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Epstein-Barr Virus Latent Membrane Protein (LMP1) and Nuclear Proteins 2 and 3C Are Effectors of Phenotypic Changes in B Lymphocytes: EBNA-2 and LMP1 Cooperatively Induce CD23

FRED WANG,[1]* CHRISTOPHER GREGORY,[2] CLARE SAMPLE,[1] MARTIN ROWE,[2] DAVID LIEBOWITZ,[1] RUTH MURRAY,[2] ALAN RICKINSON,[2] AND ELLIOTT KIEFF[1]

Departments of Medicine and Microbiology and Molecular Genetics, Harvard Medical School, 75 Francis Street, Boston, Massachusetts 02115,[1] and Cancer Research Campaign Laboratories, Department of Cancer Studies, The Medical School, The University of Birmingham, Birmingham B15 2TJ, England[2]

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Latent Epstein-Barr virus (EBV) infection and growth transformation of B lymphocytes is characterized by EBV nuclear and membrane protein expression (EBV nuclear antigen [EBNA] and latent membrane protein [LMP], respectively). LMP1 is known to be an oncogene in rodent fibroblasts and to induce B-lymphocyte activation and cellular adhesion molecules in the EBV-negative Burkitt's lymphoma cell line Louckes. EBNA-2 is required for EBV-induced growth transformation; it lowers rodent fibroblast serum dependence and specifically induces the B-lymphocyte activation antigen CD23 in Louckes cells. These initial observations are now extended through an expanded study of EBNA- and LMP1-induced phenotypic effects in a different EBV-negative B-lymphoma cell line, BJAB. LMP1 effects were also evaluated in the EBV-negative B-lymphoma cell line BL41 and the EBV-positive Burkitt's lymphoma cell line, Daudi (Daudi is deleted for EBNA-2 and does not express LMP). Previously described EBNA-2- and LMP1-transfected Louckes cells were studied in parallel. EBNA-2, from EBV-1 strains but not EBV-2, induced CD23 and CD21 expression in transfected BJAB cells. In contrast, EBNA-3C induced CD21 but not CD23, while no changes were evident in vector control-, EBNA-1-, or EBNA-LP-transfected clones. EBNAs did not affect CD10, CD30, CD39, CD40, CD44, or cellular adhesion molecules. LMP1 expression in all cell lines induced growth in large clumps and expression of the cellular adhesion molecules ICAM-1, LFA-1, and LFA-3 in those cell lines which constitutively express low levels. LMP1 expression induced marked homotypic adhesion in the BJAB cell line, despite the fact that there was no significant increase in the high constitutive BJAB LFA-1 and ICAM-1 levels, suggesting that LMP1 also induces an associated functional change in these molecules. LMP1 induction of these cellular adhesion molecules was also associated with increased heterotypic adhesion to T lymphocytes. The Burkitt's lymphoma marker, CALLA (CD10), was uniformly down regulated by LMP1 in all cell lines. In contrast, LMP1 induced unique profiles of B-lymphocyte activation antigens in the various cell lines. LMP1 induced CD23 and CD39 in BJAB; CD23 in Louckes; CD39 and CD40 in BL41; and CD21, CD40, and CD44 in Daudi. In BJAB, CD23 surface and mRNA expression were markedly increased by EBNA-2 and LMP1 coexpression, compared with EBNA-2 or LMP1 alone. This cooperative effect was CD23 specific, since no such effect was observed on another marker, CD21. S1 analyses revealed that BJAB cells express low levels of FcεRIIa CD23 mRNA, and FcεRIIb CD23 mRNA was not detectable. LMP1 preferentially increases FcεRIIb CD23 mRNA. EBNA-2 expression alone in BJAB increases the constitutively expressed FcεRIIa CD23 mRNA. However, when coexpressed with LMP1, EBNA-2 increases total CD23 mRNA without altering the high relative abundance of FcεRIIb to FcεRIIa CD23 mRNA induced by LMP1. Thus, LMP1 likely activates the FcεRIIb CD23 promoter, while EBNA-2 more likely transactivates a regulatory element common to both the FcεRIIa and FcεRIIb promoters.

Epstein-Barr virus (EBV) is associated with Burkitt's lymphoma (27), nasopharyngeal carcinoma (25), and lymphoproliferative disorders in immunocompromised individuals (4). In vitro, EBV infection results in human B-lymphocyte activation and perpetual proliferation (15, 37). EBV-infected B lymphocytes grow in tight cell clumps and express a number of B-cell activation molecules, including CD23, CD30, CD39, CD40, CD44, and cellular adhesion molecules such as ICAM-1, LFA-1, and LFA-3 (3, 19, 24, 33, 40, 42, 44, 48). The phenotype of these EBV-immortalized cells is similar to antigen- or mitogen-activated B lymphocytes (32). Thus, the mechanism by which specific EBV genes cause B lymphocyte activation and proliferation is likely to be important not only in understanding EBV-induced growth transformation and its role in oncogenesis but also in dissecting the events of B-lymphocyte activation.

EBV-induced B-lymphocyte growth transformation is associated with the expression of six nuclear proteins, EBNA-1, -2, -3A, -3B, -3C, and -LP, and three membrane proteins, LMP1, LMP2A, and LMP2B (reviewed in reference 21). The effects of EBNA-1, EBNA-2, EBNA-3A, and EBNA-LP or of LMP1 have been assayed in rodent fibroblasts (6, 49, 50) or in an EBV-negative B-lymphoma cell line, Louckes (51, 52), in which the individual EBV genes were expressed by gene transfer under control of heterologous promoters. LMP1 reduces serum dependency, contact inhibition, and anchorage-dependent growth and increases tumorigenicity of certain rodent fibroblast cell lines (49, 50). In contrast, EBNAs did not affect rodent fibroblast growth, except for EBNA-2, which slightly reduced serum dependency (6). In

* Corresponding author.

the Louckes B-lymphoma cell line, LMP1 induced broad phenotypic changes, including growth in large tight cell clumps; increased LFA-1, ICAM-1, and LFA-3 adhesion molecules; and increased CD23 and transferrin receptor expression (51). In contrast, EBNA-2 specifically induced CD23 and growth in cell clumps without increased LFA-1, ICAM-1, or LFA-3 adhesion molecule expression (52). Although EBNA-1 is necessary for EBV episome maintenance and replication (53), EBNA-1, as well as EBNA-3A or EBNA-LP, had no direct effect on B-lymphoma cell growth (52).

