Journal of General Virology (2010), **91**, 2197–2202 DOI 10.1099/vir.0.021444-0

Short Communication

Epstein–Barr virus LMP2A imposes sensitivity to apoptosis

Michelle Swanson-Mungerson,[1,2] Rebecca Bultema[2] and Richard Longnecker[2]

Correspondence
Michelle Swanson-Mungerson
mswans@midwestern.edu

[1]Department of Microbiology and Immunology, Chicago College of Osteopathic Medicine, Midwestern University, 555 31st Street, Downers Grove, IL 60516, USA

[2]Department of Microbiology and Immunology, Feinberg School of Medicine, Northwestern University, 303 E. Chicago Ave, Chicago, IL 60611, USA

Received 26 February 2010
Accepted 14 May 2010

In cell lines, the Epstein–Barr virus (EBV)-encoded protein latent membrane protein 2A (LMP2A) protects B-cells from apoptosis by blocking B-cell receptor (BCR) signalling. However, EBV-infected B-cells *in vivo* are extremely different from cell lines. This study used a murine transgenic model in which B-cells express LMP2A and a BCR specific for hen egg lysozyme to determine whether LMP2A protects resting and antigen-activated B-cells from apoptosis. LMP2A allows BCR signal transduction and induces constitutive activation of NF-κB to increase Bcl-2 levels that afford LMP2A-mediated protection from apoptosis in the absence or presence of antigen. In contrast, low levels of NF-κB inhibitor only affected Bcl-2 and Bcl-xL levels and increased apoptosis in LMP2A-negative B-cells after BCR cross-linking. These data suggest that LMP2A uniquely makes resting B-cells sensitive to NF-κB inhibition and apoptosis and suggest that NF-κB may be a novel target to eradicate latently EBV-infected B-cells.

Epstein–Barr virus (EBV) is a herpesvirus that infects over 90 % of the population. After an initial infection of B-cells in which many viral proteins are expressed, the virus transitions into a latent state and expresses few viral transcripts (Fields *et al.*, 2007). During latency, the virus expresses multiple gene patterns in both resting B-cells and EBV-associated tumours (Thorley-Lawson, 2001; Thorley-Lawson & Allday, 2008). Evidence indicates that not only the activation state of the B-cell, but also cytokines and host factors influence gene expression during latency (Kis *et al.*, 2006, 2010; Konforte & Paige, 2009; Konforte *et al.*, 2008; Pokrovskaja *et al.*, 2002; Uchakin *et al.*, 2007). Therefore, the expression of viral transcripts may vary due to the environment in which the latently infected B-cells reside.

Of the latency transcripts expressed by EBV, latent membrane protein 2A (LMP2A) is consistently detected (Babcock *et al.*, 1998, 2000; Babcock & Thorley-Lawson, 2000; Decker *et al.*, 1996; Hochberg *et al.*, 2004; Qu & Rowe, 1992; Tierney *et al.*, 1994; Tsai *et al.*, 2009). Previous reports demonstrate that LMP2A protects B-cells from apoptosis in LMP2A-expressing cell lines (Fukuda & Longnecker, 2005; Mancao & Hammerschmidt, 2007). One mechanism by which LMP2A performs this function is by blocking the signalling through the B-cell receptor (BCR) (Fukuda & Longnecker, 2005; Miller *et al.*, 1994). However, one caveat when using this system is that LMP2A is expressed at very high levels and the cells are immortalized, which is very different from resting, latently infected B-cells *in vivo* (Caldwell *et al.*, 2000; Thorley-Lawson, 2001). Therefore, to assess the ability of LMP2A to modulate BCR signalling and survival in resting B-cells, our laboratory generated the LMP2A/HEL-Tg mouse (Swanson-Mungerson *et al.*, 2005). The advantage of using transgenic (-Tg) HEL-Tg and LMP2A/HEL-Tg mice is that the B-cells from these mice all express a BCR specific for the antigen hen egg lysozyme (HEL). Therefore, we are able to directly compare the ability of LMP2A to modulate BCR signalling and B-cell survival when the only difference between the two B-cells is the expression of LMP2A.

Previous studies using an LMP2A transgenic line that expresses lower levels of LMP2A, the Tg6 line, indicate that LMP2A alters BCR signalling after exposure to antigen-independent BCR cross-linking (Portis & Longnecker, 2004a). To determine whether LMP2A alters BCR signalling in response to antigen, we purified B-cells from HEL-Tg and LMP2A/HEL-Tg mice and incubated these cells in the absence or presence of antigen for up to 10 min. As shown in Fig. 1(a), B-cells from both HEL-Tg and LMP2A/HEL-Tg mice demonstrate a marked increase in tyrosine phosphorylation after exposure to HEL or BCR cross-linking using a polyclonal anti-Ig antibody. Interestingly, there are slight differences in the banding pattern of some of the faster-migrating phosphorylated proteins produced when comparing the HEL-Tg B-cells with the LMP2A/HEL-Tg B-cells

Supplementary figures are available with the online version of this paper.

