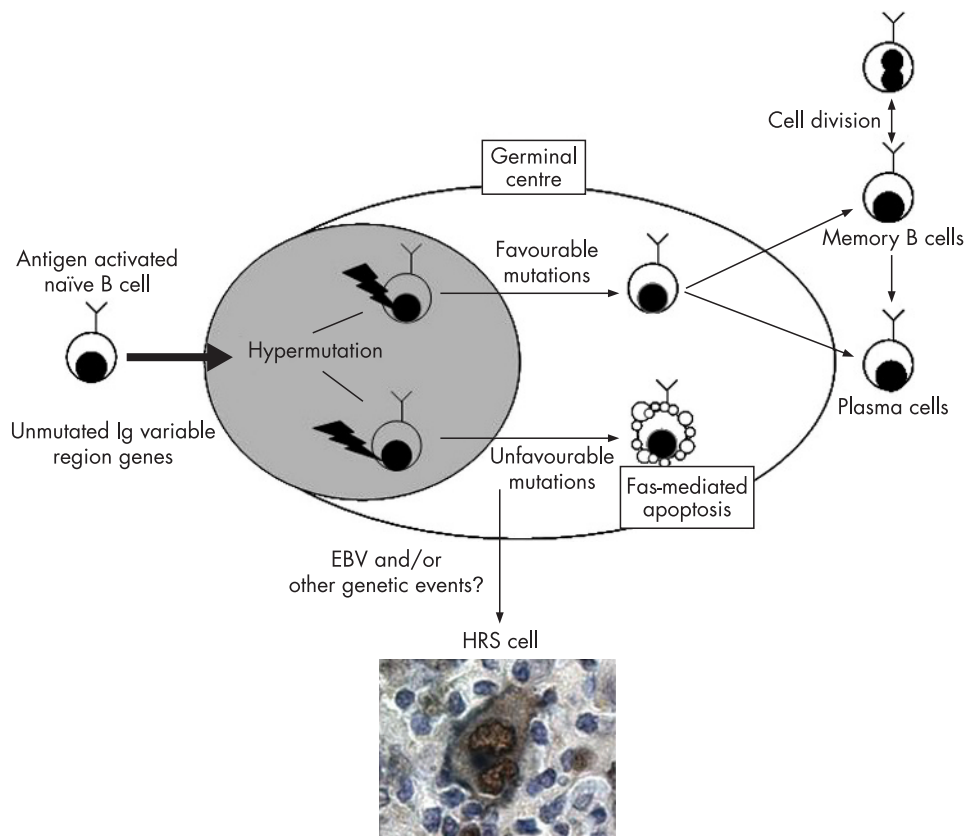


Figure 1 Hodgkin/Reed-Sternberg (HRS) cells may originate from pre-apoptotic germinal centre B cells. Naïve B cells are activated when they encounter cognate antigen. Activated B cells then migrate into B-cell follicles, proliferate and differentiate into centroblasts, thus establishing germinal centres. Germinal centre (GC) B cells undergo somatic hypermutation of the V region genes; cells with unfavourable mutations are eliminated by Fas-mediated apoptosis, whereas those carrying B-cell receptor (BCR) with high affinity for antigen will survive and leave the germinal centre as memory B cells or plasma cells. GC B cells carrying non-functional BCR genes should undergo apoptosis, but may be rescued by Epstein-Barr virus and/or still unknown genetic alterations. Such cells may be the progenitors of HRS cells.

Amplification of the *JAK2* locus is seen in some cases, providing a mechanistic explanation for the STAT activation.⁵⁹ Furthermore, HRS cells also have constitutively activated AP-1 with c-Jun and JunB overexpression.⁶⁰ AP-1 activation was also observed in anaplastic large cell lymphoma (ALCL), but not in other lymphoma types. Constitutive activation of NF- κ B is a regular feature of HRS cells; inhibition of this pathway in HL cell lines leads to their increased sensitivity to apoptosis after growth factor withdrawal and impaired tumourigenicity in severe combined immunodeficiency (SCID) mice.^{61–62}

Aberrant tyrosine kinase activity caused by various genetic alterations, such as activating point mutations, translocations or amplifications, frequently causes cellular transformation and has been observed in many different cancers. In mature B cells, several intracellular protein tyrosine kinases are essential for BCR signalling, whereas the expression of receptor tyrosine kinases (RTKs) is more limited; only expression of TRKA (tyrosine kinase receptor A) and MET have been described.^{63–65} In contrast, HL cells display aberrant activation of several RTKs, including PDGFRA, EPHB1, RON, TRKB, and TRKA, in the absence of activating mutations.⁶⁶ Recently, it has been shown that aberrant tyrosine activity is more frequent in EBV-negative HRS cells, suggesting that RTK signalling might partially replace the effects of EBV in HL pathogenesis.⁶⁷

Under normal circumstances HRS cell progenitors lacking a functional BCR should be eliminated by Fas (CD95)-mediated apoptosis in the germinal centre (GC). *In vitro* studies performed in our own laboratory and by others have demonstrated that HL-derived cell lines are resistant to Fas-mediated

apoptosis.^{68–71} It is well established that HRS cells express Fas,^{70–72–73} and the rare occurrence of mutations in the *FAS* gene suggests that the general resistance of HRS cells to Fas-induced death is due to defects further downstream in the death receptor pathway.^{74–75} c-FLIP is a proximal negative regulator of CD95-induced apoptosis that interferes with the formation of the death inducing signalling complex (DISC) required for death receptor induced death. A number of studies have shown that c-FLIP is highly expressed in HL-derived cell lines and primary HRS cells.^{68–69–71–75} We showed that c-FLIP down-regulation was accompanied by the spontaneous death of HL cells, even in the absence of challenge with Fas agonistic antibody, CH11.⁷⁶ We also showed that HRS cells express Fas ligand (FasL); simultaneous knockdown of FasL and c-FLIP expression did not cause HRS cell death, demonstrating that endogenous FasL could initiate death receptor signalling when c-FLIP was silenced. Therefore, the down-regulation of c-FLIP unmasks a mechanism whereby HRS cells undergo self-induced Fas-mediated death. It should be noted that in a similar study, Mathas *et al* observed only a small increase in apoptosis following c-FLIP knockdown alone, but much greater cell death after the addition of either CH11 or TRAIL following c-FLIP knockdown.⁶⁹ Taken together, these data suggest that abrogation of c-FLIP expression might have therapeutic benefit in HL. HRS cells also have other defects in the apoptotic machinery. For example, they are defective in caspase 3-mediated apoptosis because of their high level expression of XIAP, an inhibitor of apoptosis that binds and inhibits the proteolytic activity of caspases-3, -7 and -9.⁷⁷

