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A new model of LMP1–MYC interaction in B cell lymphoma

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Abstract

Epstein–Barr virus (EBV) is associated with aggressive B cell lymphomas (BCLs). Latent membrane protein 1 (LMP1) of EBV is an oncogenic protein required for EBV B cell transformation. However, LMP1 is a weak oncogene in mice. Mice expressing *Myc* inserted 5' of the E μ enhancer (iMyc^{E μ}), mimicking the t(8;14) translocation of endemic Burkitt lymphoma, develop delayed onset BCLs. To investigate potential cooperation between LMP1 and oncogenic MYC, we produced mice expressing the LMP1 signaling domain via a hybrid CD40–LMP1 transgene (mCD40–LMP1), and the dysregulated MYC protein of aggressive EBV+ BCLs. mCD40–LMP1/iMyc^{E μ} mice trended toward earlier BCL onset. BCLs from mCD40–LMP1/iMyc^{E μ} mice expressed LMP1 and were transplantable into immunocompetent recipients. iMyc^{E μ} and mCD40–LMP1/iMyc^{E μ} mice developed BCLs with similar immunophenotypes. LMP1 signaling was intact in BCLs as shown by inducible interleukin-6. Additionally, LMP1 signaling to tumor cells induced the two isoforms of Pim1, a constitutively active prosurvival kinase implicated in lymphomagenesis.

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Supplementary material available online
Supplementary Figure 1.

Keywords

LMP1; MYC; lymphoma

Introduction

Epstein–Barr virus (EBV), a γ -herpesvirus, infects and maintains lifelong latency in human B cells. The EBV-encoded protein LMP1 (latent membrane protein 1) is expressed early in all EBV infections. LMP1 expression is down-regulated by viral latency programs that promote a lifelong EBV carrier state, but EBV can re-emerge from its latent state and re-express LMP1. LMP1 promotes B cell survival by functioning as a dysregulated CD40 mimic, inducing abnormal cellular proliferation, activation and resistance to apoptosis. Signaling via the LMP1 C-terminal cytoplasmic domain results in amplified and sustained activation of several cell survival pathways, including nuclear factor- κ B (NF- κ B) transcriptional regulators and the kinases PI3K (phosphatidylinositol 3-kinase)/AKT, JNK (C-Jun N-terminal protein kinase), ERK (extracellular signal-regulated kinase) and p38 (reviewed by Graham *et al.* [1]). LMP1 expression is retained in a subset of EBV-associated lymphomas from both immunocompetent and immunocompromised patients; EBV lymphomas are more common in the latter (reviewed by Heslop [2]).

LMP1 *in vivo* effects have been modeled in genetically engineered mice that express different types of LMP1 transgenes [3–6]. Mice in this study expressed the mCD40–LMP1 transgenic protein, consisting of the extracellular and transmembrane portions of mouse CD40 and the C-terminal cytoplasmic domain of LMP1. The MHCII E α promoter directs the expression of this transgene to relevant immune cells (B lymphocytes, macrophages, dendritic cells) that are infected by EBV. Extensive studies of the mCD40–LMP1 chimeric protein demonstrate that it mimics wild-type LMP1 function in immune cells (reviewed in Graham *et al.* [1]; Stunz and Bishop, unpublished data). mCD40–LMP1 transgenic mice on a CD40-deficient (CD40^{−/−}) background have normal antibody responses to immunization, but develop spontaneous, disorganized germinal centers (GCs), autoantibodies and marked lymphosplenomegaly with elevated serum proinflammatory cytokines [3,7]. However, these mice do not develop lymphomas over 2 years of observation (L.L.S. and G.A.B., unpublished data). A similar mouse model that expresses mCD40–LMP1 in CD19⁺ cells on a CD40^{−/−} background lacks the autoimmune phenotype seen when the transgene is also expressed in myeloid cells, but similarly, no accelerated lymphoma development was reported [4]. A third transgene encodes full-length LMP1 behind an immunoglobulin heavy chain enhancer and a V_H promoter in immunocompetent mice. These mice develop low-grade lymphomas at > 1 year of age, with 42% disease penetrance [5]. A fourth model expresses LMP1 in CD19⁺ B cells through excision of a transcriptional/translational STOP cassette via Cre/loxP-mediated recombination in immunocompetent mice. The CD19⁺ LMP1⁺ B cells in these mice are immunologically eliminated, and mice only develop LMP1⁺ tumors after depletion of T cells [6].