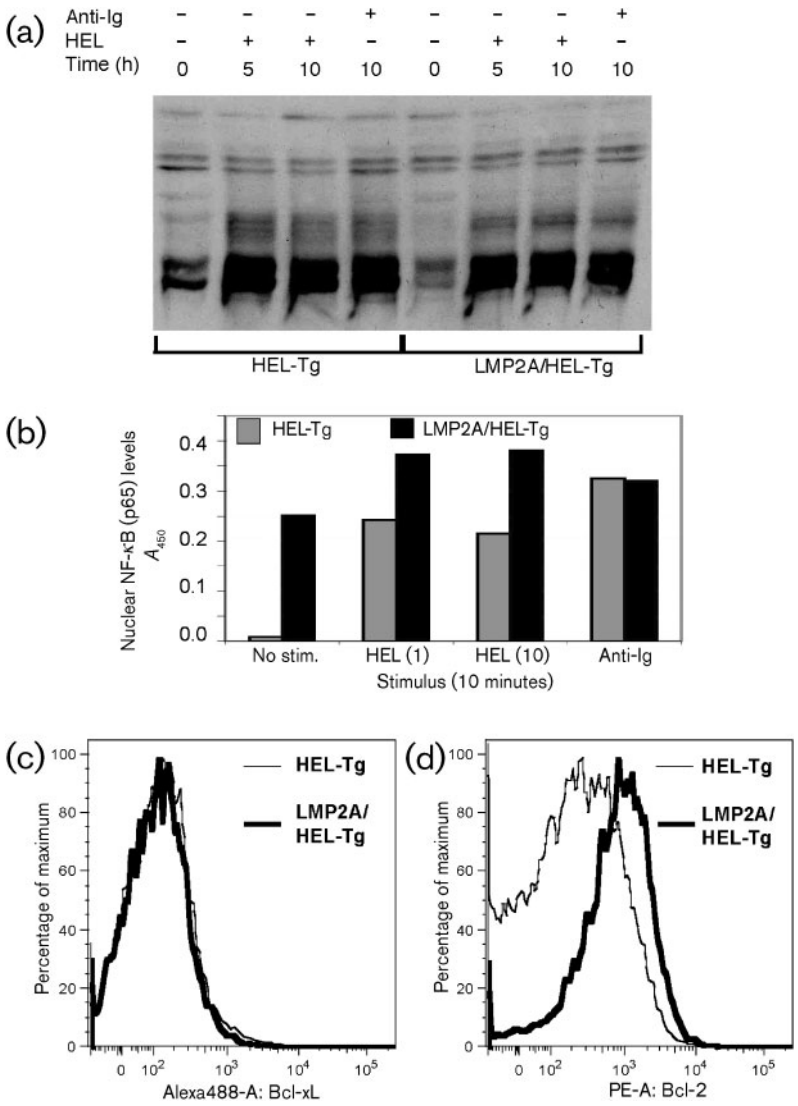

Fig. 1. LMP2A influences BCR signalling and Bcl family member expression. (a) B-cells from HEL-Tg and LMP2A/HEL-Tg mice were purified to >95 % purity as previously described (Swanson-Mungerson *et al.*, 2006). B-cells (1×10^6) were incubated in the absence or presence of HEL (1 μg ml^{-1}) or goat anti-mouse Ig (10 μg ml^{-1}) for up to 10 min before protein was isolated and analysed for tyrosine phosphorylation using Western blot analysis as described previously (Portis & Longnecker, 2004a). The data are representative of two independent experiments with similar results. (b) HEL-Tg and LMP2A/HEL-Tg purified B-cells were incubated as described in Fig. 1(a) with HEL [1 μg ml^{-1}, HEL (1); or 10 μg ml^{-1}, HEL (10)] and analysed for nuclear localization of the p65 subunit of NF-κB as described previously (Swanson-Mungerson *et al.*, 2005). The data are representative of three experiments with similar results. (c, d) HEL-Tg and LMP2A/HEL-Tg B-cells were isolated and immediately fixed with Cytofix/Cytoperm (BD Biosciences) according to the manufacturer's protocol. The cells were subsequently stained with either Alexa Fluor 488-conjugated anti-Bcl-xL or phycoerythrin-conjugated anti-Bcl-2 and analysed by flow cytometry. The data were subsequently analysed using FlowJo software and are representative of at least three mice.

after BCR cross-linking (Fig. 1a). Currently, the proteins that are differently modified are not known. Taken together, these data indicate that LMP2A alters antigen-induced BCR signalling in resting B-cells.