The B-cell activation marker CD23 has been closely associated with EBV infection. CD23 was discovered because of the high-level induction in EBV-infected lymphocytes (24, 48). However, it is also expressed early after B-cell activation by antigen, mitogen, or interleukin 4 stimulation (7, 23, 48). CD23 is closely associated with an EBV immortalizing function, since infection with the nontransforming EBV strain P3HR-1 does not induce CD23 (2) and only EBV-infected B lymphocytes which express CD23 are capable of establishing immortalized cell lines (47). EBNA-2 can specifically up regulate CD23 expression (52). Recent experiments also demonstrate that EBNA-2 is essential for EBV-induced growth transformation (5, 12). CD23 induction may, therefore, be an important pathway for EBNA-2 function and EBV infection.

Initial analysis of individual EBV gene effects in B-lymphoma cells have identified pathways by which EBV may alter B-lymphocyte growth. However, B-lymphoma cell lines vary in their stage of differentiation and may thus respond differently to specific EBV genes. We therefore undertook a series of experiments to broadly characterize LMP1 or EBNA effects in other B-lymphoma cell lines. The BJAB cell line was of particular interest since it is a B-cell lymphoma which differs from Louckes in not having a *myc* translocation (26, 31), in being unable to grow subcutaneously in nude mice (9), and in having low constitutive CD23 expression. Because of the close association of CD23 with EBV infection, we examined the CD23 induction in detail.

MATERIALS AND METHODS

Cell lines. IB4 is a latently EBV-infected lymphoblastoid cell line (LCL). Daudi is an EBV-infected Burkitt's lymphoma cell line which is deleted for EBNA-2 and a portion of EBNA-LP and fails to express LMP1 (14, 20, 28). BL41 (kindly provided by G. Lenoir [2]) and Louckes (52) are EBV-negative Burkitt's lymphoma cell lines, and BJAB (kindly provided by G. Klein [31]) is an EBV-negative B-lymphoma cell line. All cell lines were maintained in RPMI 1640 with 10% fetal calf serum.

Plasmid constructs. Recombinant derivatives of the expression vector pZipNEOSV(X) containing the open reading frames for EBNA-1, type 1 and type 2 EBNA-2, EBNA-3C, EBNA-LP, and T65 have been previously described (6, 35, 52). This vector contains the neomycin phosphotransferase gene providing resistance to the neomycin analog G418. The pSV2gpt vector used for the LMP1 expression has also been previously described in detail (49) and encodes the guanine phosphoribosyltransferase gene (*gpt*) providing resistance to mycophenolic acid.

Transfections. EBNA- and LMP1-expressing clones of the BJAB or BL41 cell line were generated by electroporation with the Progenetor II (Hoefer Scientific Instruments). Briefly, 10 μg of plasmid DNA was mixed with 5×10^6 cells in 0.5 ml of phosphate-buffered saline at 4°C for 10 min. Cells were pulsed at 200 to 300 V at 1,080 μF for 1 ms, placed on ice for an additional 5 min, and then incubated at 37°C in RPMI with 10% fetal bovine serum. Two days after electroporation, transfected cells were selected in RPMI 1640 with 10% fetal bovine serum containing either 3 mg of G418 (GIBCO) per ml or 3 μg of mycophenolic acid–10 μg of hypoxanthine–160 μg of xanthine per ml. Individual clones were screened by immunoblot and immunofluorescence for EBV gene expression. Transfected cell clones were maintained in the appropriate selective media. Dual EBNA-2 and LMP1 transfectants were derived in a similar manner. Established EBNA-2-expressing clones were electroporated with the LMP1 or vector control plasmid and then cloned in the presence of mycophenolic acid-hypoxanthine-xanthine. Dual-transfected clones were then subsequently maintained in the presence of both G418 and mycophenolic acid-hypoxanthine-xanthine.

Immunoblotting. Cell lysates, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, electrophoretic transfer to nitrocellulose, and immunoblotting were performed as previously described (6). Pooled EBV-immune human sera, followed by ^{125}I-protein A, were used for detection of EBNAs. An EBNA-2-specific monoclonal antibody, PE2, was also used to detect EBV type 1 and type 2 EBNA-2 (55). LMP1 immunoblots were developed with the monoclonal antibody S12 (30), followed by a rabbit anti-mouse immunoglobulin G antibody and ^{125}I-labeled protein A.

Surface immunofluorescence and flow cytometry. Cell surface immunofluorescence was performed on live cells by using saturating amounts of monoclonal antibodies followed by fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin. Quantitative analysis was performed on a FACS 4 flow cytometer (Becton Dickinson and Co.), and results were expressed as mean fluorescence intensity per volume ratio of 20,000 cells. Monoclonal antibodies used and their sources were as follows: CD10/CALLA (monoclonal antibody 55), III International Workshop on Leukocyte Differentiation Antigens (11); CD11a/LFA-1 alpha chain (MHM24) and CD18/LFA-1 beta chain (MHM23), A. J. McMichael (18); CD21 (HB5), T. Tedder (46) or (Bu32, Bu33, Bu34, Bu35, and Bu36) N. R. Ling (IV International Workshop on Leukocyte Differentiation Antigens); CD23 (MHM6), our (A.R.) laboratory (42) or (EBVCS 4) B. Sugden (24); CD30 (Kil), M. Stein (44); CD39 (AC2), our (M.R.) laboratory (42); CD40 (G28.5), E. A. Clark (3); CD44 (Hermes-1), E. C. Butcher (19); and CD54/ICAM-1 (RR1/1) and CD58/LFA-3 (TS2/9), T. A. Springer (40, 43).

Conjugate formation. Conjugate formation between BL41, Daudi, and BJAB target cells and EBV-specific but human leukocyte antigen class I-mismatched cytotoxic T cells was measured by a rapid in vitro assay as previously described (51).

Northern blotting. Cells were lysed in 0.5% Nonidet P-40–10 mM NaCl–10 mM Tris (pH 7.4)–3 mM $MgCl_2$. Nuclei were separated by centrifugation. The cytoplasmic fraction was adjusted to 1% sodium dodecyl sulfate–100 mM Tris–0.2 M NaCl, extracted multiple times with phenol and chloroform, and ethanol precipitated. A 15-μg portion of cytoplasmic RNA was separated on a 1% agarose-formaldehyde gel, transferred to Genescreen (Amersham Corp.), and hybridized with randomly primed ^{32}P-labeled probes (Amersham). The FcεRIIa cDNA, gamma actin DNA, and CD21 cDNA used for probes were kindly provided by H. Kikutani, Keith Robbins, and Mark Birkenbach, respectively.

