Studying Epstein–Barr Virus Pathologies and Immune Surveillance by Reconstructing EBV Infection in Mice

TOMOHARU YASUDA,¹ TRISTAN WIRTZ,¹ BAOCHUN ZHANG,² THOMAS WUNDERLICH,³
MARC SCHMIDT-SUPPRIAN,⁴ THOMAS SOMMERMAN,¹ AND KLAUS RAJEWSKY¹

¹*Immune Regulation and Cancer, Max-Delbrück-Center for Molecular Medicine,
13125 Berlin, Germany*

²*Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115*

³*Max Planck Institute for Neurological Research, 50931 Cologne, Germany*

⁴*Max Planck Institute for Biochemistry, 82152 Martinsried, Germany*

Correspondence: klaus.rajewsky@mdc-berlin.de and tomoharu.yasuda@mdc-berlin.de

Epstein–Barr virus (EBV) is a γ herpes virus endemic in humans and transforming human B lymphocytes. It causes a variety of human pathologies ranging from infectious mononucleosis upon acute infection to EBV-driven B-cell lymphomas. In humans, EBV-infected cells are under powerful immune surveillance by T and NK cells. If this immune surveillance is compromised as in immunosuppressed (AIDS- or posttransplantation) patients, the virus can spread from rare, EBV-containing cells and cause life-threatening pathologies. We have found that EBV immune surveillance and lymphomagenesis can be modeled in mice by targeted expression of key EBV proteins in the B-cell lineage. As EBV does not infect mouse B cells and mice have thus not coevolved with the virus, EBV exploits basic modes of the host immune response to optimize its coexistence with the host.

Epstein–Barr virus (EBV) and humans have coevolved over long time and come to a peaceful, although risky coexistence. This coexistence of virus and host relies on (i) the ability of the virus to persist as an infectious agent in the infected host for life in rare B cells invisible to the host's immune system and (ii) a highly efficient host immune surveillance of proliferating EBV-infected, transformed B cells leading to their rapid elimination (Babcock et al. 1998; Cohen 2000; Hislop et al. 2007).

The risk for the human host lies in the potent B-cell transforming activity of the virus, and thus EBV-driven lymphoproliferation and lymphomagenesis once immune surveillance is compromised.

How did this intricate form of coexistence evolve? We can ask a simple question with respect to immune surveillance: Has the human immune system evolved to cope with an otherwise lethal viral infection, or has the virus tricked the immune system of the host into a response mode that is favorable for virus–host coexistence? EBV clearly profits from the host immune response, which protects the host from an uncontrolled outgrowth of EBV-transformed B cells, while allowing lifelong latent infection and thus efficient spreading of EBV in the human population.

We address this issue by studying EBV infection in a host species which has *not* coevolved with the virus, but whose immune system resembles that of the human in its basic structure. A viral strategy based on the induction of immune surveillance of virus-infected cells might well reproduce in such a scenario, whereas this would not be expected if EBV immune surveillance is based on the

evolution of EBV-targeted immunological specificities in the human. Accordingly, we set out to model EBV infection in mice, a species from which EBV is absent. As EBV is unable to infect mouse B cells, we genetically targeted the expression of key EBV proteins involved in B-cell transformation and, hypothetically, the induction of immune surveillance, to the mouse B lineage.

INDUCING EBV IMMUNE SURVEILLANCE AND LYMPHOMAGENESIS BY B-CELL-SPECIFIC EXPRESSION OF EBV LATENT MEMBRANE PROTEIN 1

Although EBV encodes many functionally important proteins, we focused our initial experiments on the targeted expression of latent membrane proteins (LMPs) 1 and 2A in mouse B cells. These two proteins are biologically particularly interesting and are also known to play key roles in the activation and, ultimately, transformation of B cells by the virus (Thorley-Lawson 2001; Küppers 2003; Young and Rickinson 2004). Both LMP2A and LMP1 are constitutively active signaling molecules, delivering signals into the EBV-infected cell that mimic the physiological signals delivered by the B-cell antigen receptor (BCR) in the case of LMP2A, and the CD40 coreceptor in that of LMP1 (Fig. 1). These properties of the two LMPs are based on their peculiar transmembrane domains on the one hand, and the structures of their cytoplasmic tails on the other hand, which connect to downstream-signaling cascades resembling those activated by