One downstream effect of BCR cross-linking is the translocation of NF-κB into the nucleus (Schulze-Luehrmann & Ghosh, 2006; Weil & Israel, 2004). Previous studies by our laboratory indicated that LMP2A induces constitutive NF-κB activation in B-cells generated from the bone marrow (Swanson-Mungerson *et al.*, 2005). To determine whether LMP2A induces NF-κB nuclear localization in splenic B-cells, we analysed nuclear lysates from splenic B-cells from HEL-Tg and LMP2A/HEL-Tg mice using an NF-κB transcription factor assay (Active Motif). This assay quantifies the amount of nuclear NF-κB that binds to an NF-κB consensus DNA oligonucleotide coating on a 96-well plate (Swanson-Mungerson *et al.*, 2005). As shown in Fig. 1(b), LMP2A/HEL-Tg B-cells demonstrate constitutive nuclear localization of NF-κB in the absence of any external stimulus, which is in contrast to HEL-Tg B-cells. These findings indicate that LMP2A continues to activate NF-κB upon exit from the bone marrow and while in the peripheral organs.

Since the data in Fig. 1(a) indicate that LMP2A alters BCR signal transduction, we determined whether LMP2A also influences the translocation of NF-κB after BCR cross-linking by antigen. As shown in Fig. 1(b), HEL-Tg B-cells exposed to antigen demonstrate a marked increase in NF-κB levels compared with unstimulated HEL-Tg B-cells. The amount of NF-κB induced by antigen in HEL-Tg B-cells is equivalent to the levels of the constitutive NF-κB activation in the unstimulated LMP2A/HEL-Tg B-cells (Fig. 1b). This is interesting, since BCR cross-linking and NF-κB activation in normal B-cells induce B-cells to enter the cell cycle (Doi *et al.*, 1997), yet LMP2A/HEL-Tg B-cells do not proliferate unless they are stimulated with antigen and/or mitogen (M. Swanson-Mungerson & R. Longnecker, unpublished observation). Therefore, these data suggest that LMP2A-induced

NF-κB is not sufficient for the induction of proliferation. Therefore, it is likely that constitutively high levels of LMP2A-induced NF-κB provide a pro-survival function in the absence of BCR cross-linking, rather than a pro-activation signal. When LMP2A/HEL-Tg B-cells are stimulated with antigen, the levels of NF-κB nuclear localization are further increased, suggesting that LMP2A enhances BCR-induced NF-κB activation (Fig. 1b). Interestingly, when anti-Ig antibody cross-links the BCR as a positive control for NF-κB nuclear localization, LMP2A does not markedly increase NF-κB localization (Fig. 1b), which suggests that the stimulus used when studying the function of LMP2A on BCR cross-linking is important. Taken together, these data demonstrate that LMP2A induces constitutive NF-κB activation and further augments BCR-induced NF-κB nuclear localization after antigen exposure.

Previous data indicate that LMP2A increases the levels of NF-κB-regulated anti-apoptotic proteins, such as Bcl-2 and Bcl-xL, in the absence of a BCR (Portis & Longnecker, 2004b). Since LMP2A consistently activates NF-κB, we determined whether LMP2A also increases expression of these proteins in a B-cell that expresses a BCR. Using flow cytometric analysis, both LMP2A/HEL-Tg and HEL-Tg B-cells express very low levels of Bcl-xL (Fig. 1c). This is not surprising in the HEL-Tg B-cells, since previous data demonstrate that Bcl-xL expression is more predominant during B-cell development than in resting B-cells found in the spleen (Grillot *et al.*, 1996). In contrast, the absence of Bcl-xL expression is surprising in the LMP2A/HEL-Tg B-cells, because B-cells from TgE mice express high levels of Bcl-xL [(Portis & Longnecker, 2004b) and flow cytometric data (Supplementary Fig. S1, available in JGV Online)]. Due to the fact that B-cells from LMP2A-Tg (TgE) mice do not express a BCR (Caldwell *et al.*, 1998), it is possible that TgE B-cells are more similar to developing B-cells, which express Bcl-xL, than mature B-cells found in the spleen. Therefore, we propose that the forced expression of a BCR in the LMP2A/HEL-Tg B-cells yields mature B-cells and a return to the normal regulation of anti-apoptotic genes.

Bcl-2 is constitutively expressed at low levels in mature B-cells, presumably to maintain survival in the absence of B-cell activation (Grossmann *et al.*, 2000). Based on previous data (Portis & Longnecker, 2004b) and our observations, we expected to see Bcl-2 in the BCR-expressing B-cells of the HEL-Tg and LMP2A/HEL-Tg B mice. While both express Bcl-2, LMP2A/HEL-Tg B-cells express more Bcl-2,