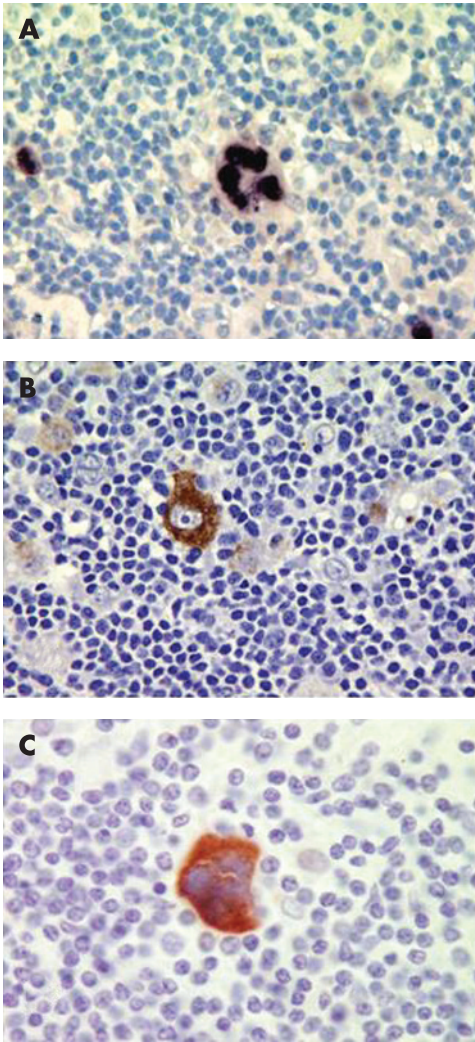


Figure 2 Gene expression in Epstein–Barr virus (EBV) associated Hodgkin lymphoma. (A) EBER expression (brown staining) in the nuclei of Hodgkin/Reed–Sternberg (HRS) cells. In situ hybridisation for EBER expression is the most reliable and sensitive method to detect the presence of latent EBV infection in clinical samples. (B) The latent membrane protein-1 (LMP1) and (C) LMP2 are both highly expressed in EBV-positive HRS cells. Not shown is the consistent expression of the EBV maintenance protein, EBNA1.

CONTRIBUTION OF EBV GENES TO THE PATHOGENESIS OF EBV-POSITIVE HODGKIN LYMPHOMA

EBV-positive HRS cells exhibit a type II form of virus latency, virus gene expression being limited to the EBERS, Epstein–Barr nuclear antigen-1 (EBNA1),⁷⁸ latent membrane protein 1 (LMP1),^{43–44, 79} LMP2,^{79–80} (fig 2), and the Bam H1A rightward transcripts (BARTs).⁷⁹ EBV may contribute to the pathogenesis of HL before the HRS cell progenitor leaves the GC because it has been shown to rescue BCR-negative human tonsillar GC cells from apoptosis, and because HRS cells with crippling *IGH* mutations are almost exclusively found in EBV-positive patients.^{81–85}

LMP1 induces many of the phenotypic changes observed in EBV-infected B cells, including expression of the B-cell activation markers, CD23 and CD40, interleukin (IL)-10 production and up-regulation of cell adhesion molecules such as ICAM1, LFA1 and LFA3. LMP1 also protects B cells from cell death by the up-regulation of several anti-apoptosis genes including *BCL2*, *MCL1* and *A20*.^{86–89} LMP1 functions as a constitutively activated member of the tumour necrosis factor receptor (TNFR) superfamily activating cell signalling pathways

in a ligand-independent manner. LMP1 can engage the MAP kinase cascade resulting in the activation of ERK, JNK and p38 and can stimulate the JAK/STAT pathway.^{90–94} Two distinct functional domains referred to as C-terminal activation regions 1 and 2 (CTAR1 and CTAR2) have been identified on the basis of their ability to activate these signalling pathways. CTAR1 activates NF- κ B mainly through the non-canonical pathway (TRAF3/NIK/IKK- α). In contrast, CTAR2 activates the canonical pathway by utilising TRAF6 and TAK1 to activate IKK- β .⁹⁵ Although NF- κ B activation can be induced by LMP1 in EBV-positive HRS cells, other routes to its activation must exist in EBV-negative cases. IKK β mutations have been reported in HL and might be more frequent in EBV-negative cases.^{96–99} Amplification of the NF- κ B/RelA locus at 2p13-16 is also frequent in HL.^{100–101}

LMP1 can induce expression of DNA methyltransferases and mediate hypermethylation of the E-cadherin promoter in epithelial cells.^{102–103} We have recently shown that LMP1 can up-regulate the Polycomb gene, *BMI1*, in HL cells.¹⁰⁴ Bmi-1 is a component of the polycomb repressive complex, PRC1; it is required for the initiation of gene silencing and induces lymphoid proliferation and the development of lymphomas in transgenic mice.¹⁰⁵ We showed that *BMI1* and LMP1 can regulate a shared subgroup of HL-associated genes, including the *ATM* tumour suppressor.¹⁰⁴ Therefore, it is likely that EBV mediates some its transforming effects through Bmi-1.

The identification that a virus strain carrying a 30 bp deletion in the LMP1 gene was more tumourigenic than the prototype B95.8 LMP1¹⁰⁶ led to numerous studies of the prevalence of this virus strain within EBV-associated cancers, including HL. In general, virus strains carrying this 30 bp deletion occur with a similar frequency in virus-positive tumour patients and in healthy donors from the same geographical region.¹⁰⁷ The exception to this is HL,¹⁰⁸ where some studies have shown an increased incidence of this deletion variant in HIV-positive HL compared to HIV-negative HL,¹⁰⁹ and in paediatric HL compared to normal controls.¹¹⁰

LMP2A is also highly expressed in EBV-positive HRS cells.⁷⁹ LMP2A was shown to compete for the binding of the Src and Syk protein tyrosine kinases, thereby blocking BCR signalling and induction of lytic cycle in B cells.¹¹¹ Paradoxically, expression of LMP2A in the B cells of transgenic mice abrogates normal B-cell development, allowing immunoglobulin-negative cells to colonise peripheral lymphoid organs,¹¹² suggesting that LMP2A can provide a BCR-like signal. LMP2A expression in transgenic mouse B cells down-regulates expression of many of the B-cell lineage genes that are absent or expressed at low levels in HRS cells (eg early B cell factor, PU.1, CD19, CD20).¹¹³ In addition, LMP2A expression induces the up-regulation of genes involved in proliferation (eg MKI67, *PCNA*), protection from apoptosis (*BCLXL*, *BIRC5* (survivin)) and suppression of cell-mediated immunity (eg *IL13R*, *EBI3*).¹¹³

We recently showed that EBV infection of HL cells up-regulates autotaxin, a secreted tumour-associated factor with lysophospholipase-D activity. Up-regulation of autotaxin increased the generation of lysophosphatidic acid (LPA) and led to the enhanced growth and survival of Hodgkin lymphoma cells.¹¹⁴ This is the first demonstration that virus infection leads directly to the synthesis of the growth-promoting lipid LPA and could represent a more general pathway utilised by herpesviruses during their normal life cycle or during the initiation and maintenance of virus-associated tumours. At this time it is unknown which EBV gene is responsible for this effect.