Thus, while LMP1 is essential for B cell immortalization after EBV infection, it is a weak B cell oncogene in mouse models. LMP1 may require cofactors such as immune suppression

and/or cooperation with another host or viral oncogene to induce lymphomas. *MYC* is a host oncogene frequently dysregulated due to chromosomal translocations in aggressive EBV+ lymphomas, including endemic Burkitt lymphoma and some cases of diffuse large B-cell lymphoma (reviewed by Slack and Gascoyne [8]). *iMyc^{Eμ}* transgenic mice used in this study carry a gene insertion that mimics the *MYC*-activating t(8;14)(q24;q32) translocation commonly found in endemic Burkitt lymphoma [9]. *iMyc^{Eμ}* mice develop predominately lymphoblastic lymphomas without V_H gene somatic mutation, at the relatively late age of 6–21 months [9,10]. *MYC*-driven lymphomagenesis is tightly correlated to the expression of Pim kinases (Pim1, Pim2, Pim3), which are implicated in prostate cancer progression, leukemogenesis and lymphomagenesis (reviewed by Nawijn *et al.* [11]). LMP1 influences Pim1 function by increasing Pim1 cytoplasmic retention, which increases resistance to the chemotherapeutic agent doxorubicin [12]. In this study, we explored the potential oncogenic interaction of LMP1 with *MYC*, a host oncogene frequently implicated in human B cell malignancies.

Methods

Mice and tumor histology

CD40^{-/-} C57Bl/6 (B6) mice expressing the hybrid mCD40–LMP1 transgene (mCD40–LMP1 mice) are described elsewhere [3]. *iMyc^{Eμ}* B6 mice [9] were intercrossed with CD40^{-/-} B6 mice for 12 generations to generate transgenic mice heterozygous for the *iMyc^{Eμ}* allele and homozygous for the CD40 null allele: CD40^{-/-} *iMyc^{Eμ}* B6 mice (*iMyc^{Eμ}* mice). These mice were crossed with CD40^{-/-} mCD40–LMP1 B6 mice to generate compound transgenic mice: CD40^{-/-} mCD40–LMP1/*iMyc^{Eμ}* B6 mice (mCD40–LMP1/*iMyc^{Eμ}* mice). mCD40–LMP1/*iMyc^{Eμ}* mice and control mice were observed for 12 months for tumor development. Mice were housed in a pathogen-free barrier facility with restricted access, and all procedures were performed as approved by the University of Iowa Animal Care and Use Committee, Iowa City, IA. Mice were euthanized at the time of visible cachexia or tumor development. Spleen, bone marrow, enlarged lymph nodes, kidney, brain, lungs and visible tumors were formalin-fixed and paraffin-embedded for immunohistochemistry. Flow cytometry of cell lines and cells isolated from spleens, livers or lymph nodes was performed using an Accuri C6 flow cytometer (Accuri Cytometers, Inc., Ann Arbor, MI).