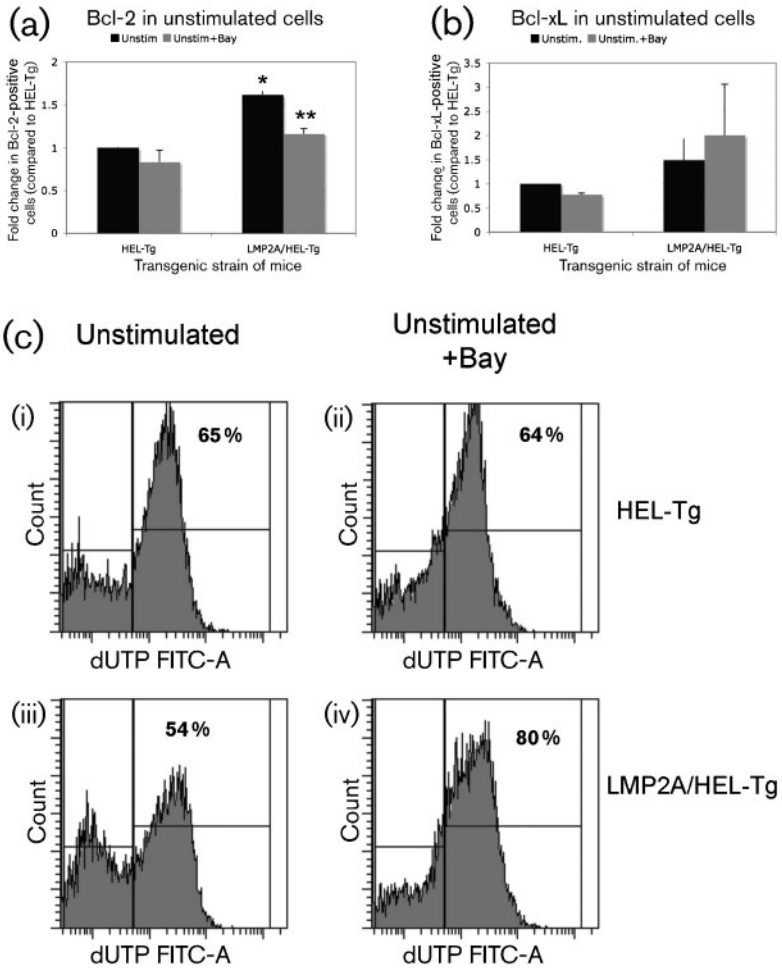

Fig. 2. NF-κB inhibition affects Bcl-2 levels and apoptosis in unstimulated LMP2A/HEL-Tg B-cells. (a, b) HEL-Tg and LMP2A/HEL-Tg B-cells were incubated for 4 h in the presence of either DMSO (unstimulated) or 5 μg ml^{-1} of the NF-κB inhibitor Bay 11-7085 (unstimulated + Bay). At the end of this incubation, cells were fixed and stained as described for Fig. 1(c, d). The data represent the fold increase in the percentage of Bcl-expressing B-cells and were derived by combining the data from three or four mice. *Indicating $P<0.05$ by unpaired Student's *t*-test when compared with DMSO-exposed HEL-Tg B-cells. **Indicating $P<0.05$ by unpaired Student's *t*-test when compared with DMSO-exposed LMP2A/HEL-Tg B-cells. (c, d) HEL-Tg and LMP2A/HEL-Tg B-cells were incubated for 18 h in the presence of either DMSO (unstimulated) or Bay 11-7085 (unstimulated + Bay). The B-cells were subsequently fixed and stained for the presence of DNA double-stranded breaks using an ApoDirect kit (BD Biosciences) as described previously (Portis & Longnecker, 2004b). Additional NF-κB inhibitors (PDTC and SN50) were used and generated similar results (unpublished data). After flow cytometry, the data were analysed using Diva software. The data are representative of three or four mice with similar results.

as indicated by a shift in Bcl-2 fluorescence when comparing the histogram of the LMP2A/HEL-Tg cells with HEL-Tg B-cells (Fig. 1d; thick line, LMP2A/HEL-Tg; thin line, HEL-Tg). We subsequently measured whether LMP2A-mediated increases in Bcl-2 require the activation of NF-κB. The addition of an NF-κB inhibitor (Bay 11-7085) blocks the LMP2A-mediated increase in Bcl-2 in LMP2A/HEL-Tg B-cells (Fig. 2a). However, the NF-κB inhibitor does not significantly affect the levels of Bcl-2-positive HEL-Tg B-cells (Fig. 2a). Furthermore, the addition of the NF-κB inhibitor had no significant effect on Bcl-xL levels in either resting HEL-Tg or LMP2A/HEL-Tg B-cells (Fig. 2b). Taken together, these data suggest that LMP2A increases NF-κB activation to selectively increase Bcl-2 in resting, mature B-cells.