Figure 1. Schematic representation of the structure and signaling function of LMP1 and LMP2A. LMP1 has six transmembrane domains and a long carboxy-terminal cytoplasmic tail containing two critical functional domains, the carboxy-terminal activation regions (CTAR) 1 and 2. Similar to the CD40 costimulatory receptor, LMP1 interacts with tumor necrosis factor receptor–associated factor (TRAF) family proteins to activate NF- κ B and JNK signaling. The ERK and JAK/STAT signaling pathways are also activated. LMP1 self-aggregates in the plasma membrane forming a constitutively active signaling complex (not shown). This is also true for LMP2A, which has an amino-terminal intracellular region containing an immunoreceptor tyrosine-based activation motif (ITAM) and 12 transmembrane domains. Phosphorylation at Tyr112 (Y¹¹²) is critical for Lyn kinase recruitment and phosphorylation of the ITAM motif, leading in turn to the recruitment of Syk and PI3K-dependent Akt activation, in analogy to ITAM-mediated signal transduction from the BCR.

BCR and CD40 engagement, respectively. Indeed, the signal-transducing ITAM motif in the cytoplasmic tail of the BCR-associated Ig α / β heterodimer (Reth 1989) is identically reproduced in the LMP2A cytoplasmic tail, and expression of LMP2A in mouse B cells instead of the BCR allows normal B-cell development to proceed, which physiologically is strictly dependent on BCR expression (Caldwell et al. 1998; Casola et al. 2004).

In line with its assumed function as a constitutively active CD40 coreceptor (which under physiological conditions delivers T-cell help to B cells in T-cell-dependent B-cell responses), LMP1 expression in human and mouse B cells leads to massive, chronic B-cell activation, and proliferation, with the induction of NF- κ B, ERK, JNK, and JAK/STAT signaling. LMP1 expression by itself is able to transform mouse B cells as well as rodent and human fibroblasts (Wang et al. 1985; Kulwichit et al. 1998; Mainou et al. 2005), and transgenic expression of an LMP1/CD40 chimeric protein comprised the LMP1 transmembrane domain and the cytoplasmic tail of CD40 promotes B-cell lymphomagenesis in mice (Homig-Holzel et al. 2008).

Surprisingly, however, B-cell-specific expression of an LMP1 transgene in mice resulted in only sporadic lymphoma development, restricted to aged animals. In young animals, transgene expression was barely detectable (Kulwichit et al. 1998). We re-addressed this issue by expressing a conditional signal-on allele of LMP1 in mouse B cells, using B-cell-specific (CD19-driven) Cre-mediated recombination (Zhang et al. 2012). In the com-

pound mutant animals, B-cell development was essentially abrogated; the few B cells that could be detected had escaped Cre-mediated recombination and thus did not express LMP1. Although we initially suspected that LMP1 expression was simply toxic to the cells, a careful analysis of the mice revealed that their B-cell deficiency was due to the elimination of the LMP1-expressing cells by T cells. Just as EBV-infected B cells in the human, the LMP1⁺ B cells in the mouse were subject to powerful immune surveillance, resulting in their elimination!

Following up on this finding, we studied the development of LMP1⁺ B cells on a T-cell-deficient background and found that after an initial period of limited cellular expansion, presumably contained by activated NK cells, aggressive LMP1-driven lymphomas began to develop at 2 mo of age, to which the animals rapidly succumbed. These were monoclonal tumors and thus must have acquired additional genetic alterations. Although these are still under investigation, we can conclude from the above results that the expression of a single EBV protein, LMP1, in mouse B cells results in the induction of a potent T- and NK-cell response through which the cells are eliminated, just like human EBV-infected B cells; and that like in the human, breaking immune surveillance leads to the development of fatal B-cell lymphomas (Zhang et al. 2012).