S1 analysis. S1 analysis for CD23 mRNA forms was performed as described by Yokota et al. (54). The 5′ *Eco*RI-

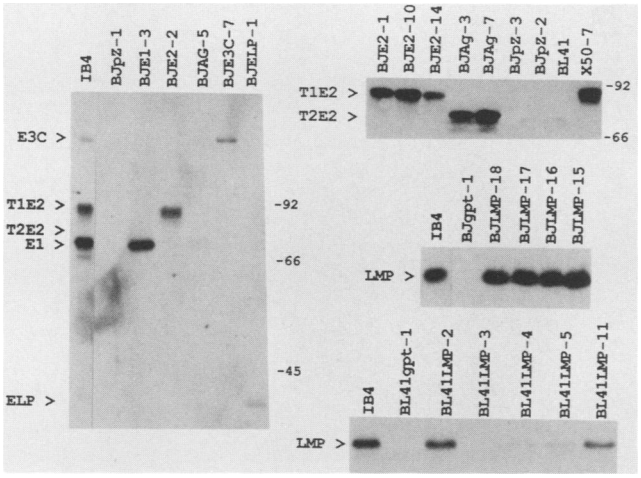

FIG. 1. Immunoblot of EBNA and LMP1 expression in BJAB and BL41 cells. The left panel is an immunoblot with EBV-immune human sera for EBNA expression. The upper right immunoblot was probed with the PE2 monoclonal antibody, which detects the 87-kilodalton EBV type 1 EBNA-2 (T1E2) and the 72-kilodalton EBV type 2 EBNA-2 (T2E2). The two panels at the lower right were probed with the S12 monoclonal antibody for LMP1. IB4 and X50-7 are latently EBV-infected lymphoblastoid cell lines. BJAB and BL41 are EBV-negative B-cell lines. Representative BJAB clones expressing vector control (BJpZ-1), EBNA-1 (BJE1-3), EB1NA-2 (BJE2-2), EB2NA-2 (BJAg-1), EBNA-3C (BJE3C-7), and EBNA-LP (BJELP-1) are shown. EB2NA-2 expression was underrepresented in the left immunoblot by using type 1 EBV-immune human sera. Comparable EB1NA-2 (BJE2-1, -10, and -14) and EB2NA-2 (BJAg-3 and -7) expression levels are demonstrated in the upper right immunoblot probed with an EBNA-2 monoclonal antibody. Transfected BJAB clones expressing the 60-kilodalton LMP1 (BJLMP-15, -16, -17, and -18) are shown in the middle right immunoblot. Vector control (BL41gpt-1), LMP1-nonexpressing (BL41LMP-3), LMP1-low-expressing (BL41LMP-4 and -5), and LMP1-high-expressing (BL41LMP-2 and -11) BL41 clones are shown in the lower right immunoblot.

*Hin*dIII fragment of the FcεRIIa cDNA (22) was cloned into Bluescript (Stratagene), and a single-stranded probe was synthesized by using an oligonucleotide primer and Klenow fragment in the presence of [^{32}P]dCTP. Approximately 30 μg of cytoplasmic RNA was incubated with the single-stranded probe overnight at 30°C; it was digested with S1 nuclease the following day and was separated by polyacrylamide-urea gel electrophoresis.

RESULTS

EBNA effects in BJAB lymphoma cells. Type 1 and 2 EBV strains differ in their EBNA-2 genes. The type 1 EBV latent nuclear protein EBNA-1, EBNA-2 (EB1NA-2), EBNA-3C or EBNA-LP or the type 2 EBV EBNA-2 (EB2NA-2) was expressed in BJAB cells by transfection of the pZipNEO expression vector. By immunoblotting, EBNA-1, EBNA-3C, and EBNA-LP expression levels were equal to or slightly greater than in latently infected LCLs. EB1NA-2 and EB2NA-2 were expressed at approximately 50 to 75% LCL levels (Fig. 1; see also Fig. 4). These clones are subsequently designated by their EBNA expression, which was monitored by immunoblotting in parallel with phenotypic characterization. At least three independently derived BJAB clones expressing each of the nuclear proteins or vector control were assayed for B-lymphocyte activation

TABLE 1. Surface phenotype of EBNA-expressing BJAB cells[a]

Transfected clone	B-cell antigens		Activation antigens			
	CD10	CD21	CD23	CD30	CD39	CD44
Control						
pZ-1	23.7	2.3	0.3	0.0	0.3	0.0
pZ-2	2.8	1.7	0.9	0.1	2.0	0.1
pZ-3	34.4	3.0	0.5	0.0	0.7	0.1
EBNA-1						
E1-2	10.0	0.9	0.4	0.2	1.8	0.1
E1-3	23.4	1.5	0.3	0.0	0.9	0.0
E1-4	23.2	1.5	1.0	0.0	0.2	0.2
EB1NA-2						
E2-2	10.7	107.6	16.9	0.2	2.9	0.7
E2-11	28.7	120.0	14.8	0.8	4.3	1.7
E2-15	29.8	72.5	9.5	0.0	2.5	1.6
EB2NA-2						
AG-1	28.8	8.6	2.0	0.0	1.6	1.3
AG-2	30.0	8.2	2.0	0.1	0.9	0.1
AG-7	17.6	12.9	3.5	0.0	0.9	0.0
EBNA-3C						
E3C-3	25.0	25.1	1.3	0.2	2.6	0.0
E3C-4	34.8	43.2	0.0	0.0	1.6	0.0
E3C-7	27.1	41.0	0.0	0.0	2.2	0.0
EBNA-LP						
T65-4	33.6	6.3	1.7	0.0	1.4	0.0
T65-8	36.7	3.9	1.7	0.0	1.6	0.5
ELP-1	18.1	6.3	1.1	0.0	1.2	0.0
ELP-4	14.3	3.9	2.6	0.4	2.4	1.1
ELP-9	36.5	6.7	1.2	0.0	0.9	0.0
ELP-12	20.2	5.3	0.1	0.0	0.5	0.0

[a] Values are expressed as mean fluorescence intensities. BJAB cells expressing EBNA-LP only were derived either with a vector containing the T65 cDNA (T65-4 and -8) or the EBNA-LP open reading frame only (ELP-1, -4, -9, and -12).

marker (CD21, CD23, CD30, CD39, CD40, and CD44), cell adhesion molecule (LFA-1, ICAM-1, and LFA-3), and the Burkitt's lymphoma marker, CD10, surface expression by indirect immunofluorescence with monoclonal antibodies and fluorescence-activated cell surface analysis. EB1NA-2 (total 8 clones) consistently induced low-level CD23 expression, compared with essentially undetectable levels in vector control cells (3 representative clones are shown in Table 1). This level is markedly less than the CD23 level ordinarily expressed in LCLs (mean fluorescence intensity of 6 LCLs = 163). EB1NA-2 also induced high-level CD21 expression in all clones (Table 1). Although we had previously reported no CD21 induction in EB1NA-2 Louckes cells (52), Louckes cells express higher constitutive CD21 levels. Reexamination of the EB1NA-2 Louckes cells revealed that there was indeed an EB1NA-2 induction of CD21 above the elevated baseline values (mean fluorescence intensity = 136 versus vector control mean fluorescence intensity = 66). In contrast, EB2NA-2 (total, 5 clones) had only a slight effect on CD21 expression and no significant effect on CD23 expression in BJAB cells (Table 1). EBNA-3C (total, 4 clones) induced moderate-level CD21 expression but had no effect on CD23 (Table 1). Other surface markers, including CD10, CD30, CD39, CD40, CD44, LFA-1, ICAM-1, and LFA-3 were unaffected by EBNA expression (Table 1 and data not shown). The surface marker profiles of EBNA-1 and EBNA-LP clones were essentially indistinguishable from vector control cells.