Previous studies indicate that LMP2A protects B-cells from apoptosis (Fukuda & Longnecker, 2005; Mancao & Hammerschmidt, 2007; Portis & Longnecker, 2004b). To determine whether LMP2A protects B-cells from apoptosis in our transgenic mouse model, we analysed the percentage of apoptotic cells by using an ApoDirect kit from BD Biosciences. This assay fluorescently tags double-stranded DNA breaks, thus allowing the analysis of the percentage of cells that are in the irreversible stages of apoptosis by flow cytometry (Portis & Longnecker, 2004b). LMP2A/HEL-Tg B-cells have significantly fewer apoptotic cells (54 %) than HEL-Tg B-cells (65 %), further supporting the role of LMP2A in protecting cells from apoptosis [Fig. 2c(i), (iii)]. Similar experiments using Annexin V staining also demonstrate that LMP2A protects B-cells from apoptosis (Supplementary Fig. S2, available in JGV Online). We determined that the increases in NF-κB and Bcl-2 are important to protect unstimulated LMP2A/HEL-Tg B-cells from apoptosis by incubating purified HEL-Tg and LMP2A/HEL-Tg B-cells in the absence or presence of the NF-κB inhibitor. The addition of the NF-κB inhibitor significantly increases the percentage of apoptotic LMP2A/HEL-Tg B-cells [Fig. 2c(i)–(iv)], as determined by the shift of the population from being negative to positive for double-stranded DNA breaks [LMP2A/HEL-Tg + Bay (80 % positive) versus LMP2A/HEL-Tg + DMSO (54 % positive)], while the NF-κB inhibitor has little effect on the HEL-Tg B-cells [(HEL-Tg + Bay (64 % positive) versus LMP2A/HEL-Tg + DMSO (65 % positive)] [Fig. 2c(i), (ii)]. These data indicate that resting LMP2A-expressing B-cells are uniquely sensitive to NF-κB inhibition. Since inhibition of NF-κB results in reduced expression of Bcl-2 and not Bcl-xL in

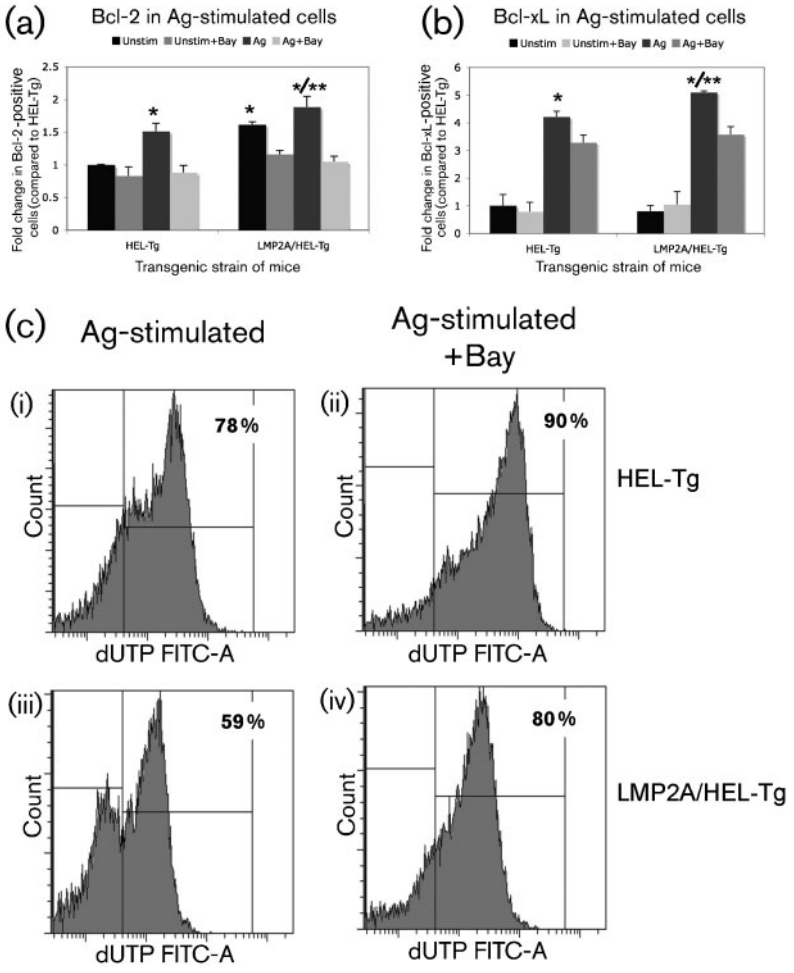

Fig. 3. NF-κB inhibition affects levels of Bcl family members and apoptosis in both LMP2A/HEL-Tg and HEL-Tg B-cells after antigen exposure. (a, b) HEL-Tg and LMP2A/HEL-Tg B-cells were incubated as described above (Fig. 2a, b) and in the presence of HEL (1 μg ml^{-1}). The data represent the fold increase in the percentage of Bcl-expressing B-cells compared with resting, non-antigen-exposed HEL-Tg B-cells and are a combination of three or four mice. *Indicating $P<0.05$ by unpaired Student's *t*-test when compared with DMSO-exposed HEL-Tg B-cells. **Indicating $P<0.05$ by unpaired Student's *t*-test when compared with DMSO-exposed LMP2A/HEL-Tg B-cells. (c) HEL-Tg and LMP2A/HEL-Tg B-cells were incubated for 18 h as described above for Fig. 2(c) using 1 μg HEL ml^{-1}. The B-cells were analysed as described above for Fig. 2(c). The data are representative of three or four mice with similar results.