MODELING ACUTE EBV INFECTION

EBV infection usually occurs early in life or young adulthood and may pass unnoticed or cause infectious

mononucleosis. The latter is characterized by a dramatic expansion of EBV-infected B cells, a fraction of which actively produce (and are ultimately lysed by) the virus. This process, which involves B-cell transformation by the virus, would be fatal for the host (and indeed is so in patients bearing mutations that interfere with the defense against EBV—the X-linked lymphoproliferative [XLP] syndrome [Morra et al. 2001]), were there not the induction of a rapid T- and NK-cell response against the EBV-infected B cells, reflected by an equally dramatic T-cell expansion and leading to the elimination of the virus-infected targets. A few weeks after primary EBV infection, T- and B-cell counts in the blood have largely returned to normal, but now long-term T-cell memory has been induced and persists for life, to prevent reinfection or the spreading of the virus from the few cells in which it persists in a form invisible to the host immune system (Hislop et al. 2007).

Can we reconstruct primary EBV infection accompanied by induction of protective immunological memory in a mouse model? We used for this purpose a system of inducible B-cell-specific gene expression, which we had developed in a different context (T Wunderlich and M Schmidt-Suppran, unpubl.). In this system, a transgene encoding a Cre-ERT2 fusion protein (Feil et al. 1997) was knocked into the second exon of the CD19 locus, leading to its expression selectively in B cells; application of the ERT2-binding ligand tamoxifen results in translocation of the fusion protein into the nucleus, elimination of a loxP-flanked STOP cassette in the LMP1 signal-on allele, and subsequent LMP1 expression driven by the ROSA26 promoter. To our advantage, induction of Cre-mediated recombination through the CD19creERT2 knock-in allele is a rare event, occurring only in a few percent of B cells and thus perfectly mimicking EBV infection of just a few B cells in its initial phase.

In the compound mutant mice tamoxifen-induced LMP1 expression in CD19⁺ B cells led to an explosive expansion of these cells over a period of 6–8 d, resulting in an enlarged spleen with LMP1 expressing B-cell blasts outnumbering normal splenic B cells about 20-fold, corresponding to roughly 10 rounds of division (Fig. 2A). Two weeks later, cell counts had returned to baseline and LMP1⁺ B cells were no longer detectable (Fig. 2A). Studying the process of the expansion and disappearance of the LMP1⁺ B cells upon tamoxifen induction in more detail, we found that the expansion of these cells was accompanied, indeed presumably shortly followed, by an equally massive expansion of CD4⁺ and CD8⁺ T cells (Fig. 2B). The expanding T cells had an activated phenotype (Fig. 2C; and data not shown), and when exposed to LMP1-transduced B cells in vitro, responded vigorously in a degranulation assay (T Wirtz, unpubl.). By day 8 after tamoxifen application, the LMP1⁺ B cells had largely disappeared, and T-cell numbers, although still somewhat elevated, had sharply decreased (Fig. 2B). A significant fraction of the persisting T cells had an effector/memory (CD44⁺CD62L[−]) phenotype (Fig. 2C), and accordingly, a second injection of tamoxifen did not result in a second wave of B-cell expansion (T Yasuda, unpubl.).

We interpret these results to mean that like EBV-infected B cells in humans undergoing a primary EBV infection, LMP1 expressing B-cell blasts in the mouse initially expand in an uncontrolled fashion, but concomitantly induce a potent T-cell response, which leads to the rapid clearance of the “infected” B cells. As a consequence of this process, T-cell memory is established: LMP1-expressing B cells are under immune surveillance, as we had already found in our earlier work (Zhang et al. 2012).

CONCLUSIONS

The present results show that the basic features of acute EBV infection—rapid, uncontrolled expansion of infected B cells, induction of a vigorous T-cell response, clearance of the infected cells, and induction of specific immune surveillance—can be modeled in the mouse by induced expression of a single EBV protein, LMP1, in a small fraction of its B cells. Given that EBV is not endemic in mice, the virus has apparently evolved a strategy to trick the host immune system into a response mode that is favorable for virus–host coexistence.

