Epstein–Barr virus and the B cell: a secret romance

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Infection by the Epstein–Barr virus (EBV) in immunocompetent individuals seems mainly confined to antigen-experienced memory B cells. However, a recent report shows that EBV$^+$ post-transplant lymphoproliferative disease might arise not only from memory B cells but also from naïve and germinal center (GC) B cells. Intriguingly, some of the EBV-positive B-cell clones seem to carry non-functional Ig-V-region genes as a result of deleterious somatic mutations acquired during the GC reaction. Given that such GC B cells are destined to die by apoptosis in the absence of EBV, these findings suggest that transformation by EBV might bypass negative selection of B cells within GCs.

Unlike other types of viral infection, the Epstein–Barr virus (EBV) establishes a long-term relationship with its host, the B cell. This long-term companion relies on the principle of latency, that is, the capacity of human B cells to host the virus and hide it from EBV-specific T cells. In turn, long-term persistence requires the virus to express viral homologs of human genes promoting activation (*LMP1* and *CD40*) [1] and survival (*BHRF* and *BCL2*) [2] of the transformed B cell. The receptor for EBV – CD21 – is expressed with particular abundance on memory B cells [3]. Unlike naïve or germinal center (GC) B cells, memory B cells are long-lived [4] and upon EBV-infection exhibit a very restricted expression-pattern of EBV-encoded genes [5] rendering them practically invisible to EBV-specific cytotoxic T cells (Table 1). For these reasons, memory B cells constitute the primary target for EBV infection in immunocompetent individuals [6]. Accordingly, patients with X-linked agammaglobulinemia, who lack mature B cells, cannot be infected by EBV *in vivo* [7] (Fig. 1).

Post-transplant lymphoproliferative disease: a spectrum ranging from polymorphic immunoblastic hyperplasia to monomorphic B-cell lymphoma

In a recent report, Timms *et al.* [8] present a comprehensive analysis of EBV–B-cell interactions in an immunocompromised situation, namely post-transplant lymphoproliferative disease (PTLD). The authors correlate the expression pattern of EBV-encoded genes (so-called latency programs; see Table 1) with the differentiation stage of EBV-transformed tumor clones, as assessed by sequence analysis of rearranged immunoglobulin (Ig)-V-region genes, immunophenotyping of B-cell differentiation markers, and Ig heavy (H)- and light-chain expression. In accordance with previous findings [9], this analysis revealed a heterogeneous picture, including all EBV-latency programs and a spectrum of B-cell lymphoproliferation ranging from polyclonal immunoblastic or plasmacytic hyperplasia to monomorphic B-cell lymphoma. Malignant B-cell populations were also heterogeneous with respect to their genotype. Some of the atypical B cells were classified as polyclonal B-cell proliferations based on immunoreactivity with multiple anti-Ig-isotype-specific antibodies and a diffuse 'smear' pattern of PCR amplification products using V_H- and J_H-specific primers. Two other cases were designated 'bi-clonal' based on the amplification of two potentially productive IgH-V–diversity joining (DJ) gene rearrangements. Although the process of allelic