Cell lines

The B cell lymphoma line Hal2G1 was derived from a transplanted tumor, harvested from a wild-type B6 mouse injected intraperitoneally (i.p.) 4 weeks earlier with ~10⁶ cells of a primary lymphoma arising in a mCD40–LMP1/*iMyc^{Eμ}* mouse. Hal14, Hal16, Hal17, Hal19 and Hal20 lymphoma cell lines were derived from individual tumor-bearing *iMyc^{Eμ}* mice, whereas Hal12 and Hal18 were derived from individual tumor-bearing mCD40–LMP1/*iMyc^{Eμ}* mice. Mouse CH12.LX B cells stably expressing the human CD40 (hCD40)–LMP1 molecule have been previously described [13]. All B lymphoma cell lines were maintained in RPMI 1640 supplemented with 2 mM L-glutamine, 10 μM 2-mercaptoethanol (2-ME) (Life Technologies), 10% heat-inactivated fetal calf serum (FCS) (Atlanta Biologicals) and antibiotics. This medium is referred to as BCM-10. High Five (Hi-5) insect cells, uninfected

or infected with recombinant baculovirus expressing mouse CD154, were prepared as previously described [14]. Hi-5 cells grow at room temperature, and die to form membrane fragments when cultured at 37°C, so they do not grow in the B cell co-cultures as used here.

Antibodies

S12, a mouse anti-LMP1 immunoglobulin G (IgG) antibody (Ab) directed to the C-terminus of LMP1, was a gift from Dr. Fred Wang (Harvard University, Cambridge, MA). Monoclonal antibodies (mAbs) specific for rat IgG2a (eBR2a), mouse IgG2a κ (isotype control) (eBM2a), mouse/human CD45R (B220) (RA3-6B2), mouse CD21/CD35 (ebio4E3), mouse IgD (11–26), mouse CD11b (M1/70), mouse IgG2b κ control (eBMG2b), mouse CD5 (53-7.3), human/mouse terminal deoxynucleotidyl transferase TdT (19-3), mouse CD19 (MB19-1), mouse IgM (II/41), rat IgG1 control (eBRG1), mouse T- and B-cell activation antigen (GL-7), mouse CD16/32 (to block FcR binding), mouse CD23 (B3B4) and mouse C1qRp (AA4.1) were purchased from eBioscience (San Diego, CA). mAbs specific for mouse CD43 (S7), mouse Ig λ_1 , λ_2 and λ_3 light chain (R26-46) and mouse Ig κ light chain (187.1) were purchased from BD Biosciences (San Jose, CA). The anti-mouse CD40 mAb, 1C10, was produced from a hybridoma provided by Dr. Frances Lund (University of Alabama, Birmingham, AL). The isotype control mAb for 1C10, mAb72, was produced from the 72-2 hybridoma (rat IgG2a isotype control) obtained from the American Type Culture Collection (ATCC, Manassas, VA). Anti-mouse interleukin-6 (IL-6) mAbs (MP5-20F3 and MP5-32C11, biotin-conjugated) used for enzyme-linked immunosorbent assay (ELISA) were obtained from eBioscience.

IL-6 production

To quantify IL-6 production, lymphoma cell lines (1×10^6 cells/mL, 1 mL total volume in a 24-well plate) were cultured with BCM-10 alone, CD154-expressing or parental Hi-5 insect cells (5 mouse:1 insect cell) for 48 h in BCM-10, and the supernatants were analyzed for IL-6 by ELISA as previously described [15].

Pim1 expression assay

Hal2G1, Hal16, Hal17 and Hal18 cells at a concentration of 1×10^6 /mL were stimulated with 5 μ g/mL 1C10 (anti-CD40 mAb) or isotype control Abs for indicated times. Cell lysates were prepared as previously described [16] and lysate proteins separated using 12% sodium dodecylsulfate (SDS)-polyacrylamide gels. Proteins were then transferred onto Immobilon-P polyvinylidene fluoride (PVDF) membranes from Millipore (Billerica, MA). Western blots were probed for the p33 and p44 isoforms of Pim1 kinase using anti-Pim-1 mAb (clone 12H8) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Blotting for mouse actin (anti-mouse actin Ab; Sigma) was used as a loading control. Binding of peroxidase-labeled secondary Abs was visualized using the chemiluminescent detection reagent Supersignal West Pico (Pierce, Rockford, IL). Chemiluminescence was quantified using a Fujifilm LAS-1000 imaging system (Fujifilm Medical Systems, Ltd., Stamford, CT) and Image-Gauge software (FujiFilm Life Sciences, Edison, NJ).