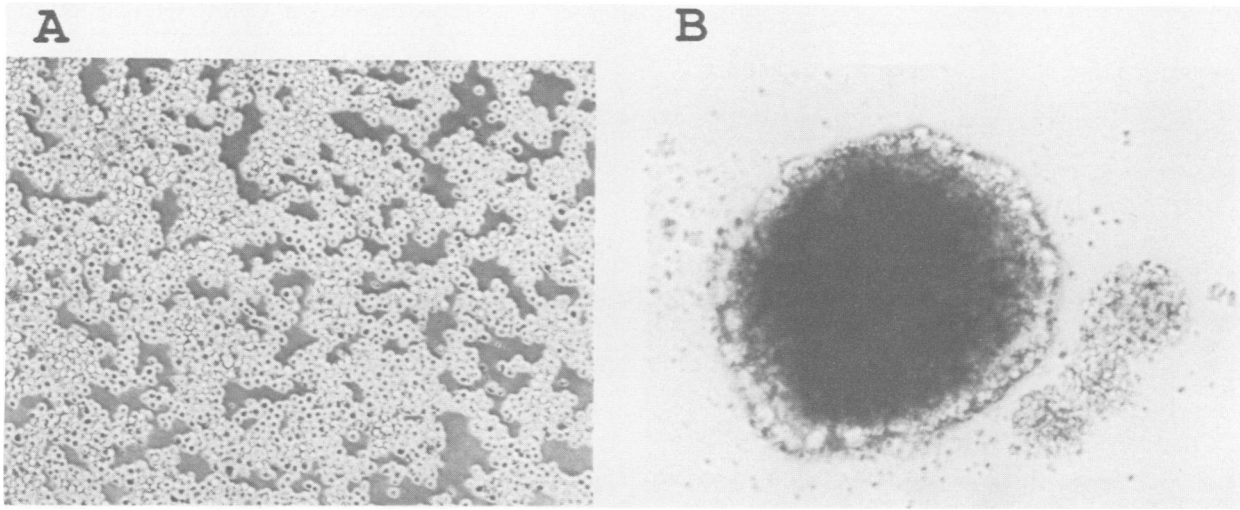

FIG. 2. Homotypic adhesion of LMP1-expressing and vector control BJAB cells. LMP1-expressing (BJLMP-15) or vector control (BJgpt-2) BJAB cells were suspended as single cells, cultured overnight, and then photographed under phase-contrast microscopy. All BJAB LMP1 and vector control clones demonstrated phenotypes similar to these representative clones.

LMP1 effects in BJAB lymphoma cells. An appropriate level of LMP1 expression in BJAB cells required use of the human metallothionein type II promoter. This vector uses the guanine phosphoribosyltransferase gene (*gpt*) as a positive selection marker, and a different set of vector control clones was derived in parallel (BJgpt). These controls (BJgpt) did not differ in phenotype from the EBNA vector control clones (BJpZ). LMP1 expression caused growth of BJAB cells in large, dense, tight cell clumps (Fig. 2), as had previously been observed for Loukes LMP1 cells. The dramatic LMP1-induced homotypic adhesion in all clones was associated with minimally increased LFA-1 or ICAM-1 adhesion molecule expression above the high background level already expressed in BJAB cells (Table 2). LMP1 also induced moderate CD23 and CD39 expression (Table 2). The LMP1-induced CD23 and CD39 levels were still significantly less than normally observed in LCLs. CD21, CD30, and CD44 were not induced. Surprisingly, LMP1 markedly and consistently down regulated CD10 expression (Table 2).

LMP1 effects in other B-lymphoma cell lines. BL41 is an EBV-negative Burkitt's lymphoma cell line and expresses low levels of activation antigens similar to BJAB (2). However, BL41 differs from BJAB by its constitutively lower CD40 and cellular adhesion molecule expression and higher CD10 expression. LMP1 and control BL41 clones were derived, two of which (BL41LMP-2 and -11; Fig. 1) expressed high LMP1 levels comparable to LCL levels and two of which (BL41LMP-4 and -5; Fig. 1) expressed approximately 50% of the LCL LMP1 level. As previously noted with Loukes cells (51), the LMP1 level directly correlated with the degree of homotypic adhesion. High-level LMP1 clones (BL41LMP-2 and -11) grew in large tight cell clumps, while low-level LMP1 clones (BL41LMP-4 and -5) showed moderate homotypic adhesion (data not shown). This was accompanied by a significant ICAM-1 induction and, to a lesser extent, LFA-1 induction (Table 2). LFA-3 was also markedly induced. Among activation markers, CD40 was induced in all LMP1 clones (Table 2) and CD39 was induced slightly by high LMP1 expression levels (Table 2; BL41LMP-2 and -11). CD21, CD23, CD30, and CD44 were unaffected by LMP1 expression in BL41 cells. CD10 was again down regulated by LMP1 expression, most markedly by high-level LMP1 expression (Table 2; BL41LMP-2 and -11).

These analyses were also extended to previously described Loukes (51) and Daudi (28) high-level LMP1-expressing clones. In both cell lines LMP1 also induced marked homotypic adhesion accompanied by high-level LFA-1, ICAM-1, and LFA-3 expression (Table 2). CD23 was induced in Loukes, while CD21, CD40, and CD44 were induced in Daudi. CD10 was again down regulated in all LMP1-expressing clones.

The LMP1-induced increases in BL41, Daudi, and Loukes cellular adhesion molecule expression was also associated with increased heterotypic adhesion with T lymphocytes (Fig. 3; [51]). BJAB cells constitutively demonstrate high-level conjugate formation which does not increase with LMP1 expression (data not shown). These interactions, which are independent of specific immune recognition through the T-cell receptor, are mediated by LFA-1:ICAM-1 and LFA-3:CD2 pathways, since conjugate formation can be completely blocked by monoclonal antibodies to LFA-1 and LFA-3 (Fig. 3).