Studies of B cell subsets from healthy carriers have revealed that EBV persists in the peripheral blood in IgD-negative memory B cells (CD19+, CD23–, CD80/B7–) where viral protein expression may be restricted to LMP2A.¹¹⁵ Following

primary infection of naïve or memory B cells, the virus expresses the EBNA2-dependent lymphoblastoid growth programme. The detection of both LMP1 and LMP2 in purified tonsillar memory B cells and germinal centre B cells¹¹⁶ suggests that these viral proteins, through surrogate T-cell help (LMP1) and BCR engagement (LMP2A), provide the necessary signals for EBV-infected B cells to undergo antigen-independent proliferation in the germinal centre, in turn leading to replenishment of the pool of EBV-positive memory B cells. However, the recent demonstration that EBV-positive memory B cells are antigen selected suggests that these cells do not arise from a B-cell differentiation programme driven only by the virus. In this context LMP1 and LMP2 expression might favour the selection of EBV-infected B cells in the highly competitive antigen-dependent environment of the GC.¹¹⁷ Together with as yet undefined cellular alterations, LMP1 and LMP2 could also favour the neoplastic transformation of GC B cells, leading to the development of EBV-positive HL.

Although this model is appealing, other studies suggest that the influence of LMP1 during B-cell differentiation is more complicated. LMP1 expression cannot be detected within GC of lymphoid tissues by immunohistochemistry¹¹⁸ and has been shown to induce extra-follicular B-cell differentiation but not GC formation.¹¹⁹ Therefore, it is possible that EBV accesses the memory-B-cell pool following an extra-follicular B-cell maturation programme which is driven by LMP1, and not through the conventional GC stage. CD40 can control the exit of B cells from the GC, and inhibit their further differentiation into plasma cells.¹²⁰ If LMP1 has similar effects on GC B-cell differentiation, an LMP1-expressing post-GC/pre-plasma cell may emerge from which the HRS cell develops. This would be consistent with the finding that HRS cells often express some of the markers of plasma cell differentiation; and also with the observation that the completion of plasma cell differentiation may be prejudicial to the maintenance of viral latency because it can result in the reactivation of viral replication.¹²¹

FAILURE OF THE IMMUNE RESPONSE TO ELIMINATE EBV-INFECTED HRS CELLS AND ATTEMPTS TO DEVELOP IMMUNOTHERAPEUTIC STRATEGIES

LMP2A and LMP1 are targets for cytotoxic T lymphocytes (CTLs) in association with different MHC class I restriction elements *in vitro*.^{122–123} *In vitro* HL cells can process and present epitopes from LMP1 and LMP2A in the context of multiple class I alleles and are sensitive to lysis by EBV-specific CTLs.^{131–132} However, EBV-infected HRS cells survive *in vivo*. HRS cells express the thymus and activated regulated chemokine (TARC), IL-10, IL-13 and TGF β which could counteract EBV-specific CTL responses.^{124–127} Surprisingly, EBV-positive cases of HL have been shown to contain more activated CTLs and express higher levels of MHC class I than EBV-negative cases.^{128–131}

EBV-specific CTLs can be generated from patients with advanced HL, albeit at lower frequency than normal controls.¹³³ These EBV-specific CTLs survived and had antiviral activity *in vivo*; however, despite the resolution of some symptoms and the stabilisation of disease, all HL patients failed to recover from their disease. Successful CTL therapy for EBV-positive HL will require not only EBV-specific CTL with improved cytotoxicity, but probably also modification of the tumour microenvironment to remove the inhibitory barriers which inhibit CTL function. Antigen presentation systems may improve the cytolytic activity of CTLs. For example, exposure to AdE1-LMPpoly (a replication-deficient adenoviral system which encodes a glycine alanine repeat-deleted EBNA1 covalently linked to multiple CD8⁺ T cell epitopes from LMP1 and LMP2) was effective in expanding T cells specific for LMP1, LMP2A, and EBNA1, across a range of HLA types.¹³⁴ A recombinant

Take-home messages

- Epstein-Barr virus (EBV) is associated with only a proportion of cases of Hodgkin lymphoma (HL), where the virus is present in the tumour cell populations. The virus-negative and virus-positive forms of the disease are morphologically similar but appear to differ in terms of their precise underlying molecular changes.
- EBV contributes to the aberrant activation of key signalling pathways in HL; understanding how EBV subverts cellular physiology can also inform our understanding of EBV-negative HL.
- The development of EBV-associated HL may be regarded as an unintentional consequence of the normal viral life cycle which is designed to enable EBV to persist in the B cell pool of normal carriers.
- Understanding the mechanisms by which EBV transforms cells and escapes the immune system will be important for the development of strategies to treat patients with EBV-associated malignancies.

poxvirus vaccine encoding a polypeptide protein comprising six HLA A2 restricted epitopes derived from LMP1 ("LMP1 polypeptide vaccine"), can generate stronger anti-LMP1 responses.¹³⁶ CTLs generated by exposure to LMP2A-expressing dendritic cells demonstrate increased cytotoxicity.¹³⁵

There are a number of possibilities to alleviate the CTL inhibitory effect of the microenvironment,^{137–138} but the depletion of regulatory T cells (Tregs) is likely to be of prime importance. Recent studies show that both tumour infiltrating lymphocytes and peripheral blood mononuclear cells from HL patients contain many Tregs.¹³⁹ A recent study showed that Tregs which express LAG-3 are more frequent in EBV-positive compared with EBV-negative HL.¹³⁹ Furthermore, the level of LAG-3 expression on the tumour-infiltrating lymphocytes was coincident with impairment of T-cell activity against LMP1 and LMP2, whereas deletion of CD4⁺ LAG-3⁺ T-cells enhanced LMP-specific immunity.

SUMMARY

A proportion of HL tissues harbour EBV within tumour cells. Superficially at least, there are surprisingly few differences between EBV-positive and EBV-negative HRS cells. Emerging evidence suggests that while EBV is able to subvert cellular processes in favour of growth and survival, cellular genetic events are required to do this when EBV is absent. The challenge is to unravel this complexity by detailed consideration of the function of latent EBV genes in the appropriate cellular context. It is hoped that this approach will reveal more fundamental aspects of HL pathogenesis and pave the way for more targeted therapies for HL patients.