Statistical analysis

Lymphoma-free survival was plotted using Kaplan–Meier curves. A two-sample Kolmogorov–Smirnov test was performed to compare lymphoma-free survival between iMyc^{Eμ} mice and mCD40–LMP1/iMyc^{Eμ} mice.

Results

Impact of co-expression of LMP1 and MYC on lymphoma-free survival

mCD40–LMP1/iMyc^{Eμ} mice observed for up to 12 months showed a trend toward accelerated lymphomagenesis compared to iMyc^{Eμ} mice on the same background: 227 days vs. 271 days, respectively, although this trend did not achieve statistical significance ($p = 0.6953$) (Figure 1). Lymphoma-free survival at < 180 days was not statistically different, although we observed an early separation of the curves ($p = 0.3001$) (Figure 1). mCD40–LMP1/iMyc^{Eμ} mice developed predominantly B220 + lymphoblastic lymphomas [Figures 2(A) and 2(B)], the predominant neoplasm in iMyc^{Eμ} mice [9]. There was one documented case of a CD3 + CD4 + CD5 + CD1d + T cell lymphoma in an iMyc^{Eμ} mouse (data not shown). Importantly, mCD40–LMP1 and CD40^{−/−} littermates did not develop any lymphomas (Figure 1). Lymphoma cells from mCD40–LMP1/iMyc^{Eμ} infiltrated extranodal sites including kidneys [Figure 2(C)], ovaries, liver and bone marrow; no central nervous system (CNS) infiltration was observed (data not shown). Lymphomas from mCD40–LMP1/iMyc^{Eμ} mice retained uniform membrane expression of LMP1 [Figure 2(C)].

Primary LMP1 + tumors arising in mCD40–LMP1/iMyc^{Eμ} mice were easily transplantable into pristane-primed immunocompetent B6 hosts. The Hal2G1 cell line was derived from a lymphoma that developed in a wild-type B6 mouse following pristane-priming and injection i.p. with fresh tumor cells from a lymphoma arising spontaneously in a mCD40–LMP1/iMyc^{Eμ} mouse. Cell lines were also easily developed from lymphomas that arose spontaneously in both mCD40–LMP1/iMyc^{Eμ} and iMyc^{Eμ} mice (Table I). Cell lines derived from mCD40–LMP1/iMyc^{Eμ} mice were cultured *in vitro* for at least 100 passages. Of three cell lines tested, none showed evidence of somatic hypermutation of Ig V_H genes (data not shown). The majority of primary lymphomas and lymphoma cell lines derived from mCD40–LMP1/iMyc^{Eμ} and iMyc^{Eμ} mice expressed IgM and CD5 with either evidence of a B1a B cell origin (IgMhiIgD+CD23–CD5+CD11b+) or aberrant expression of B1a and transitional B cell markers (CD21 and CD23; Hal16 and Hal19) (Figure 2, Table I), Supplementary Figure 1 to be found online at <http://informahealthcare.com/doi/abs/10.3109/10428194.2014.900762>. Additional lymphomas had a mixed B cell phenotype not consistent with a single B cell subtype of origin (Table I).

LMP1 signaling in lymphomas

Spontaneous IL-6 production did not differ significantly between cell lines derived from iMyc^{Eμ} versus mCD40–LMP1/iMyc^{Eμ} mice (Figure 3). However, when mCD40–LMP1-expressing B cell lines (Hal2G1 and Hal18) were incubated with cell membrane-bound CD154, which stimulates the mCD40–LMP1 molecule, we observed significantly increased production of IL-6 (Figure 3).