Mechanistically this strategy appears to critically involve the LMP1 protein, a constitutively active mimic of the CD40 B-cell coreceptor. When expressed in B cells, LMP1 hyperactivates these cells and drives their expansion, by setting in motion signaling cascades promoting B-cell survival and proliferation. An essential element of the viral strategy is to confer immunogenicity to the infected cells, so that after their initial expansion the virus-transformed cells are contained by the host's immune system. This feature of EBV infection is faithfully reproduced in the LMP1-based mouse model, suggesting that it is not the recognition of the multitude of EBV-derived peptide epitopes by the human T-cell system that drives EBV immune surveillance, but rather the LMP1-dependent immunogenicity of the infected cells. An important element in bringing this immunogenicity about is likely the strong up-regulation of a multitude of surface molecules involved in antigen presentation, cell adhesion, and costimulation on the LMP1⁺ B-cell blasts (Cahir-McFarland et al. 2004; Zhang et al. 2012); but additional, more specific mechanisms may well be involved. The end result is a vigorous T-cell response, where the responding T cells recognize epitopes on the EBV/LMP1⁺ B cells in an MHC-restricted manner (i.e., through their TCR [Hislop et al. 2007; Zhang et al. 2012]). Whereas in the human these epitopes are known to comprise peptides derived from the various EBV proteins, they are still elusive in the mouse model. LMP1-derived peptides may be involved, although our attempts to identify such peptides have failed (Zhang et al. 2012). T-cell “superantigens” are an interesting additional possibility in this context.

Taken together, modeling EBV infection, pathologies, and immune surveillance in mice has shed new light on the mechanisms controlling virus–host interaction, offers unique opportunities to study the immune surveillance of

Figure 2. Expansion of LMP1-expressing B cells induces T-cell proliferation and activation. (A) Mice of the indicated genotypes were treated with 4 mg of tamoxifen by intragastric gavage. R26YFP and R26LMP1 designate alleles of the Rosa26 locus, from which YFP or LMP1 together with a human CD2 reporter can be expressed upon deletion if a loxP-flanked STOP cassette. B-cell numbers in spleens from these mice or untreated mice (–) were analyzed by flow cytometry on different time points after treatment (d6–d37). Numbers of LMP1-expressing B cells were determined by human CD2 reporter and Fas expression. Cell numbers are shown as means from groups of mice (CD19^{cre}ERT2;R26YFP, (–): *n* = 2, d6: *n* = 3; CD19^{cre}ERT2;R26LMP1, d6: *n* = 6, d8–9: *n* = 3) with SD, or represent individual mice. (B) Splenic CD4⁺ and CD8⁺ T cell numbers in the same mice as in A, determined by flow cytometry. (C) Activation status of splenic CD4⁺ and CD8⁺ T cells in CD19^{cre}ERT2;R26LMP1 mice at various times after tamoxifen treatment, determined by flow-cytometric measurements of CD44 and CD62L expression. Percentage of cells in the various gated populations are shown. Untreated: no tamoxifen treatment.

EBV-infected cells, and has provided preclinical models of EBV-driven B-cell lymphomas in immunosuppressed (AIDS and posttransplantation) patients and, perspective-ly, the XLP syndrome in humans. With respect to the latter, we have found in yet unpublished experiments that upon T-cell depletion concomitant induction of LMP1 and LMP2A in a small fraction of mouse B cells in vivo results in uncontrolled expansion of the LMP-expressing cells and rapid death of the animals. Whether this will also occur upon ablation of the adaptor molecule SAP, whose loss results in XLP syndrome in humans (Engel et al. 2003; Cannons et al. 2011), is presently under investigation.

ACKNOWLEDGMENTS

We are grateful to Dr. D. Calado for advice; J. Russ and C. Grosse for technical assistance; and S. Knäspel, M. Bezohra, and R. Lauhkonen-Seitz for administrative help. This work was supported by the European Research Council (ERC Advanced Grant ERC-AG-LS6 to K.R.).

REFERENCES

- Babcock GJ, Decker LL, Volk M, Thorley-Lawson DA. 1998. EBV persistence in memory B cells in vivo. *Immunity* 9: 395–404.