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REFERENCES

- 1 Harris NL, Jaffe ES, Stein H, *et al*. A Revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994;**84**:1361–92.

- 2 Pinto A, Gattei V, Zagonel V, *et al.* Hodgkin's disease: a disorder of dysregulated cellular cross-talk. *Biotherapy* 1998;**10**:309–20.
- 3 Harris NL, Jaffe ES, Diebold J, *et al.* The World Health Organization Classification of Neoplastic Diseases of the Hematopoietic and Lymphoid Tissues. *Ann Oncol* 1999;**10**:1419–32.
- 4 Küppers R, Yahalom J, Josting A. Advances in biology, diagnostics, and treatment of Hodgkin's disease. *Biol Blood Marrow Transplant* 2006;**12**:66–76.
- 5 Buettner M, Greiner A, Avramidou A, *et al.* Evidence of abortive plasma cell differentiation in Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma. *Hematol Oncol* 2005;**23**:127–32.
- 6 Küppers R, Rajewsky K, Zhao M, *et al.* Hodgkin disease: Hodgkin and Reed-Sternberg cells picked from histological sections show clonal immunoglobulin gene rearrangements and appear to be derived from B cells at various stages of development. *Proc Natl Acad Sci USA* 1994;**91**:10962–6.
- 7 Kanzler H, Kuppers R, Hansmann ML, *et al.* Hodgkin and Reed-Sternberg cells in Hodgkin's disease represent the outgrowth of a dominant tumor clone derived from (crippled) germinal center B cells. *J Exp Med* 1996;**184**:1495–505.
- 8 Re D, Muschen M, Ahmadi T, *et al.* Oct-2 and Bob-1 deficiency in Hodgkin and Reed-Sternberg cells. *Cancer Res* 2001;**61**:2080–4.
- 9 Jundi F, Kley K, Anagnostopoulos I, *et al.* Loss of PU.1 expression is associated with defective immunoglobulin transcription in Hodgkin and Reed-Sternberg cells of classical Hodgkin disease. *Blood* 2002;**99**:3060–2.
- 10 Torlakovic E, Tierens A, Dang HD, *et al.* The transcription factor PU.1, necessary for B-cell development is expressed in lymphocyte predominance, but not classical Hodgkin's disease. *Am J Pathol* 2001;**159**:1807–14.
- 11 Marafioti T, Hummel M, Foss H-D, *et al.* Hodgkin and Reed-Sternberg cells represent an expansion of a single clone originating from a germinal center B-cell with functional immunoglobulin gene rearrangements but defective immunoglobulin transcription. *Blood* 2000;**95**:1443–50.
- 12 Jox A, Zander T, Kuppers R, *et al.* Somatic mutations within the untranslated regions of rearranged Ig genes in a case of classical Hodgkin's disease as a potential cause for the absence of Ig in the lymphoma cells. *Blood* 1990;**93**:3964–72.
- 13 Theil J, Laumen H, Marafioti T, *et al.* Defective octamer-dependent transcription is responsible for silenced immunoglobulin transcription in Reed-Sternberg cells. *Blood* 2001;**97**:3191–6.
- 14 Ushmorov A, Ritz O, Hummel M, *et al.* Epigenetic silencing of the immunoglobulin heavy-chain gene in classical Hodgkin lymphoma-derived cell lines contributes to the loss of immunoglobulin expression. *Blood* 2004;**104**:3326–34.
- 15 Schwering I, Brauning A, Klein U, *et al.* Loss of the B-lineage-specific gene expression program in Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma. *Blood* 2003;**101**:1505–12.
- 16 Renne C, Martin-Subero JI, Eickernjager M, *et al.* Aberrant expression of ID2, a suppressor of B-cell specific gene expression in Hodgkin's lymphoma. *Am J Pathol* 2006;**169**:655–64.
- 17 Mathas S, Janz M, Hummel F, *et al.* Intrinsic inhibition of transcription factor E2A by HLH proteins ABF-1 and Id2 mediates reprogramming of neoplastic B cells in Hodgkin lymphoma. *Nat Immunol* 2006;**7**:207–15.
- 18 Radtke F, Wilson A, Mancini SJ, *et al.* Notch regulation of lymphocyte development and function. *Nat Immunol* 2004;**5**:247–53.
- 19 Kuppers R, Hansmann M. The Hodgkin and Reed/Sternberg cell. *Int J Biochem Cell Biol* 2005;**37**:511–7.
- 20 Müschen M, Rajewsky K, Bräuninger A, *et al.* Rare occurrence of classical Hodgkin's disease as a T cell lymphoma. *J Exp Med* 2000;**191**:387–94.
- 21 Cohen JL. Epstein-Barr virus infection. *N Engl J Med* 2000;**343**:481–92.
- 22 Levine PH, Ablashi DV, Berard CW, *et al.* Elevated antibody titers to Epstein-Barr virus in Hodgkin's disease. *Cancer* 1971;**27**:416–21.
- 23 Mueller N, Evans A, Harris NL, *et al.* Hodgkin's disease and Epstein-Barr virus: altered antibody pattern before diagnosis. *N Engl J Med* 1989;**320**:689–95.
- 24 Weiss LM, Strickler JG, Warnke RA, *et al.* Epstein-Barr viral DNA in tissues of Hodgkin's disease. *Am J Pathol* 1987;**129**:86–91.
- 25 Anagnostopoulos I, Herbst H, Niedobitek G, *et al.* Demonstration of monoclonal EBV genomes in Hodgkin's disease and Ki-1 positive anaplastic large cell lymphoma by combined Southern blot and in situ hybridization. *Blood* 1989;**74**:810–6.
- 26 Weiss LM, Movahed LA, Warnke RA, *et al.* Detection of Epstein-Barr viral genomes in Reed-Sternberg cells of Hodgkin's disease. *N Engl J Med* 1989;**320**:502–6.
- 27 Wu TC, Mann RB, Charache P, *et al.* Detection of EBV gene expression in Reed-Sternberg cells of Hodgkin's disease. *Int J Cancer* 1990;**46**:801–4.
- 28 Coates PJ, Slavin G, D'Ardenne AJ. Persistence of Epstein-Barr virus in Reed-Sternberg cells throughout the course of Hodgkin's disease. *J Pathol* 1991;**164**:291–7.
- 29 Gutensohn N, Cole P. Epidemiology of Hodgkin's disease. *Semin Oncol* 1980;**7**:92–102.
- 30 Hjalgrim H, Asklung J, Rostgaard K, *et al.* Characteristics of Hodgkin's lymphoma after infectious mononucleosis. *N Engl J Med* 2003;**349**:1324–32.
- 31 Hjalgrim H, Ekstrom Smedby K, Rostgaard K, *et al.* Infectious mononucleosis, childhood social environment and risk of Hodgkin lymphoma. *Cancer Res* 2007;**67**:2382–8.
- 32 Razzouk BI, Srinivas S, Sample CE, *et al.* Epstein-Barr virus DNA recombination and loss in sporadic Burkitt's lymphoma. *J Infect Dis* 1996;**173**:529–35.
- 33 Gan YJ, Razzouk BI, Su T, *et al.* A defective, rearranged Epstein-Barr virus genome in EBV-negative and EBV-positive Hodgkin's disease. *Am J Pathol* 2002;**160**:781–6.