LMP2A/HEL-Tg B-cells (Fig. 2), it is likely that Bcl-2, and not Bcl-xL, mediates the enhanced survival of LMP2A/HEL-Tg B-cells when compared to HEL-Tg B-cells (Fig. 2c).

Stimulation through the BCR is known to increase NF-κB activation with subsequent activation of Bcl-2 and Bcl-xL to promote cell survival (Gugasyan *et al.*, 2000). HEL-Tg B-cells exposed to antigen show significantly increased Bcl-2 and Bcl-xL levels, which is reversed by the addition of the NF-κB inhibitor (Figs 3a, b). Since these B-cells now express higher levels of Bcl family members, we determined whether exposure of HEL-Tg B-cells to antigen now made the survival of these cells NF-κB-dependent. Antigen-exposed HEL-Tg B-cells demonstrate an increase in apoptosis when exposed to an NF-κB inhibitor [Ag: 78 % versus Ag + Bay: 90 %; Fig. 3c(i), (ii)], suggesting that, in contrast to resting B-cells, antigen-activated B-cells are dependent on NF-κB signalling for survival.

We determined whether antigen-activated LMP2A/HEL-Tg B-cells also require NF-κB-dependent increases in Bcl-2 and Bcl-xL levels for survival. As shown in Fig. 3(a), antigen-activated LMP2A/HEL-Tg B-cells increase Bcl-2 levels that are then reversed by the addition of the NF-κB inhibitor. Additionally, the antigen-induced increase in Bcl-xL levels in LMP2A/HEL-Tg B-cells is reversed by the NF-κB inhibitor (Fig. 3b). Consequently, when these cells are analysed for levels of apoptosis, antigen-activated LMP2A/HEL-Tg B-cells demonstrate an increased level of apoptosis in the presence of the NF-κB inhibitor [Ag: 59 % versus Ag + Bay: 80 %; Fig. 3c(iii), (iv)]. Taken together, these data demonstrate that LMP2A requires NF-κB signalling, and probably also the subsequent activation of Bcl-2 and/or Bcl-xL for survival in the presence of BCR-cross-linking.

The data presented here identify a surprising and unique dependence of LMP2A-expressing B-cells for NF-κB activation. In comparison with previously published findings, the data presented here indicate that the mechanisms by which LMP2A protects non-transformed B-cells from apoptosis may be slightly different from the mechanisms employed by LMP2A in cell lines. In cell lines, LMP2A blocks the induction of apoptosis by blocking BCR signalling (Fukuda & Longnecker, 2005). However, in non-transformed cells LMP2A may constitutively activate NF-κB and Bcl-2 to protect cells from apoptosis.

The idea that LMP2A blocks BCR signalling to inhibit the induction of the lytic cycle provides a model for the role of LMP2A in the maintenance of latency (Longnecker & Miller, 1996). However, more recent evidence indicates that the maintenance of latency and regulation of EBV latency genes are much more complex. Latency genes and the genes for the induction of the lytic cycle can be regulated by cytokines (Kis *et al.*, 2006, 2010; Konforte & Paige, 2009; Konforte *et al.*, 2008; Uchakin *et al.*, 2007), other viral proteins and microRNAs (Dawson *et al.*, 2001; Guasparri *et al.*, 2008; Lung *et al.*, 2009; Stewart *et al.*, 2004) and autoregulation (Anderson & Longnecker, 2008). Therefore, it is likely that many different environmental factors, not just antigens, are important in the regulation of EBV latent infection and the switch to lytic virus replication.

Our data suggest that LMP2A requires NF-κB for the transcription and production of Bcl family members to promote survival. We think that it would be interesting to determine whether LMP2A uses Bcl-2 exclusively in both resting and activated B-cells to protect B-cells from apoptosis. There is evidence that LMP2A may use different Bcl family members under different conditions *in vivo*, since LMP2A only upregulates Bcl-xL in the presence of Myc overexpression (Bultema *et al.*, 2009). However, it is conceivable that the environment in which the latently infected B-cells reside will dictate the usage and/or skewing in favour of using one anti-apoptotic protein or another.

Taken together, these data provide results indicating that LMP2A makes unstimulated B-cells uniquely sensitive to NF-κB inhibition. This provides the basis for the therapeutic targeting of resting, latently infected B-cells expressing LMP2A for eradication of EBV prior to the development of EBV-associated lymphomas.