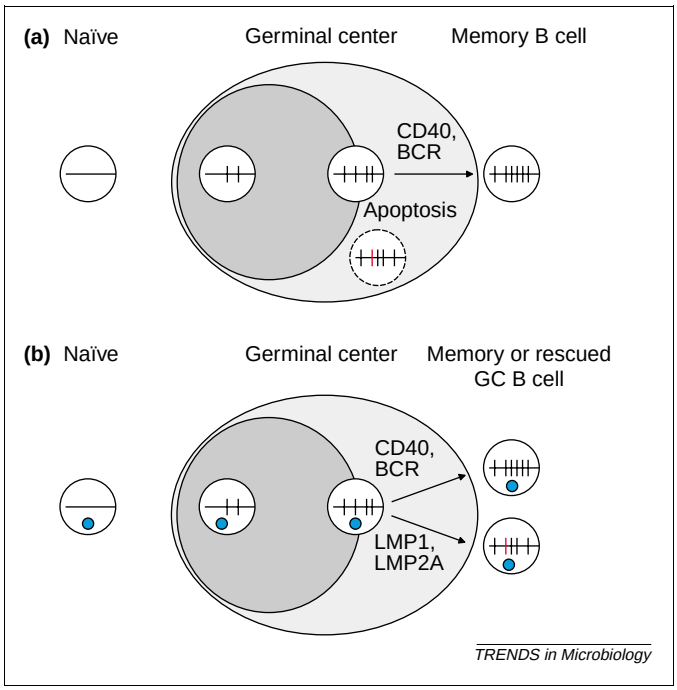

Fig. 1. Rescue of pre-apoptotic germinal center (GC) B cells by Epstein–Barr-virus (EBV)-encoded latent membrane proteins. The figure depicts a germinal center consisting of a dark zone and a light zone. Closed circles (blue) represent the EBV genome. Horizontal lines within cells indicate immunoglobulin (Ig)-variable-region genes. Vertical lines within cells indicate somatic mutations in the variable regions of Ig genes, which are generated during the GC reaction. Only GC B cells that acquire advantageous mutations [i.e. those that increase the affinity of the B-cell receptor (BCR) for its cognate antigen] receive survival signals through engagement of CD40 and the BCR. These cells can subsequently differentiate further to memory B cells (and long-lived plasma cells; not shown). (a) Germinal center B cells with 'crippling' mutations (red) normally undergo apoptosis. (b) However, transformation by EBV might rescue these cells as CD40- and BCR-dependent survival signals can be mimicked by the EBV-encoded oncoproteins LMP1 and LMP2A, respectively.

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Table 1. Latency programs of Epstein–Barr virus in human B cells

Latency	Expressed genes	B-cell differentiation	Lymphoma entity
0	Low-level LMP2	Memory B cell	None
I	EBNA1	Germinal center (GC) B cell	Burkitt's lymphoma
II[a]	EBNA1, LMP1, LMP2	GC B cell	Hodgkin's disease
III[b]	All nine latent genes	Naïve and GC B cell	Immunoblastic lymphoma

[a]Also referred to as 'default program' [20].
[b]Also referred to as 'growth program' [20].

exclusion effectively prevents productive rearrangement of both IgH alleles during normal B-cell development, this control can be bypassed in malignant B cells. For instance, in B-cell chronic lymphocytic leukemia, lack of allelic exclusion, and thus simultaneous expression of two IgH chains, can be repeatedly found [10]. Therefore, it remains open whether the two PTLD populations described here are indeed 'bi-clonal', or whether they represent tumor clones in which the mechanism of allelic exclusion was not active.

Although some B-cell clones carried Ig-V-region gene rearrangements devoid of somatic mutations – thereby suggesting a naïve B cell as the precursor of the proliferating clone – highly mutated Ig-V regions were amplified in the majority of PTLD cases. In these cases, the B-cell clones were derived from antigen-experienced GC or memory B cells. Unlike GC B cells, memory B cells have undergone a rigorous selection process for the expression of a functional B-cell receptor (BCR) with high affinity for its cognate antigen. As replacement (R) mutations within the framework regions (FRs) of Ig genes would often destabilize the Ig molecule, memory B cells carry preferentially silent (S) mutations within FRs, and the R:S ratio within FRs in GC B cells is relatively random. Based on this criterion, the authors could distinguish GC-B-cell-type PTLD from memory-B-cell-type PTLD.

Can EBV substitute defective BCR signaling in 'crippled' GC B cells?