- 34 Staratschek-Jox A, Kotkowski S, Belge G, *et al.* Detection of Epstein-Barr virus in Hodgkin-Reed-Sternberg cells: no evidence for the persistence of integrated viral fragments in latent membrane protein-1 (LMP-1)-negative classical Hodgkin's disease. *Am J Pathol* 2000;**156**:209–16.
- 35 Gallagher A, Perry J, Freeland J, *et al.* Hodgkin lymphoma and Epstein-Barr virus (EBV): no evidence to support hit-and-run mechanism in cases classified as non-EBV-associated. *Int J Cancer* 2003;**104**:624–30.
- 36 Weiss LM, Chen Y-Y, Liu X-F, *et al.* Epstein-Barr virus and Hodgkin's disease: a correlative in situ hybridization and polymerase chain reaction study. *Am J Pathol* 1991;**139**:1259–65.
- 37 Herbst H, Steinbrecher E, Niedobitek G, *et al.* Distribution and phenotype of Epstein-Barr virus-harboring cells in Hodgkin's disease. *Blood* 1992;**80**:484–91.
- 38 Hummel M, Anagnostopoulos I, Dallenbach F, *et al.* EBV infection patterns in Hodgkin's disease and normal lymphoid tissue: expression and cellular localization of gene products. *Brit J Haematol* 1992;**82**:689–94.
- 39 Zhou X-G, Hamilton-Dutoit SJ, Yan Q-H, *et al.* The association between Epstein-Barr virus and Chinese Hodgkin's disease. *Int J Cancer* 1993;**55**:359–63.
- 40 Chang KL, Albuja PF, Chen Y-Y, *et al.* High prevalence of Epstein-Barr virus in the Reed-Sternberg cells of Hodgkin's disease occurring in Peru. *Blood* 1993;**81**:496–501.
- 41 Weinreb M, Day PJR, Niggl F, *et al.* The consistent association between Epstein-Barr virus and Hodgkin's disease in children in Kenya. *Blood* 1996;**87**:3828–36.
- 42 Uccini S, Monardo F, Stoppacciaro A, *et al.* High frequency of Epstein-Barr virus-genome detection in Hodgkin's disease of HIV-positive patients. *Int J Cancer* 1990;**46**:581–5.
- 43 Pallesen G, Hamilton-Dutoit SJ, Rowe M, *et al.* Expression of Epstein-Barr virus latent gene products in tumour cells of Hodgkin's disease. *Lancet* 1991;**337**:320–2.
- 44 Murray PG, Young LS, Rowe M, *et al.* Immunohistochemical demonstration of the Epstein-Barr virus-encoded latent membrane protein in paraffin sections of Hodgkin's disease. *J Pathol* 1992;**166**:1–5.
- 45 Chan WC. Cellular origin of nodular lymphocyte-predominant Hodgkin's lymphoma: immunophenotypic and molecular studies. *Semin Hematol* 1999;**36**:242–52.
- 46 Glaser SL, Lin RJ, Stewart SL, *et al.* Epstein-Barr virus-associated Hodgkin's disease: epidemiologic characteristics in international data. *Int J Cancer* 1997;**70**:375–82.
- 47 Jarrett RF, Gallagher A, Jones DB, *et al.* Detection of Epstein-Barr virus genomes in Hodgkin's disease: relation to age. *J Clin Pathol* 1991;**44**:844–8.
- 48 Armstrong AA, Alexander FE, Cartwright R, *et al.* Epstein-Barr virus and Hodgkin's disease: further evidence for the three disease hypothesis. *Leukemia* 1998;**12**:1272–6.
- 49 Khan G, Lake A, Shield L, *et al.* Phenotype and frequency of Epstein-Barr virus-infected cells in pre-treatment blood samples from patients with Hodgkin lymphoma. *Br J Haematol* 2005;**129**:511–9.
- 50 Wilson KS, Gallagher A, Freeland JM, *et al.* Viruses and Hodgkin lymphoma: no evidence of polyomavirus genomes in tumor biopsies. *Leuk Lymphoma* 2006;**47**:1315–21.
- 51 Gallagher A, Perry J, Shield L, *et al.* Virus and Hodgkin disease: no evidence of novel herpesviruses in non-EBV-associated lesions. *Int J Cancer* 2002;**101**:259–64.
- 52 Armstrong AA, Shield L, Gallagher A, *et al.* Lack of involvement of known oncogenic DNA viruses in Epstein-Barr virus-negative Hodgkin's disease. *Br J Cancer* 1998;**77**:1045–7.
- 53 Flavell KJ, Biddulph JP, Powell JE, *et al.* South Asian ethnicity and material deprivation increase the risk of Epstein-Barr virus infection in childhood Hodgkin's disease. *Br J Cancer* 2001;**85**:350–6.
- 54 Dutton A, Reynolds GM, Dawson CW, *et al.* Constitutive activation of phosphatidylinositol 3 kinase contributes to the survival of Hodgkin's lymphoma cells through a mechanism involving Akt kinase and mTOR. *J Pathol* 2005;**205**:498–506.
- 55 Zheng B, Fiumara P, Li YV, *et al.* MEK/ERK pathway is aberrantly active in Hodgkin's disease: a signalling pathway shared by CD30, CD40 and RANK that regulates cell proliferation and survival. *Blood* 2003;**102**:1019–27.
- 56 Kube D, Hollick U, Vockerodt M, *et al.* STAT3 is constitutively activated in Hodgkin cell lines. *Blood* 2001;**98**:762–70.
- 57 Skinnider BF, Elia AJ, Gascoyne RD, *et al.* Signal transducer and activator of transcription 6 is frequently activated in Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma. *Blood* 2002;**99**:618–26.
- 58 Hinz M, Lemke P, Anagnostopoulos I, *et al.* Nuclear factor kappaB-dependent gene expression profiling of Hodgkin's disease tumor cells, pathogenetic significance, and link to constitutive signal transducer and activator of transcription 5a activity. *J Exp Med* 2002;**196**:605–17.
- 59 Joos S, Granzow M, Holtgreve-Grez H, *et al.* Hodgkin's lymphoma cell lines are characterized by frequent aberrations on chromosomes 2p and 9p including REL and JAK2. *Int J Cancer* 2003;**103**:489–95.
- 60 Mathas S, Hinz M, Anagnostopoulos I, *et al.* Aberrantly expressed c-Jun and JunB are a hallmark of Hodgkin lymphoma cells, stimulate proliferation and synergize with NF-kappa B. *EMBO J* 2001;**21**:4104–13.
- 61 Bargou RC, Leng C, Krappmann D, *et al.* High-level nuclear NF-kappa B and Oct-2 is a common feature of cultured Hodgkin/Reed-Sternberg cells. *Blood* 1996;**87**:4340–7.
- 62 Bargou RC, Emmerich F, Krappmann D, *et al.* Constitutive nuclear factor-kappaB-RelA activation is required for proliferation and survival of Hodgkin's disease tumor cells. *J Clin Invest* 1997;**100**:2961–9.
- 63 Kronfeld I, Kazimirsky G, Gelfand EW, *et al.* NGF rescues human B lymphocytes from anti-IgM induced apoptosis by activation of PKC ζ . *Eur J Immunol* 2002;**32**:136–43.