Acknowledgements

M. S.-M. is supported in part by Midwestern University intramural funds. R. L. is a John Edward Porter Professor in Biomedical Research and supported by the Public Health Service grants CA62234 and CA73507 from the National Cancer Institute.

References

Anderson, L. J. & Longnecker, R. (2008). An auto-regulatory loop for EBV LMP2A involves activation of Notch. *Virology* **371**, 257–266.

Babcock, G. J. & Thorley-Lawson, D. A. (2000). 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 tumors. *Proc Natl Acad Sci U S A* **97**, 12250–12255.

Babcock, G. J., Decker, L. L., Volk, M. & Thorley-Lawson, D. A. (1998). EBV persistence in memory B cells *in vivo*. *Immunity* **9**, 395–404.

Babcock, G. J., Hochberg, D. & Thorley-Lawson, A. D. (2000). 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.

Bultema, R., Longnecker, R. & Swanson-Mungerson, M. (2009). Epstein-Barr virus LMP2A accelerates MYC-induced lymphomagenesis. *Oncogene* **28**, 1471–1476.

Caldwell, R. G., Wilson, J. B., Anderson, S. J. & 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.

Caldwell, R. G., Brown, R. C. & Longnecker, R. (2000). Epstein-Barr virus LMP2A-induced B-cell survival in two unique classes of EmuLMP2A transgenic mice. *J Virol* **74**, 1101–1113.

Dawson, C. W., George, J. H., Blake, S. M., Longnecker, R. & Young, L. S. (2001). The Epstein-Barr virus encoded latent membrane protein 2A augments signaling from latent membrane protein 1. *Virology* **289**, 192–207.

Decker, L. L., Klaman, L. D. & Thorley-Lawson, D. A. (1996). Detection of the latent form of Epstein-Barr virus DNA in the peripheral blood of healthy individuals. *J Virol* **70**, 3286–3289.

Doi, T. S., Takahashi, T., Taguchi, O., Azuma, T. & Obata, Y. (1997). NF-κB RelA-deficient lymphocytes: normal development of T cells and B cells, impaired production of IgA and IgG1 and reduced proliferative responses. *J Exp Med* **185**, 953–961.

Fields, B. N., Knipe, D. M. & Howley, P. M. (2007). In *Field's Virology*, 5th edn, pp. 2656. Philadelphia, PA: Lippincott Williams & Wilkins.

Fukuda, M. & Longnecker, R. (2005). Epstein-Barr virus (EBV) latent membrane protein 2A regulates B-cell receptor-induced apoptosis and EBV reactivation through tyrosine phosphorylation. *J Virol* **79**, 8655–8660.

Grillot, D. A., Merino, R., Pena, J. C., Fanslow, W. C., Finkelman, F. D., Thompson, C. B. & Nunez, G. (1996). bcl-x exhibits regulated expression during B cell development and activation and modulates lymphocyte survival in transgenic mice. *J Exp Med* **183**, 381–391.

Grossmann, M., O'Reilly, L. A., Gugasyan, R., Strasser, A., Adams, J. M. & Gerondakis, S. (2000). The anti-apoptotic activities of Rel and RelA required during B-cell maturation involve the regulation of Bcl-2 expression. *EMBO J* **19**, 6351–6360.

Guasparri, I., Bubman, D. & Cesarman, E. (2008). EBV LMP2A affects LMP1-mediated NF-κB signaling and survival of lymphoma cells by regulating TRAF2 expression. *Blood* **111**, 3813–3820.

Gugasyan, R., Grumont, R., Grossmann, M., Nakamura, Y., Pohl, T., Nesic, D. & Gerondakis, S. (2000). Rel/NF-κB transcription factors: key mediators of B-cell activation. *Immunol Rev* **176**, 134–140.

Hochberg, D., Middeldorp, J. M., Catalina, M., Sullivan, J. L., Luzuriaga, K. & Thorley-Lawson, D. A. (2004). Demonstration of the Burkitt's lymphoma Epstein-Barr virus phenotype in dividing latently infected memory cells *in vivo*. *Proc Natl Acad Sci U S A* **101**, 239–244.

Kis, L. L., Takahara, M., Nagy, N., Klein, G. & Klein, E. (2006). IL-10 can induce the expression of EBV-encoded latent membrane protein-1 (LMP-1) in the absence of EBNA-2 in B lymphocytes and in Burkitt lymphoma- and NK lymphoma-derived cell lines. *Blood* **107**, 2928–2935.

Kis, L. L., Salamon, D., Persson, E. K., Nagy, N., Scheeren, F. A., Spits, H., Klein, G. & Klein, E. (2010). IL-21 imposes a type II EBV gene expression on type III and type I B cells by the repression of C- and activation of LMP-1-promoter. *Proc Natl Acad Sci U S A* **107**, 872–877.

Konforte, D. & Paige, C. J. (2009). Interleukin-21 regulates expression of the immediate-early lytic cycle genes and proteins in Epstein-Barr virus infected B cells. *Virus Res* **144**, 339–343.