LMP1 induction of endogenous Pim1 in B cell lymphomas

When stimulated with anti-mouse CD40 mAb, which induces signals delivered via the cytoplasmic domain of LMP1, mCD40–LMP1/iMyc^{Eμ} lymphoma cell lines produced a 2.5-fold increase in Pim1 expression, both p44 and p33 isoforms (Figure 4). Similar LMP1 induction of both isoforms of Pim1 was seen in another mouse B cell lymphoma cell line transfectant, CH12.hCD40–LMP1, which expresses a similar molecule that consists of the extracellular and transmembrane regions of hCD40 with the same LMP1 intracellular C-terminal domain [17] (data not shown). Cell lines derived from iMyc^{Eμ} mice that did not express endogenous CD40 or mCD40–LMP1 had low baseline levels of endogenous Pim1 expression (data not shown). Pim1 levels increased in all cell lines after lipopolysaccharide (LPS) stimulation, a known Pim1 inducer (data not shown).

Discussion

Prior reports demonstrate that LMP1 expression alone is insufficient to induce aggressive B cell lymphomas in mice. We used a biologically relevant oncogene pairing to investigate whether LMP1, a viral oncogene, cooperates with the host oncogene *MYC*, which is commonly dysregulated in EBV+ lymphomas. The combination resulted in a trend toward accelerated lymphoma development. Lymphomas derived from mCD40–LMP1/iMyc^{Eμ} mice retained strong LMP1 expression on a mouse genetic background that permits high-affinity antibody production and normal T cell function [3]. The CD40^{−/−} background of our mice was chosen to enhance the mCD40–LMP1 phenotype of lymphosplenomegaly, but was not necessary to allow emergence of LMP1 + tumors. Mice bred to express a functional CD40 transgene as well as the mCD40–LMP1 and *MYC* transgenes also develop LMP1 + lymphomas (unpublished results). Thus, LMP1 immunogenicity did not prevent development of mature LMP1 + B cells, as in the Zhang mouse model that expressed full-length LMP1 from an early stage of B cell development [6]. The immunogenic portion of LMP1 in Zhang's mouse model was not identified. Results in our new model suggest that the C-terminal intracellular portion of LMP1 does not contain peptides immunogenic for B6 mice. In humans, LMP1 is considered a weak CD8 and CD4 T cell immunogen compared to EBV nuclear antigen (EBNA) proteins, and the predominant CD4 T cell LMP1 epitope is found in the C-terminal portion [18,19]. Evidence for the low immunogenicity of LMP1 in humans is supported by LMP1 expression in EBV-associated lymphomas in immunocompetent individuals (i.e. EBV+ Hodgkin lymphomas, diffuse large B-cell lymphomas, T and natural killer [NK] lymphomas) [2,20].

Similar to prior *Myc* mouse lymphoma models, lymphomas from LMP1/iMyc^{Eμ} mice lack evidence of Ig class switch recombination or somatic hypermutation and express surface markers that suggest they derive from pre-GC B cells. This suggests that *MYC* expression dominates over LMP1 in influencing B cell tumor origin in LMP1/iMyc^{Eμ} mice. Human EBV+ lymphomas are thought to predominantly derive from GC B cells. In a natural EBV infection of B cells, LMP1 is expressed with another oncogene, LMP2a, which mimics B-cell receptor signaling [21]. Double-transgenic LMP1/LMP2A mice do not develop accelerated spontaneous lymphomas [22], but demonstrate a restoration of LMP1 + cells trafficking into the GC. Lymphoma development after natural EBV infection may thus be

facilitated in part by LMP1, which confers a B cell survival advantage, and by LMP2a, which allows the EBV-infected B cell to traffic to the GC. The ability of LMP1 to enhance the expression of activation-induced deaminase [23] could then promote *MYC* gene translocations in EBV infected GC B cells, resulting in the *MYC* dysregulation frequently seen in EBV+ lymphomas.