- 64 **Skibinski G**, Skibinska A, James K. The role of hepatocyte growth factor and its receptor *c-met* in interactions between lymphocytes and stromal cells in secondary human lymphoid organs. *Immunology* 2001;**102**:506–14.
- 65 **Torcia M**, Bracci-Laudiero L, Lucibello M, *et al*. Nerve growth factor is an autocrine survival factor for memory B lymphocytes. *Cell* 1996;**85**:345–56.
- 66 **Renne C**, Willenbrock K, Kuppers R, *et al*. Autocrine- and paracrine-activated receptor tyrosine kinases in classic Hodgkin's lymphoma. *Blood* 2005;**105**:4051–9.
- 67 **Renné C**, Hirsch N, Willenbrock K, *et al*. The aberrant coexpression of several receptor tyrosine kinases is largely restricted to EBV-negative cases of classical Hodgkin's lymphoma. *Int J Cancer* 2007;**120**:2504–9.
- 68 **Dutton A**, Burns AT, Young LS, *et al*. Targeting cellular FLICE-like inhibitory protein as a novel approach to the treatment of Hodgkin's lymphoma. *Expert Rev Anticancer Ther* 2006;**6**:911–9.
- 69 **Mathas S**, Lietz A, Anagnostopoulos I, *et al*. C-Flip mediates resistance of Hodgkin/Reed-Sternberg cells to death receptor-induced apoptosis. *J Exp Med* 2004;**199**:1041–52.
- 70 **Re D**, Hofmann A, Wolf J, *et al*. Cultivated H-RS cells are resistant to CD95L-mediated apoptosis despite expression of wild-type CD95. *Exp Hematol* 2000;**28**:31–5.
- 71 **Thomas RK**, Kallenborn A, Wickenhauser C. Constitutive expression of c-FLIP in Hodgkin and Reed-Sternberg cells. *Am J Pathol* 2002;**160**:1521–8.
- 72 **Xerri L**, Carbuca N, Parc P, *et al*. Frequent expression of Fas/APO-1 in Hodgkin's disease and anaplastic large cell lymphomas. *Histopathology* 1995;**27**:235–41.
- 73 **Meikar SS**, Naresh KN, Redkar AA, *et al*. Expression of Fas and Fas ligand in Hodgkin's disease. *Leuk Lymphoma* 1999;**33**:521–30.
- 74 **Muschen M**, Re D, Brauninger A, *et al*. Somatic mutations of the CD95 gene in Hodgkin and Reed-Sternberg cells. *Cancer Res* 2000;**60**:5640–3.
- 75 **Maggio EM**, Van Der Berg A, De Jong D, *et al*. Low frequency of Fas mutations in Reed-Sternberg cells of Hodgkin's lymphoma. *Am J Pathol* 2003;**162**:29–35.
- 76 **Dutton A**, O'Neil JD, Milner AE, *et al*. Expression of the cellular FLICE-inhibitory protein (c-FLIP) protects Hodgkin's lymphoma cells from autonomous Fas-mediated death. *Proc Natl Acad Sci USA* 2004;**101**:6611–6.
- 77 **Kashkar H**, Haefs C, Shin H, *et al*. XIAP-mediated caspase inhibition in Hodgkin's lymphoma-derived B cells. *J Exp Med* 2003;**198**:341–7.
- 78 **Grässer FA**, Murray PG, Kremmer E, *et al*. Monoclonal antibodies directed against the Epstein-Barr virus-encoded nuclear antigen 1 (EBNA 1): immunohistologic detection of EBNA 1 in the malignant cells of Hodgkin's disease. *Blood* 1994;**84**:3792–8.
- 79 **Deacon EM**, Pallesen G, Niedobitek G, *et al*. Epstein-Barr virus and Hodgkin's disease: transcriptional analysis of virus latency in the malignant cells. *J Exp Med* 1993;**177**:339–49.
- 80 **Niedobitek G**, Kremmer E, Herbst H, *et al*. Immunohistochemical detection of the Epstein-Barr virus-encoded latent membrane protein 2A in Hodgkin's disease and infectious mononucleosis. *Blood* 1997;**90**:1664–72.
- 81 **Vockeroth M**, Belge G, Kube D, *et al*. An unbalanced translocation involving chromosome 14 is the probable cause for loss of potentially functional rearranged immunoglobulin heavy chain genes in the Epstein-Barr virus-positive Hodgkin's lymphoma-derived cell line L591. *Br J Haematol* 2002;**119**:640–6.
- 82 **Brauninger A**, Schmitz R, Bechtel D, *et al*. Molecular biology of Hodgkin's and Reed/Sternberg cells in Hodgkin's lymphoma. *Int J Cancer* 2006;**118**:1853–61.
- 83 **Bechtel D**, Kurth J, Unkel C, *et al*. Transformation of BCR-deficient germinal centre B cells by EBV supports a major role of the virus in the pathogenesis of Hodgkin's and posttransplant lymphoma. *Blood* 2005;**106**:4345–50.
- 84 **Chaganti S**, Bell AI, Beque-Pastor N, *et al*. Epstein-Barr virus infection in vitro can rescue germinal centre B cells with inactivated immunoglobulin genes. *Blood* 2005;**106**:4249–52.
- 85 **Mancao C**, Altmann M, Jungnickel B, *et al*. Rescue of 'crippled' germinal centre B cells from apoptosis by Epstein-Barr virus. *Blood* 2005;**106**:4339–44.
- 86 **Henderson S**, Rowe M, Gregory C, *et al*. Induction of bcl-2 expression by Epstein-Barr virus latent membrane protein 1 protects infected B cells from programmed cell death. *Cell* 1991;**65**:1107–15.
- 87 **Laherty CD**, Hu HM, Oipari AW, *et al*. The Epstein-Barr virus LMP1 gene product induces A20 zinc finger protein expression by activating nuclear factor kappa B. *J Biol Chem* 1992;**267**:24157–60.
- 88 **Wang S**, Rowe M, Lundgren E. Expression of the Epstein Barr virus transforming protein LMP1 causes a rapid and transient stimulation of the Bcl-2 homologue Mcl-1 levels in B-cell lines. *Cancer Res* 1996;**56**:4610–3.
- 89 **Rowe M**, Peng-Pilon M, Huen DS, *et al*. Upregulation of bcl-2 by the Epstein-Barr virus latent membrane protein LMP1: a B-cell-specific response that is delayed relative to NF-kappa B activation and to induction of cell surface markers. *J Virol* 1994;**68**:5602–12.
- 90 **Eliopoulos AG**, Gallagher, NJ, Blake SM, *et al*. Activation of the p38 mitogen-activated protein kinase pathway by Epstein-Barr virus-encoded latent membrane protein 1 coregulates interleukin-6 and interleukin-8 production. *J Biol Chem* 1999;**274**:16085–96.
- 91 **Eliopoulos AG**, Young LS. Activation of the cJun N-terminal kinase (JNK) pathway by the Epstein-Barr virus-encoded latent membrane protein 1 (LMP1). *Oncogene* 1998;**16**:1731–42.
- 92 **Gires O**, Kohlhuber F, Kilger E, *et al*. Latent membrane protein 1 of Epstein-Barr virus interacts with JAK3 and activates STAT proteins. *EMBO J* 1999;**18**:3064–73.
- 93 **Roberts ML**, Cooper NR. Activation of a ras-MAPK-dependent pathway by Epstein-Barr virus latent membrane protein 1 is essential for cellular transformation. *Virology* 1998;**240**:93–9.