Intriguingly, in two of the PTLD cases, Timms *et al.* only amplified IgH-V-region genes rendered non-functional by somatic mutations. In one case, point mutations generated a pre-terminal translation stop; in the other, a large 187-bp deletion resulted in loss of the IgH-V–DJ gene rearrangement reading frame. The authors claim that these two B-cell clones were unable to express a functional BCR. As shown by an earlier landmark study based on conditional gene targeting, *in vivo* ablation of the BCR is a condition that mature B cells cannot survive or cannot even be rescued by the concomitant expression of a *BCL2* transgene [11]. Therefore, it is tempting to speculate that PTLD exemplifies the capacity of EBV to provide mature B cells with crucial survival signals, even in the absence of a functional BCR. Supporting this notion, the EBV-encoded latent protein LMP2A not only contains the crucial immunoreceptor tyrosine-based activation motif (ITAM) of the BCR in a constitutively active form, but also prevents the BCR from signal transduction by excluding it from lipid rafts [12]. Besides PTLD, the survival of GC-derived B cells harboring 'crippled' Ig-V-region genes has already been described for two other types of EBV-associated B-cell malignancy, namely classical Hodgkin's disease (HD) [13,14] and angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) [15]. These studies used single-cell PCR to prove that malignant B cells in some cases of HD and AILD did not carry a productive Ig-V-region gene rearrangement on either allele. Only by characterization of the IgH loci configuration on both alleles at the single-cell level, including amplification of IgH germline alleles and IgH–DJ and IgH-V–DJ rearrangements, can the presence of a productively rearranged IgH allele be formally excluded. However, this proof is missing in the study by Timms and colleagues [8]. For example, in one case of PTLD the authors amplified two rearranged IgH-V–DJ alleles, one of which was rendered non-functional by deleterious somatic mutations. Given that somatic mutations can also affect PCR primer binding sites, it is indeed possible that, in the two cases where only non-functional IgH-V–DJ joints were amplified, a second productive IgH allele was missed owing to technical reasons. Consistent with lack of a productive IgH chain, malignant B cells in PTLD could not be stained using antibodies against IgH chains. However, it is worth noting that EBV-encoded genes can down-regulate Ig expression [16]. In addition, malignant B cells in HD lack Ig expression in almost all cases, regardless of whether they harbor a 'crippled' or productive IgH allele [17]. The lack of Ig expression in EBV-negative cases of HD – even in the presence of a productive IgH allele – is most probably caused by a defect in the transcription factors *OCT2* and *OBF1* [18].

Timms and colleagues [8] propose the exciting yet unproven scenario that GC B cells that acquire deleterious somatic mutations (and are hence destined to die by apoptosis) can be rescued by EBV. Indeed, several lines of evidence, including findings in HD and AILD, and, notably, the capacity of the EBV-encoded LMP2A protein to replace BCR signaling, are in direct support of this view. With respect to PTLD, future studies could distinguish between EBV-mediated survival signals and the role of frequent genetic alterations in PTLD predominantly involving the *TP53*, *MYC* and *NRAS* genes [9].

The findings by Timms and colleagues [8] could also have clinical consequences: PTLD expressing the EBV growth program (i.e. tumor cells expressing all EBV latent genes) might respond to treatment by adoptive immune transfer with EBV-specific allogeneic cytotoxic T cells [19]. By contrast, PTLD with restricted or absent EBV latent gene expression might require aggressive chemotherapy.

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References

1 Kilger, E. *et al.* (1998) Epstein–Barr virus-mediated B-cell proliferation is dependent upon latent membrane protein 1, which simulates an activated CD40 receptor. *EMBO J.* 17, 1700–1709
2 Lee, M.A. and Yates, J.L. (1992) BHRF1 of Epstein–Barr virus, which