Cooperative effect of EBNA-2 and LMP1 on CD23. Since both EBNA-2 and LMP1 induced modest CD23 expression in BJAB and Loukes cells, experiments were undertaken to investigate whether EBNA-2 and LMP1 can act cooperatively to induce higher CD23 levels. Two stable EB1NA-2-expressing BJAB clones were transfected with the LMP1-expressing or control vector. Nine clones resistant to both mycophenolic acid (linked to LMP1) and G418 (linked to EBNA-2) were studied. By immunoblotting, six clones expressed EB1NA-2 but no detectable LMP1. Three clones expressed both EB1NA-2 and high-level LMP1 (BJE2-11/LMP-7 and BJE2-15/LMP-1 and -5), as demonstrated by representative clones in Fig. 4. CD23 surface expression of all three EB1NA-2/LMP1-expressing clones was markedly increased when compared with cells expressing either EB1NA-2 or LMP1 alone (Table 3). Comparison of the mean fluorescence intensities revealed higher CD23 levels than expected from simply additive EB1NA-2 and LMP1 effects alone. The LMP1-mediated down regulation of CD10 was not affected by concomitant EB1NA-2 expression. How-

TABLE 2. Surface phenotype of LMP-expressing BJAB, BL41, Daudi, and Louckes cells[a]

Transfected clones[b]	B-cell antigens			Activation antigens				Adhesion molecules		
	CD10	CD21	CD40	CD23	CD30	CD39	CD44	LFA1	ICAM1	LFA3
LCL	1.3	15.4	125.9	163.7	38.4	117.5	125.6	80.4	91.2	50.1
BJgpt-1	14.0	9.4	218.3	2.2	0.0	6.1	3.0	19.9	83.3	98.9
BJgpt-2	21.9	6.3	227.2	5.4	0.0	4.6	1.4	28.2	82.1	105.3
BJLMP-6	3.6	10.3	197.2	42.1	2.7	22.9	3.4	57.4	183.7	134.7
BJLMP-15	2.4	5.1	202.6	30.1	1.2	13.5	0.4	26.4	126.5	102.1
BJLMP-16	0.6	2.3	201.1	13.0	1.4	10.3	0.0	28.2	127.2	88.0
BJLMP-17	0.7	7.7	203.3	24.3	0.4	19.1	0.0	30.8	87.6	124.5
BJLMP-18	1.6	3.8	201.8	53.4	0.0	22.2	0.0	37.8	99.1	119.1
BL41gpt-1	90.8	18.8	46.6	0.3	0.0	0.9	0.0	1.6	22.5	5.6
BL41gpt-2	101.3	33.1	57.3	0.5	0.0	0.6	0.0	1.6	41.7	3.8
BL41gpt-3	150.8	25.6	69.4	4.8	0.0	1.6	0.0	2.3	36.3	5.5
BL41LMP-4	74.0	60.0	154.2	0.6	0.1	1.6	0.0	7.6	82.9	11.0
BL41LMP-5	56.2	25.0	116.6	4.3	0.1	5.4	0.1	10.1	143.1	32.5
BL41LMP-2	1.8	32.2	144.9	1.1	0.7	26.8	0.5	7.6	138.8	79.0
BL41LMP-11	32.9	28.3	147.7	4.9	4.4	26.4	0.3	12.6	151.3	97.2
Louckes	51.8	63.8	168.2	10.2	3.4	2.8	0.1	10.5	40.9	7.7
Logpt-1	71.6	66.8	122.7	10.0	2.2	1.9	0.0	10.9	28.6	3.5
Logpt-2	48.9	20.4	162.5	17.0	2.0	2.5	0.2	9.8	39.6	3.9
LoLMP-4	41.5	59.2	130.4	105.6	2.1	3.6	0.5	26.3	52.0	24.1
LoLMP-5	14.9	55.7	177.3	34.2	1.5	5.2	0.1	19.3	100.2	32.1
LoLMP-6	25.7	48.1	169.8	80.6	1.9	4.5	0.1	33.9	163.6	69.5
LoLMP-8	15.8	36.2	197.2	55.1	4.1	6.5	0.2	37.4	94.2	57.9
DAgpt-1	85.1	24.2	74.9	0.2	0.2	0.2	0.2	2.5	59.8	6.9
DAgpt-2	97.1	33.2	95.6	0.4	0.3	0.4	0.1	10.5	73.4	9.0
DALMP-2	79.3	72.2	179.7	0.9	0.4	1.2	13.6	28.5	179.1	35.5
DALMP-3	37.1	98.4	170.9	1.4	1.1	2.6	89.8	35.0	166.1	71.0
DALMP-17	12.4	105.7	165.1	1.2	0.6	1.0	79.1	23.3	153.6	52.2

[a] Cell surface marker expression is expressed as mean fluorescence intensity. The average values of six EBV-infected LCLs are shown. BJAB cells expressing vector control (BJgpt-1 and -2) or high-level LMP1 (BJLMP-6, -15, -16, -17, and -18); BL41 cells expressing vector control (BL41gpt-1, -2, and -3), low-level LMP1 (BL41LMP-4 and -5), or high-level LMP1 (BL41LMP-2 and -11); Louckes cells expressing vector control (Logpt-1 and -2) or high-level LMP1 (LoLMP-4, -5, -6, and -8); and Daudi cells expressing vector control (DAgpt-1 and -2) or high-level LMP1 (DALMP-2, -3, and -17) are shown. CD23, LFA-1, ICAM-1, and LFA-3 expression of vector and LMP1-expressing Louckes (51) and Daudi cells (29) have been previously reported and are shown here for comparative purposes.

[b] Values for EBV-infected LCLs are included for comparison.

ever, the EB1NA-2-induced CD21 expression was abrogated by LMP1 expression. Thus, the EB1NA-2 and LMP1 cooperative effect is specific for CD23.