- 94 **Kieser A**, Kilger E, Gires O, *et al*. Epstein-Barr virus latent membrane protein-1 triggers AP-1 activity via the c-Jun N-terminal kinase cascade. *EMBO J* 1997;**16**:6478–85.
- 95 **Wu L**, Nakano H, Wu Z. The CTAR2 domain of the Epstein-Barr virus-encoded latent membrane protein 1 activated NF-kappa B through TRAF6 and TAK1. *J Biol Chem* 2005;**281**:2162–9.
- 96 **Emmerich F**, Meiser M, Hummel M, *et al*. Overexpression of I kappa B alpha without inhibition of NF-kappaB activity and mutations in the I kappa B alpha gene in Reed-Sternberg cells. *Blood* 1999;**94**:3129–34.
- 97 **Cabannes E**, Khan G, Aillet F, *et al*. Mutations in the I kappa B gene in Hodgkin's disease suggest a tumour suppressor role for I kappa B alpha. *Oncogene* 1999;**18**:3063–70.
- 98 **Jungnickel B**, Staratschek-Jox A, Bräuninger A, *et al*. Clonal deleterious mutations in the I kappa B gene in the malignant cells in Hodgkin's lymphoma. *J Exp Med* 2000;**191**:395–401.
- 99 **Jarrett RF**, Lake A, Andrew L, *et al*. Somatic I kappa B mutations are a frequent occurrence in Hodgkin's lymphoma [abstract]. *Blood* 2002;**100**:4333.
- 100 **Martin-Subero JI**, Gesk S, Harder L, *et al*. Recurrent involvement of the REL and BCL11A loci in classical Hodgkin lymphoma. *Blood* 2002;**99**:1474–7.
- 101 **Barth TF**, Martin-Subero JI, Joos S, *et al*. Gains of 2p involving the REL locus correlate with nuclear c-Rel protein accumulation in neoplastic cells of classical Hodgkin lymphoma. *Blood* 2003;**101**:3681–6.
- 102 **Tsai CN**, Tsai CL, Tse KP, *et al*. The Epstein-Barr virus oncogene product, latent membrane protein 1, induces the down-regulation of E-cadherin gene expression via activation of DNA methyltransferases. *PNAS* 2002;**99**:10084–9.
- 103 **Sparmann A**, van Lohuizen M. Polycomb silencers control cell fate, development and cancer. *Nat Rev Cancer* 2006;**6**:846–56.
- 104 **Dutton A**, Woodman CB, Chukvuma MB, *et al*. Bmi-1 is induced by the Epstein-Barr oncogene LMP1 and regulates the expression of viral target genes in Hodgkin lymphoma cells. *Blood* 2006;**109**:2597–603.
- 105 **Tsai CL**, Li HP, Lu YJ, *et al*. Activation of DNA methyltransferase 1 by EBV LMP1 involves c-Jun NH2-terminal kinase signalling. *Cancer Res* 2006;**66**:11668–76.
- 106 **Hu LF**, Chen F, Zheng X, *et al*. Clonability and tumorigenicity of human epithelial cells expressing the EBV encoded membrane protein LMP1. *Oncogene* 1993;**8**:1575–83.
- 107 **Khanim F**, Yao Q-Y, Niedobitek G, *et al*. Analysis of Epstein-Barr virus gene polymorphisms in normal donors and in virus-associated tumors from different geographic locations. *Blood* 1996;**88**:3491–501.
- 108 **Knecht H**, Bachmann E, Brousset P, *et al*. Deletions within the LMP1 oncogene of Epstein-Barr virus are clustered in Hodgkin's disease and identical to those observed in nasopharyngeal carcinoma. *Blood* 1993;**82**:2937–42.
- 109 **Bellas C**, Santon A, Manzanal A, *et al*. Pathological, immunological, and molecular features of Hodgkin's disease associated with HIV infection comparison with ordinary Hodgkin's disease. *Am J Surg Pathol* 1996;**20**:1520–4.
- 110 **Santon A**, Martin C, Manzanal A, *et al*. Paediatric Hodgkin's disease in Spain: association with Epstein-Barr virus strains carrying latent membrane protein-1 oncogene deletions and high frequency of dual infections. *Br J Haematol* 1998;**103**:129–36.
- 111 **Miller CL**, Burkhardt AL, Lee JH, *et al*. Integral membrane protein 2 of Epstein-Barr virus regulates reactivation from latency through dominant negative effects on protein-tyrosine kinases. *Immunity* 1995;**2**:155–66.
- 112 **Caldwell RG**, Wilson JB, Anderson SJ, *et al*. Epstein-Barr virus LMP2A drives B cell development and survival in the absence of normal B cell receptor signals. *Immunity* 1998;**9**:405–11.
- 113 **Portis T**, Dyck P, Longnecker R. Epstein-Barr Virus (EBV) LMP2A induces alterations in gene transcription similar to those observed in Reed-Sternberg cells of Hodgkin lymphoma. *Blood* 2003;**102**:4166–78.
- 114 **Baumforth KRN**, Flavell JR, Reynolds GM, *et al*. Induction of autotoxin by the Epstein-Barr virus promotes the growth and survival of Hodgkin lymphoma cells. *Blood* 2005;**106**:2138–46.
- 115 **Babcock GJ**, Decker LL, Volk M, *et al*. EBV persistence in memory B cells in vivo. *Immunity* 1998;**9**:395–404.
- 116 **Babcock GJ**, Thorley-Lawson DA. Tonsillar memory B cells, latently infected with Epstein-Barr virus, express the restricted pattern of latent genes previously found only in Epstein-Barr virus-associated tumours. *Proc Natl Acad Sci USA* 2000;**97**:12250–5.
- 117 **Thorley-Lawson DA**. EBV the prototypical human tumor virus—just how bad is it? *J Allergy Clin Immunol* 2005;**116**:251–6.
- 118 **Niedobitek G**, Agathangelou A, Herbst H, *et al*. Epstein-Barr virus (EBV) infection in infectious mononucleosis: virus latency, replication and phenotype of EBV-infected cells. *J Pathol* 1997;**182**:151–9.
- 119 **Uchida J**, Yasui T, Takaoka-Scichijo Y, *et al*. Mimicry of CD40 signals by Epstein-Barr virus LMP1 in B lymphocyte responses. *Science* 1999;**286**:300–3.
- 120 **Basso K**, Klein U, Niu H, *et al*. Tracking CD40 signalling during germinal centre development. *Blood* 2004;**104**:4088–96.
- 121 **Laichalk LL**, Thorley-Lawson DA. Terminal differentiation into plasma cells initiates the replicative cycle of Epstein-Barr virus in vivo. *J Virol* 2005;**79**:1296–307.
- 122 **Khanna R**, Burrows SR, Nicholls J, *et al*. Identification of cytotoxic T cell epitopes within Epstein-Barr virus (EBV) oncogene latent membrane protein 1 (LMP1): evidence for HLA A2 supertype-restricted immune recognition of EBV-infected cells by LMP1-specific cytotoxic T lymphocytes. *Eur J Immunol* 1998;**28**:451–8.
- 123 **Lee SP**, Tierney RJ, Thomas WA, *et al*. Conserved CTL epitopes within EBV latent membrane protein 2: a potential target for CTL-based tumor therapy *J Immunol* 1997;**158**:3325–34.
- 124 **Herbst H**, Foss HD, Samol J, *et al*. Frequent expression of interleukin-10 by Epstein-Barr virus-harboring tumor cells of Hodgkin's disease. *Blood* 1996;**87**:2918–29.