- Cahir-McFarland ED, Carter K, Rosenwald A, Giltane JM, Henrickson SE, Staudt LM, Kieff E. 2004. Role of NF- κ B in cell survival and transcription of latent membrane protein 1-expressing or Epstein-Barr virus latency III-infected cells. *J Virol* **78**: 4108–4119.
- Caldwell RG, Wilson JB, Anderson SJ, Longnecker R. 1998. Epstein-Barr virus LMP2A drives B cell development and survival in the absence of normal B cell receptor signals. *Immunity* **9**: 405–411.
- Cannons JL, Tangye SG, Schwartzberg PL. 2011. SLAM family receptors and SAP adaptors in immunity. *Annu Rev Immunol* **29**: 665–705.
- Casola S, Otipoby KL, Alimzhanov M, Humme S, Uyttersprot N, Kutok JL, Carroll MC, Rajewsky K. 2004. B cell receptor signal strength determines B cell fate. *Nat Immunol* **5**: 317–327.
- Cohen JI. 2000. Epstein-Barr virus infection. *N Engl J Med* **343**: 481–492.
- Engel P, Eck MJ, Terhorst C. 2003. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nat Rev Immunol* **3**: 813–821.
- Feil R, Wagner J, Metzger D, Chambon P. 1997. Regulation of Cre recombinase activity by mutated estrogen receptor ligand-binding domains. *Biochem Biophys Res Commun* **237**: 752–757.
- Hislop AD, Taylor GS, Sauce D, Rickinson AB. 2007. Cellular responses to viral infection in humans: Lessons from Epstein-Barr virus. *Annu Rev Immunol* **25**: 587–617.
- Homig-Holzel C, Hojer C, Rastelli J, Casola S, Strobl LJ, Muller W, Quintanilla-Martinez L, Gewies A, Ruland J, Rajewsky K, et al. 2008. Constitutive CD40 signaling in B cells selectively activates the noncanonical NF- κ B pathway and promotes lymphomagenesis. *J Exp Med* **205**: 1317–1329.
- Kulwichit W, Edwards RH, Davenport EM, Baskar JF, Godfrey V, Raab-Traub N. 1998. Expression of the Epstein-Barr virus latent membrane protein 1 induces B cell lymphoma in transgenic mice. *Proc Natl Acad Sci* **95**: 11963–11968.
- Küppers R. 2003. B cells under influence: Transformation of B cells by Epstein-Barr virus. *Nat Rev Immunol* **3**: 801–812.
- Mainou BA, Everly DN Jr, Raab-Traub N. 2005. Epstein-Barr virus latent membrane protein 1 CTAR1 mediates rodent and human fibroblast transformation through activation of PI3 K. *Oncogene* **24**: 6917–6924.
- Morra M, Howie D, Grande MS, Sayos J, Wang N, Wu C, Engel P, Terhorst C. 2001. X-linked lymphoproliferative disease: A progressive immunodeficiency. *Annu Rev Immunol* **19**: 657–682.
- Reth M. 1989. Antigen receptor tail clue. *Nature* **338**: 383–384.
- Thorley-Lawson DA. 2001. Epstein-Barr virus: Exploiting the immune system. *Nat Rev Immunol* **1**: 75–82.
- Wang D, Liebowitz D, Kieff E. 1985. An EBV membrane protein expressed in immortalized lymphocytes transforms established rodent cells. *Cell* **43**: 831–840.
- Young LS, Rickinson AB. 2004. Epstein-Barr virus: 40 years on. *Nat Rev Cancer* **4**: 757–768.
- Zhang B, Kracker S, Yasuda T, Casola S, Vanneman M, Homig-Holzel C, Wang Z, Derudder E, Li S, Chakraborty T, et al. 2012. Immune surveillance and therapy of lymphomas driven by Epstein-Barr virus protein LMP1 in a mouse model. *Cell* **148**: 739–751.

Cold Spring Harbor Symposia on Quantitative Biology

Studying Epstein–Barr Virus Pathologies and Immune Surveillance by Reconstructing EBV Infection in Mice

Tomoharu Yasuda, Tristan Wirtz, Baochun Zhang, et al.

Cold Spring Harb Symp Quant Biol 2013 78: 259-263 originally published online November 15, 2013

Access the most recent version at doi:[10.1101/sqb.2013.78.020222](https://doi.org/10.1101/sqb.2013.78.020222)

References

This article cites 19 articles, 3 of which can be accessed free at:
<http://symposium.cshlp.org/content/78/259.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

To subscribe to *Cold Spring Harbor Symposia on Quantitative Biology* go to:
<http://symposium.cshlp.org/subscriptions>