IL-6 cooperates with *MYC* in an IL-6/iMyc^{Eμ} transgenic Balb/c mouse model to accelerate the development of plasma cell neoplasms and increase disease penetrance to 100% [24]. mCD40–LMP1 mice have elevated basal levels of serum IL-6 [3], but levels are 10–50-fold lower than in IL-6 transgenic mice [25]. Thus, very high serum IL-6 may be needed to achieve 100% lymphoma penetrance, accounting for the more modest trend toward increased disease penetrance in mCD40–LMP1/iMyc^{Eμ} mice. Additionally, B6 mice are less susceptible than Balb/c to IL-6-induced tumorigenesis, and the latter are particularly prone to the development of plasmacytomas [26,27].

The prosurvival effects of the Pim kinases are mediated through inactivation of proapoptotic and cell cycle inhibitory proteins (reviewed by Brault *et al.* [28]). Wild-type LMP1 constitutively signals when assembled at cellular membranes and has a half-life of 4–8 h [29]. Using our inducible model of mCD40–LMP1-induced signaling, we show LMP1-mediated expression of both isoforms of Pim1 after 2 h, well within the surface membrane half-life of wild-type LMP1, and with similar kinetics to CD40 induction of Pim1 expression (2 h) [30]. Thus, Pim1 may be an important *in vivo* mediator of LMP1-mediated prosurvival effects that contribute to B cell tumorigenesis.

This study investigated the biologically relevant pairing of a virally derived oncogene, LMP1, believed to be the most potent EBV oncogene, with a commonly dysregulated host oncogene, *MYC*, demonstrating LMP1 expression in an aggressive B cell lymphoma model in immunocompetent hosts. We show for the first time that LMP1 can induce endogenous B cell Pim1, a prosurvival kinase, implicated in lymphoma development. The consistent histology and transplantability of derived LMP1 + lymphoma cell lines represent a robust model to test small molecule inhibitors, such as Pim inhibitors, for EBV-associated lymphomas characterized by LMP1 expression and *MYC* dysregulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Lymphoma-free survival of mCD40-LMP1/iMyc^{Eμ} mice. Kaplan-Meier survival analysis of mice of indicated genotypes. Median lymphoma-free survival for mCD40-LMP1/iMyc^{Eμ} mice and iMyc^{Eμ} mice was 227 and 271 days, respectively ($p = 0.6953$ as analyzed by two-sample Kolmogorov-Smirnov test). None of the mCD40-LMP1 or CD40^{-/-} littermates developed lymphoma. All mice in the cohort were on a CD40^{-/-} background.

Figure 2.

Representative lymphoma from a mCD40-LMP1/iMyc^{Eμ} transgenic mouse. (A) Lymphoblastic lymphoma histology. Splenic white pulp is expanded and red pulp infiltrated by neoplastic round cells. Neoplastic cells are round to polygonal with large nuclei containing 1–2 large nucleoli, often adhered to the nuclear membrane. The mitotic rate is high and macrophages containing apoptotic bodies are present. Hematoxylin and eosin (HE), magnification $\times 200$, bar = 100 μm . (Inset: HE, $\times 600$.) (B) B220 staining. Neoplastic cells are B220 +. B220, magnification $\times 200$, bar = 100 μm . (Inset: B220, $\times 600$.) (C) LMP1 staining. LMP1 + tumor cells are effacing and infiltrating the kidney. LMP1, magnification $\times 200$, bar = 100 μm . (Inset: LMP1, $\times 600$.) All images were obtained with an Olympus BX51 microscope, attached Olympus DP72 camera and MicroSuite Pathology Software (Olympus). (D) Immunophenotyping of representative lymphoma. Representative lymphoma flow cytometry from a mCD40-LMP1/iMyc^{Eμ} mouse. Black solid lines indicate antibody-specific staining and gray dashed lines indicate antibody isotype controls.

Figure 3.