Mechanism of EBNA-2 and LMP1 cooperative effect on CD23. In order to determine whether surface CD23 induction was associated with changes in CD23 mRNA levels, cytoplasmic RNA from various transfected clones was analyzed by Northern (RNA) blot analyses. BJAB cells expressing EB1NA-2 (BJE2-7 and -15; BJE2-11/LMP-4, -6, and -10; BJE2-15/gpt-1; and BJE2-15/LMP-3; Fig. 5) or LMP1 (BJLMP-15, -16, and -17; Fig. 5) contain slightly increased CD23 mRNA levels (Fig. 5, top panel) relative to actin control mRNA (Fig. 5, lower panel) compared with vector control cells (BJpz-1 and -2 and BJgpt-1, -2, and -3; Fig. 5). However, all three dual-transfected clones expressing EB1NA-2 and high-level LMP1 (BJE2-11/LMP-7 and BJE2-15/LMP-1 and -5; Fig. 5) contained much higher CD23 mRNA levels relative to actin control mRNA. The CD23 mRNA levels in BJAB clones expressing both EB1NA-2 and LMP1 were comparable to the CD23 mRNA level in EBV-infected LCLs (IB4; Fig. 5). Determination of the CD23-to-actin mRNA ratios by densitometry scanning of autoradiograms revealed that dual EB1NA-2/LMP1-expressing clones had an average CD23 to actin ratio 1.8 times that of a control LCL (IB4). This was more than an additive effect of EB1NA-2 and LMP1, since EB1NA-2 had a CD23-to-actin

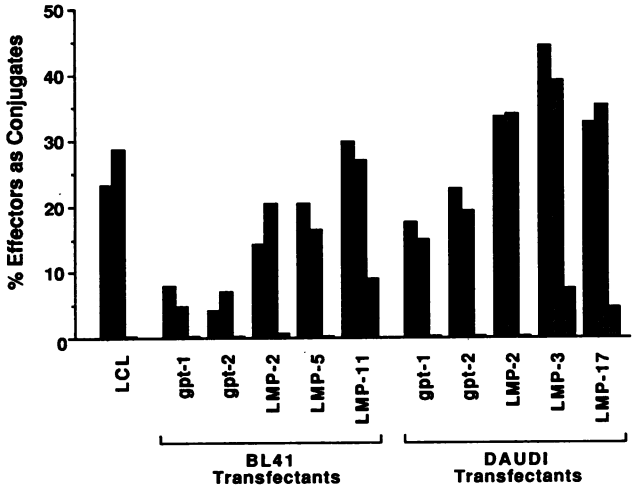

FIG. 3. Heterotypic adhesion of LMP1-expressing BL41 and Daudi cells with T lymphocytes. Conjugate formation between an EBV-infected LCL, vector control (*gpt*), or LMP1-expressing BL41 and Daudi cells is expressed as the percentage of effector T lymphocytes forming conjugates with the target B lymphocytes in the presence of medium alone (■), a control anti-CD10 monoclonal antibody 55 (▩), or anti-LFA-1 plus anti-LFA-3 monoclonal antibodies MHM23 and TS2/9 (▩).

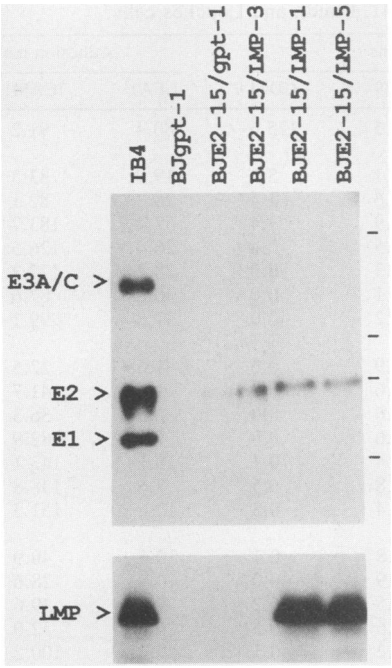

FIG. 4. Immunoblot of dual EB1NA-2/LMP1 transfectants. EBNA expression is demonstrated in the top panel, and LMP1 expression of the identical samples is demonstrated in the bottom panel. EB1NA-2-expressing BJAB clones were transfected with either vector control or LMP1. Representative dual-transfected clones which either expressed EB1NA-2 and no LMP1 (BJU2-15/gpt-1 and BJU2-15/LMP-3) or EB1NA-2 and high-level LMP1 (BJU2-15/LMP-1 and BJU2-15/LMP-3) are shown. Molecular mass markers shown are 200, 116, 92, and 66 kilodaltons.

mRNA ratio of 0.2 (average of six clones) compared with the control LCL, while LMP1 gave an average CD23-to-actin ratio of 0.4 (average of three clones). Hybridization with a CD21 cDNA probe revealed that CD21 mRNA was induced by EB1NA-2 but was similar to vector control levels when LMP1 was coexpressed with EB1NA-2 (Fig. 5). Thus, EB1NA-2 and LMP1 can act cooperatively to specifically induce high-level CD23 mRNA and cell surface expression.

Recently, two CD23 mRNA structures which initiate at different sites, possibly demarcating unique promoters (54), have been described. Type a CD23 mRNA (FcεRIIa) is restricted to B lymphocytes and is constitutively expressed.

TABLE 3. Surface phenotype of EB1NA-2/LMP1 dual-expressing BJAB cells[a]

Transfected clones	EBV protein expression		Cell surface antigens			
	EBNA-2	LMP1	CD10	CD21	CD23	CD39
BJpZ-1	−	−	31.1	3.1	4.0	2.8
BJE2-15	+++	−	45.6	55.6	23.2	10.2
BJE2-15/LMP-3	++	−	22.1	21.4	11.0	15.2
BJLMP-17	−	++	0.7	7.7	24.3	19.1
BJLMP-18	−	+++	1.6	3.8	53.4	22.2
BJE2-15/LMP-1	+++	+++	4.7	9.8	89.7	45.9
BJE2-15/LMP-5	+++	+++	4.0	8.4	116.7	22.9

[a] EB1NA-2 and LMP1 expression determined by immunoblotting at the time of cell surface marker analysis is shown, with +++ equal to levels expressed in EBV-infected LCLs. Mean fluorescence intensities of CD10, CD21, CD23, and CD39 expression are shown.

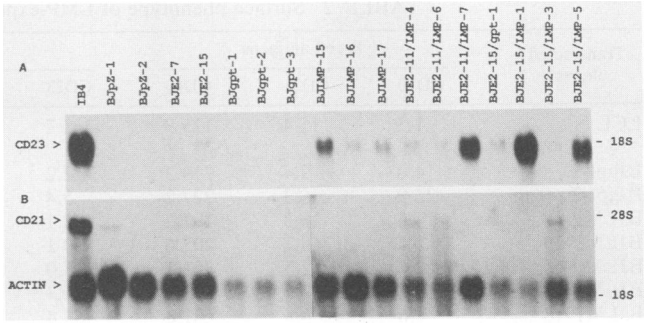

FIG. 5. Northern blots of EB1NA-2- and LMP1-expressing BJAB cells for CD23, actin, and CD21. The blot was first probed with a full-length FcεRIIa cDNA probe, which detects both FcεRIIa and FcεRIIb (A), stripped, and then reprobed with actin and CD21 probes (B). Vector control (BJpZ-1 and -2 and BJgpt-1, -2, and -3), EB1NA-2 only (BJE2-7 and -15; BJE2-11/LMP-4 and -6; BJE2-15/gpt-1; and BJE2-15/LMP-3), LMP1 only (BJLMP-15, -16, and -17), and dual EB1NA-2/LMP1 (BJE2-11/LMP-7 and BJE2-15/LMP-1 and -3)-expressing clones are shown.