is homologous to human proto-oncogene BCL2, is not essential for transformation of B cells or for virus replication *in vitro*. *J. Virol.* 66, 1899–1906
3 Feldhahn, N. *et al.* (2002) Silencing of B cell receptor signals in human naïve B cells. *J. Exp. Med.* 196, 1291–1305
4 Maruyama, M. *et al.* (2000) Memory B-cell persistence is independent of persisting immunizing antigen. *Nature* 407, 636–642
5 Babcock, G.J. *et al.* (2002) The expression pattern of Epstein–Barr virus latent genes *in vivo* is dependent upon the differentiation stage of the infected B cell. *Immunity* 13, 497–506
6 Babcock, G.J. *et al.* (1998) EBV persistence in memory B cells *in vivo*. *Immunity* 9, 395–404
7 Faulkner, G.C. *et al.* (1999) X-Linked agammaglobulinemia patients are not infected with Epstein–Barr virus: implications for the biology of the virus. *J. Virol.* 73, 1555–1564
8 Timms, J.M. *et al.* (2003) Target cells of Epstein–Barr-virus (EBV)-positive post-transplant lymphoproliferative disease: similarities to EBV-positive Hodgkin's lymphoma. *Lancet* 361, 217–223
9 Hopwood, P. and Crawford, D.H. (2000) The role of EBV in post-transplant malignancies: a review. *J. Clin. Pathol.* 53, 248–254
10 Rassenti, L.Z. and Kipps, T.J. (1997) Lack of allelic exclusion in B cell chronic lymphocytic leukemia. *J. Exp. Med.* 185, 1435–1445
11 Lam, K.P. *et al.* (1997) In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. *Cell* 90, 1073–1083
12 Dykstra, M.L. *et al.* (2001) Epstein–Barr virus coopts lipid rafts to block the signaling and antigen transport functions of the BCR. *Immunity* 14, 57–67
13 Kanzler, H. *et al.* (1996) 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.* 184, 1495–1505
14 Müschen, M. *et al.* (2000) Rare occurrence of classical Hodgkin's disease as a T cell lymphoma. *J. Exp. Med.* 191, 387–394
15 Bräuninger, A. *et al.* (2001) Survival and clonal expansion of mutating 'forbidden' (immunoglobulin receptor-deficient) Epstein–Barr virus-infected B cells in angioimmunoblastic T cell lymphoma. *J. Exp. Med.* 194, 927–940
16 Jochner, N. *et al.* (1996) Epstein–Barr virus nuclear antigen 2 is a transcriptional suppressor of the immunoglobulin mu gene: implications for the expression of the translocated *c-myc* gene in Burkitt's lymphoma cells. *EMBO J.* 15, 375–382
17 Marafioti, T. *et al.* (2000) 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* 95, 1443–1450
18 Re, D. *et al.* (2001) Oct-2 and Bob-1 deficiency in Hodgkin and Reed–Sternberg cells. *Cancer Res.* 61, 2080–2084
19 Haque, T. *et al.* (2002) Treatment of Epstein–Barr-virus-positive post-transplantation lymphoproliferative disease with partly HLA-matched allogeneic cytotoxic T cells. *Lancet* 360, 436–442
20 Thorley-Lawson, D.A. (2001) Epstein–Barr virus: exploiting the immune system. *Nat. Rev. Immunol.* 1, 75–82

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Molecular call-and-response: how *Salmonella* learns the gospel from its host

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Host–microbe interactions are often portrayed as a game of molecular hide-and-seek or tug-of-war where one partner seeks to establish an upper-hand over the other. Perhaps a more useful analogy is the traditional call-and-response preaching method used so effectively in churches of the southern USA to encourage participation by the assembled parishioners. The preacher calls out a line of a gospel or hymn and the congregation responds as one to the cue. A recent paper identifies Nramp as a potential molecular preacher, and *Salmonella*, and probably other pathogenic bacteria, are singing back full-throated.

Salmonella enterica serovar Typhimurium normally causes self-limiting gastroenteritis. This pathogen uses type III pili to deliver effector proteins to its preferred host cell, the macrophage. The bacterial colony proliferates in an intracellular vacuole. As macrophages wander the body, the bacteria are disseminated by escape into various cellular compartments. Death can occur, but only in host mice that lack the product of the *Nramp* gene. Here, bacterial loads in the liver and spleen reach lethal levels. This *nramp* loss-of-function mutation was first defined as a natural variation between strains of inbred mice. The strain lacking *Nramp* was killed by a variety of bacterial pathogens, including several species of *Mycobacterium* and *Leishmania*, and *Candida albicans* and *Toxoplasma gondii* (reviewed in Ref. [1]). In fact, *Nramp,* like many an itinerant preacher, went by several names in the inbred mouse strain literature (*Ity*/*Bcg*/*Lsh*). *Nramp* was cloned based on its genomic position in one of the earliest chromosome walks in mouse [2], and was subsequently shown to encode a protein that resides in a late endocytic vacuole of macrophages, the same subcellular address inhabited by *S. enterica* Typhimurium. The function of Nramp is still somewhat unclear, but it is thought to pump divalent ions pleiotropically. It might also function to scavenge iron from dead red blood cells in the infected host.

Nramp gathers the congregation

A quirk of the cell biology edifice of *Salmonella* that has emerged over the past ten years is the heavy reliance on

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