Konforte, D., Simard, N. & Paige, C. J. (2008). Interleukin-21 regulates expression of key Epstein-Barr virus oncoproteins, EBNA2 and LMP1, in infected human B cells. *Virology* **374**, 100–113.

Longnecker, R. & Miller, C. L. (1996). Regulation of Epstein-Barr virus latency by latent membrane protein 2. *Trends Microbiol* **4**, 38–42.

Lung, R. W., Tong, J. H., Sung, Y. M., Leung, P. S., Ng, D. C., Chau, S. L., Chan, A. W., Ng, E. K., Lo, K. W. & To, K. F. (2009). Modulation of LMP2A expression by a newly identified Epstein-Barr virus-encoded microRNA miR-BART22. *Neoplasia* **11**, 1174–1184.

Mancao, C. & Hammerschmidt, W. (2007). Epstein-Barr virus latent membrane protein 2A is a B-cell receptor mimic and essential for B-cell survival. *Blood* **110**, 3715–3721.

Miller, C. L., Lee, J. H., Kieff, E., Burkhardt, A. L., Bolen, J. B. & Longnecker, R. (1994). Epstein-Barr virus protein LMP2A regulates reactivation from latency by negatively regulating tyrosine kinases involved in sIg-mediated signal transduction. *Infect Agents Dis* **3**, 128–136.

Pokrovskaja, K., Ehlin-Henriksson, B., Kiss, C., Challa, A., Gordon, J., Gogolak, P., Klein, G. & Szekely, L. (2002). CD40 ligation downregulates EBNA-2 and LMP-1 expression in EBV-transformed lymphoblastoid cell lines. *Int J Cancer* **99**, 705–712.

Portis, T. & Longnecker, R. (2004a). Epstein-Barr virus (EBV) LMP2A alters normal transcriptional regulation following B-cell receptor activation. *Virology* **318**, 524–533.

Portis, T. & Longnecker, R. (2004b). Epstein-Barr virus (EBV) LMP2A mediates B-lymphocyte survival through constitutive activation of the Ras/PI3K/Akt pathway. *Oncogene* **23**, 8619–8628.

Qu, L. & Rowe, D. T. (1992). Epstein-Barr virus latent gene expression in uncultured peripheral blood lymphocytes. *J Virol* **66**, 3715–3724.

Schulze-Luehrmann, J. & Ghosh, S. (2006). Antigen-receptor signaling to nuclear factor κB. *Immunity* **25**, 701–715.

Stewart, S., Dawson, C. W., Takada, K., Curnow, J., Moody, C. A., Sixbey, J. W. & Young, L. S. (2004). Epstein-Barr virus-encoded LMP2A regulates viral and cellular gene expression by modulation of the NF-κB transcription factor pathway. *Proc Natl Acad Sci U S A* **101**, 15730–15735.

Swanson-Mungerson, M. A., Caldwell, R. G., Bultema, R. & Longnecker, R. (2005). Epstein-Barr virus LMP2A alters *in vivo* and *in vitro* models of B-cell anergy, but not deletion, in response to autoantigen. *J Virol* **79**, 7355–7362.

Swanson-Mungerson, M., Bultema, R. & Longnecker, R. (2006). Epstein-Barr virus LMP2A enhances B-cell responses *in vivo* and *in vitro*. *J Virol* **80**, 6764–6770.

Thorley-Lawson, D. A. (2001). Epstein-Barr virus: exploiting the immune system. *Nat Rev Immunol* **1**, 75–82.

Thorley-Lawson, D. A. & Allday, M. J. (2008). The curious case of the tumour virus: 50 years of Burkitt's lymphoma. *Nat Rev Microbiol* **6**, 913–924.

Tierney, R. J., Steven, N., Young, L. S. & Rickinson, A. B. (1994). Epstein-Barr virus latency in blood mononuclear cells: analysis of viral gene transcription during primary infection and in the carrier state. *J Virol* **68**, 7374–7385.

Tsai, S. C., Lin, S. J., Chen, P. W., Luo, W. Y., Yeh, T. H., Wang, H. W., Chen, C. J. & Tsai, C. H. (2009). EBV Zta protein induces the expression of interleukin-13, promoting the proliferation of EBV-infected B cells and lymphoblastoid cell lines. *Blood* **114**, 109–118.

Uchakin, P. N., Stowe, R. P., Paddon-Jones, D., Tobin, B. W., Ferrando, A. A. & Wolfe, R. R. (2007). Cytokine secretion and latent herpes virus reactivation with 28 days of horizontal hypokinesia. *Aviat Space Environ Med* **78**, 608–612.

Weil, R. & Israel, A. (2004). T-cell-receptor- and B-cell-receptor-mediated activation of NF-κB in lymphocytes. *Curr Opin Immunol* **16**, 374–381.