Type b CD23 mRNA (FcεRIIb) is expressed in monocytes and eosinophils and is induced in B lymphocytes by the lymphokine interleukin-4 (54). FcεRIIa initiates approximately 3 kilobases upstream from FcεRIIb and utilizes two unique first exons (Fig. 6B). The remaining 9 exons of FcεRIIa and b are identical. To determine how EBNA-2 and LMP1 affect FcεRIIa or FcεRIIb mRNA expression, cytoplasmic RNA from EB1NA-2-, LMP1-, or EB1NA-2/LMP1-expressing BJAB clones were compared with controls by S1 analysis by using a single-stranded probe derived from the 5′ *Eco*RI-*Hin*dIII fragment of the FcεRIIa cDNA. FcεRIIa mRNA protects either a 588-base fragment corresponding to full-length FcεRIIa or a 488-base fragment due to a variant splice in the 5′ untranslated region of FcεRIIa mRNAs. FcεRIIb mRNA protects only a 381-base fragment, since it utilizes a different first exon which initiates in the second FcεRIIa intron (45a). EBV-infected LCLs have more FcεRIIb than FcεRIIa (Fig. 6A; IB4). Vector control BJAB cells have barely detectable FcεRIIa, which is evident only on prolonged autoradiogram exposures (data not shown). Thus, BJAB resembles other EBV-negative B-lymphoma cells and normal B lymphocytes by preferentially expressing FcεRIIa mRNA (54). FcεRIIa and, to a lesser extent, FcεRIIb are readily detectable in EB1NA-2 BJAB cells. The ratio of FcεRIIa to FcεRIIb in EB1NA-2 clones is similar to that in vector control cells, with a predominance of FcεRIIa (BJE2-7 and -15, BJE2-11/LMP-4, BJE2-11/LMP-6, and BJE2-15/gpt-1; Fig. 6A). By contrast, LMP1-expressing BJAB cells have predominately FcεRIIb and small amounts of FcεRIIa (BJLMP-15, -16, and -17; Fig. 6A). The marked induction of total CD23 mRNA by coexpression of EB1NA-2 and LMP1 is associated with a predominance of FcεRIIb (BJE2-15/LMP-1, BJE2-11/LMP-7, and BJE2-15/LMP-5; Fig. 6A). The FcεRIIa-to-FcεRIIb ratio appears similar to that induced by LMP1 expression alone. Thus, EB1NA-2 appears to increase the total amount of CD23 mRNA without preference for a specific CD23 mRNA form. EB1NA-2 expression increases whichever CD23 mRNA is being expressed, i.e., predominately FcεRIIa in BJAB cells alone or FcεRIIb in LMP1-expressing BJAB cells.

DISCUSSION

These experiments significantly extend knowledge of the phenotypic effects induced by specific EBV latent genes on

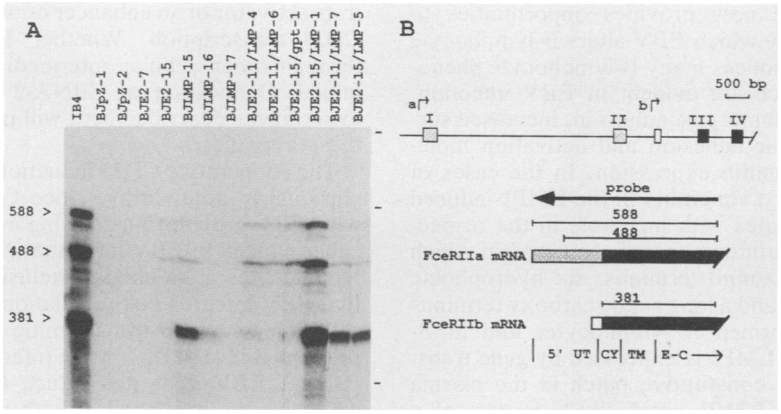

FIG. 6. S1 analyses of EB1NA-2- and LMP1-expressing BJAB cells (A) and structure of CD23 mRNAs (B). The genomic location of the 5′ exons and presumed promoters (↱) of the CD23 mRNAs are shown at the top of panel B. FcεRIIa and FcεRIIb both utilize identical exons 3 though 11 (■). The unique 5′ exons for FcεRIIa (▩) and FcεRIIb (□) are shown relative to the 5′ untranslated region (UT) of the mRNAs and the cytoplasmic (CY), transmembrane (TM), and extracytoplasmic (E-C) domains of the protein. (The figure was adapted from Yokota et al. [54]). A 600-base probe from the 5′ *Eco*RI-*Hin*dIII fragment of the FcεRIIa cDNA was used to determine the relative amounts of FcεRIIa (588- and 488-base pair fragments) and FcεRIIb (381-base pair fragment) mRNA-induced transfected BJAB cells. Vector control clones (BJpZ -1 and -2), EB1NA-2 clones (BJE2-7 and -15), LMP1 clones (BJLMP-15, -16, and -17), dual-transfected clones expressing only EBNA-2 (BJE2-11/LMP-4 and -6 and BJE2-15/gpt-1), and dual-transfected clones expressing EBNA-2 and LMP1 (BJE2-15/LMP-1, BJE2-11/LMP-7, and BJE2-15, LMP-5) are shown. The molecular size markers shown are 873, 603, and 310 base pairs.

human B lymphocytes. All of the effects observed are characteristic of EBV effects on normal B lymphocytes and are evident in EBV infection of B-lymphoma cells. The most important conclusions are summarized in Table 4 and are as follows. (i) EBNA-2 from type 1 EBV specifically induces CD21 and CD23 mRNA and surface expression in both BJAB and Louckes cells. EBNA-2 from type 2 EBV has little if any effect on CD21 or CD23. This likely reflects intrinsic differences between these EBNA-2 proteins, since the type 2 EBNA-2 expression levels were nearly equivalent to the type 1 EBNA-2 level. EBNA-3C induces CD21 mRNA and surface protein expression but has no effect on CD23 expression. The EBNA-3C-induced CD21 effect is less than that observed for type 1 EBNA-2. EBNA-1 or EBNA-LP do not induce significant phenotypic changes in BJAB or Louckes cells. (ii) LMP1 induces marked B-lymphocyte homotypic adhesion. This is accompanied by increased surface expression of the cellular adhesion proteins LFA-1, ICAM-1, and LFA-3 in three B-cell lines which have low constitutive adhesion protein expression. LMP1 probably also causes a functional activation of LFA-1 or ICAM-1, since BJAB cells constitutively express high adhesion molecule levels but do not clump until LMP1 is expressed. LMP1 also uniformly down regulates the Burkitt's lymphoma marker CD10. In each lymphoma cell line, LMP1 induces a characteristic array of the CD21, CD23, CD39, CD40, and CD44 activation markers. The specific pattern of activation markers induced by LMP1 in each B-lymphoma cell line probably reflects the intrinsic ability of each cell line to express various B-cell activation markers. (iii) Coexpression of EBNA-2 and LMP1 induces high-level CD23 expression. LMP1 specifically up regulates the CD23 FcεRIIb mRNA, while EBNA-2 up regulates the constitutive CD23 FcεRIIa mRNA in BJAB or the FcεRIIb mRNA in LMP1 BJAB.