- 125 **Poppema S**, Potters M, Visser L, *et al*. Immune escape mechanisms in Hodgkin's disease. *Ann Oncol* 1998;**9**(Suppl 5):S21-4.
- 126 **Kapp U**, Yeh W-C, Patterson B, *et al*. Interleukin 13 is secreted by and stimulates the growth of Hodgkin and Reed-Sternberg cells. *J Exp Med* 1999;**189**:1939-46.
- 127 **van den Berg A**, Visser L, Poppema S. High expression of the CC chemokine TARC in Reed-Sternberg cells. A possible explanation for the characteristic T-cell infiltrate in Hodgkin's lymphoma. *Am J Pathol* 1999;**154**:1685-91.
- 128 **Oudejans JJ**, Jiwa NM, Kummer JA, *et al*. Analysis of major histocompatibility complex class I expression on Reed-Sternberg cells in relation to the cytotoxic T-cell response in Epstein-Barr virus-positive and -negative Hodgkin's disease. *Blood* 1996;**87**:3844-51.
- 129 **Murray PG**, Constandinou CM, Crocker J, *et al*. Analysis of major histocompatibility complex class I, TAP expression, and LMP2 epitope sequence in Epstein-Barr virus-positive Hodgkin's disease. *Blood* 1998;**92**:2477-83.
- 130 **Oudejans JJ**, Jiwa NM, Kummer JA, *et al*. Activated cytotoxic T cells as prognostic marker in Hodgkin's disease *Blood* 1997;**89**:1376-82.
- 131 **Lee SP**, Constandinou CM, Thomas WA, *et al*. Antigen presenting phenotype of Hodgkin Reed-Sternberg cells: analysis of the HLA class I processing pathway and the effects of interleukin-10 on Epstein-Barr virus-specific cytotoxic T-cell recognition. *Blood* 1998;**92**:1020-30.
- 132 **Sing AP**, Ambinder RF, Hong DJ, *et al*. Isolation of Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes that lyse Reed-Sternberg cells: implications for immune-mediated therapy of EBV+ Hodgkin's disease. *Blood* 1997;**89**:1978-86.
- 133 **Roskrow MA**, Suzuki N, Gan YJ, *et al*. Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes for the treatment of patients with EBV-positive relapsed Hodgkin's disease. *Blood* 1998;**91**:2925-34.
- 134 **Smith C**, Cooper L, Burgess M, *et al*. Functional reversion of antigen-specific CD8+ T cells from patients with Hodgkin lymphoma following in vitro stimulation with recombinant polyepitope. *J Immunol* 2006;**177**:4897-906.
- 135 **Su Z**, Peluso MV, Raffegerst SH, *et al*. The generation of LMP2a-specific cytotoxic T lymphocytes for the treatment of patients with Epstein-Barr virus-positive Hodgkin disease. *Eur J Immunol* 2001;**31**:947-58.
- 136 **Duraiswamy J**, Sherritt M, Thomson S, *et al*. Therapeutic LMP1 polyepitope vaccine for EBV-associated Hodgkin disease and nasopharyngeal carcinoma. *Blood* 2003;**101**:3150-6.
- 137 **Wagner HJ**, Bollard CM, Vigouroux S, *et al*. A strategy for treatment of Epstein-Barr virus-positive Hodgkin's disease by targeting interleukin 12 to the tumor environment using tumor antigen-specific T cells. *Cancer Gene Ther* 2004;**11**:81-91.
- 138 **Bollard CM**, Rossig C, Calonge MJ, *et al*. Adapting a transforming growth factor beta-related tumor protection strategy to enhance antitumor immunity. *Blood* 2002;**99**:3179-87.
- 139 **Gandhi MK**, Lambley E, Duraiswamy J, *et al*. Expression of LAG-3 by tumor-infiltrating lymphocytes is coincident with the suppression of latent membrane antigen-specific CD8+ T-cell function in Hodgkin lymphoma patients. *Blood* 2006;**108**:2280-9.

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