LMP1-induced IL-6 production in lymphoma cell lines derived from mCD40–LMP1/iMyc^{Eμ} mice. Hal2G1 and Hal18 lymphoma cell lines were derived from separate mCD40–LMP1/iMyc^{Eμ} mice and express the mCD40–LMP1 transgene but no native mouse CD40. Hal2G1 and Hal18 were stimulated with cells expressing CD154, which stimulates the mCD40–LMP1 protein to signal via the cytoplasmic LMP1 tail. The Hal17 lymphoma cell line was derived from an iMyc^{Eμ} mouse, and does not express the mCD40–LMP1 transgene or endogenous mouse CD40. Triplicate replicates of each lymphoma cell line were cultured in BCM-10 alone (BCM), with control insect cells (control) or with insect cells expressing CD154 (CD154). Supernatants from 48 h time-points were collected and analyzed by mouse IL-6 ELISA. Data shown are representative of five independent experiments.

Figure 4.

Pim1 expression in lymphoma cell lines derived from mCD40–LMP1/iMyc^{Eμ} mice. (A) Inducible Pim1 expression. Hal2G1 lymphoma cells were stimulated with isotype control antibody (Iso) for 2 h or stimulated with agonistic anti-mouse CD40 antibody for the indicated times to induce the mCD40–LMP1 protein to signal via the cytoplasmic LMP1 tail. Cytoplasmic extracts were separated by SDS-PAGE and then analyzed by immunoblotting with an anti-Pim1 antibody that recognized both Pim1 p44 and p33 isoforms. As a loading control, blots were stripped and reprobed with anti-actin antibody. (B) Pim1 fold induction. Pim1 p44, Pim1 p33 and actin values were quantitated by densitometry. Pim1 densitometry values (arbitrary units [AUs]) for each isoform were

divided by actin AUs for each lane. To calculate fold induction, Pim1 AU/actin AU values were normalized to the 0 h (Pim1 AU/actin AU) value which was arbitrarily normalized to 1. Similar results were seen in two other cell lines (Hal18 and hCD40–LMP1 CH12.LX cells), which also express an inducible LMP1 transgene (data not shown). Data shown are representative of six independent experiments.

Table I

Immunophenotype of primary lymphomas and derived cell lines.

Lymphoma		4018/1*			4005/2 [†]			4003/1		
	Hal2G1	Hal12	Hal18*	Hal14	Hal16 [‡]	Hal17	Hal19	Hal20	Hal21	
Cell line Genotype	LMP1/iMyc ^{Eu}	LMP1/iMyc ^{Eu}	LMP1/iMyc ^{Eu}	iMyc ^{Eu}	iMyc ^{Eu}	iMyc ^{Eu}	iMyc ^{Eu}	iMyc ^{Eu}	iMyc ^{Eu}	
B220	+	+	+	+	+	+	+	+	+	
CD19	+	+	+	+	+	+	+	+	—	
κ lite	+	+	+	+	+	—	+	+	+	
IgM	—	hi	hi	hi	+	+/—	+	+	+	
IgD	—	—	+	—	+	+/—	+	—	—	
AA4.1	+	ND	—	ND	—	ND	—	—	—	
CD21	—	ND	—	ND	—	—	+	—	—	
CD23	lo	ND	—	ND	lo	—	+	—	—	
TdT	—	—	—	—	—	—	—	—	—	
GL7	—	—	—	—	—	—	—	—	—	
CD5	+	—	+	—	+	+	+	+	+	
CD11b	—	ND	+	—	ND	+	—	—	—	
B cell phenotype [‡]	Mixed	Imm	B1a	Imm	Mixed	B1a/T2	Mixed	B1a/T2	Mixed	

IgM, immunoglobulin M; TdT, terminal deoxynucleotidyl transferase; ND, not determined.

* Cell line Hal18 was derived from mouse 4018/1.

† Cell line Hal16 was derived from mouse 4005/2.

‡ B1a phenotype: IgMhiIgD + CD23CD5 + CD11b +, T2 phenotype: AA4.1 + CD21 + CD23 +, Immature (Imm) phenotype: B220 + κ light chain + IgM+ IgD-, Mixed B cell phenotype denotes an immunophenotype not consistent with a single B cell subtype (see text).