Description of these specific EBV gene effects on the

TABLE 4. Summary of EBNA-2, EBNA-3C, and LMP1 effects on cellular gene expression[a]

Cellular gene	EBV protein effects							
	EB1NA-2		EB2NA-2 BJAB	EBNA-3C BJAB	LMP1			
	BJAB	Louckes			BJAB	BL41	Louckes	Daudi
LFA-1	→	→	→	→	→[b]	↑	↑	↑
ICAM-1	→	→	→	→	→[b]	↑	↑	↑
LFA-3	→	→	→	→	→[b]	↑	↑	↑
CD10	→	→	→	→	↓	↓	↓	↓
CD21	↑	↑	→	↑	→	→	→	↑
CD23	↑	↑	→	→	↑	→[c]	↑	→
CD39	→	→	→	→	↑	↑	→	→
CD40	→	→	→	→	→	↑	→	↑
CD44	→	→	→	→	→	→	→	↑
Vimentin[d]	ND[e]	ND	ND	ND	ND	↑	↑	→[f]

[a] →, No change in cell surface expression versus vector control cells; ↑, increased cell surface expression versus vector control cells; ↓, decreased cell surface expression versus vector control cells.
[b] LMP1 induces homotypic adhesion but no increase of high constitutive cellular adhesion molecule surface expression.
[c] BL41 cells express high-level CD23 when infected with B95-8 EBV (2).
[d] Vimentin expression measured by immunoblot and Northern blot. Data from studies of Birkenbach et al. (1).
[f] Daudi cells are unusual because they express no detectable vimentin protein or mRNA (29).
[e] ND, Not done.

B-lymphocyte phenotype now provides opportunities to dissect the mechanisms by which EBV alters B-lymphocyte gene expression. LMP1 induces many B-lymphocyte phenotypic characteristics which are evident in EBV infection. These include increased homotypic adhesion, increased surface expression of cellular adhesion and activation molecules, and increased vimentin expression. In the cases of LFA-1 (51), CD23 (51), and vimentin (1), the LMP1-induced protein expression correlates with increases in the respective mRNAs. LMP1 is an integral membrane protein which has a short cytoplasmic amino terminus, six hydrophobic transmembrane domains, and a long acidic carboxy terminus (10, 16). In EBV-transformed B lymphocytes and in B-lymphoma cells in which LMP1 is expressed by gene transfer, LMP1 localizes to a constitutive patch in the plasma membrane (29, 51). The LMP1 patch likely is part of a plasma membrane complex which constitutively transduces activating signals through as yet undefined second-messenger molecules. These are probably normal B-lymphocyte second messengers which are important for LFA-1, CD23, and vimentin mRNA induction. LMP1's preferential CD23 FcεRIIb mRNA induction is similar to the stimulatory effect of the cytokine interleukin-4 on B-lymphoma cells (54), suggesting that these two distinct membrane interactions may use similar intracellular second messengers to induce FcεRIIb mRNA. Further biochemical definition of the LMP1 plasma membrane complex should lead to the identification of the cellular pathways mediating these LMP1-induced effects on cellular gene expression.

LMP1 actions may not be limited to distant effects on mRNA up regulation. LMP1 may also be capable of functionally activating other plasma membrane molecules. LMP1 induces homotypic BJAB adhesion, with little quantitative effect on the already elevated LFA-1 or ICAM-1 levels. Thus, although these molecules are responsible for homotypic adhesion of EBV-infected and LMP1-expressing cells (33, 51), increased LFA-1 and ICAM-1 levels are not sufficient for homotypic adhesion. Recently, a similar activating effect on adhesion molecules has been suggested from the rapid clumping which follows incubation of interleukin-4-activated B lymphocytes with phorbol esters (41). Studies of LFA-1:ICAM-1-mediated T-cell adhesion also demonstrate that there is increased LFA-1 functional activity induced by stimulation of the T-cell receptor (8). The vector control and LMP1-expressing BJAB cells provide an opportunity to characterize the LFA-1 or ICAM-1 modifications associated with the LMP1-induced changes in functional activity.

These studies also more clearly define the role of EBNA-2 in CD23 induction. Our data indicate that EBNA-2 does not affect which CD23 mRNA is expressed in B-lymphoma cells. Rather, EBNA-2 increases whatever CD23 mRNAs are already being expressed. In BJAB cells, the constitutive FcεRIIa mRNA is expressed at very low levels and EB1NA-2 amplifies FcεRIIa in preference to FcεRIIb. In LMP1-expressing cells, the FcεRIIb mRNA is preferentially expressed, and the addition of EB1NA-2 increases mostly FcεRIIb CD23 mRNA. We cannot exclude the possibility that the effects of LMP1 or EBNA-2 are posttranscriptional. However, FcεRIIa and FcεRIIb mRNAs differ only in the first 200 nucleotides (54). Thus, there is little structural basis for a putative LMP1 effect on FcεRIIb versus FcεRIIa mRNA stability. More likely, LMP1 activates the FcεRIIb promoter via cellular second messengers which are normally activated by B-cell stimuli such as interleukin-4. Since EBNA-2 is a nuclear protein which markedly up regulates either form of CD23 mRNA, EBNA-2 is more likely to be a transactivator of an enhancer or other general up regulator of CD23 transcription. Whether EBNA-2 acts directly or through other cellular intermediates remains to be determined. Delineation of EBNA-2 and LMP1 effects in the experiments described here will permit further evaluation of these hypotheses.

The cooperative CD23 induction by EBNA-2 and LMP1 is particularly noteworthy, since CD23 is closely associated with EBV growth-transforming infection. Not only is CD23 superinduced in EBV infection (24, 48), but immortalized B lymphocytes arise almost exclusively from the CD23-positive EBV-infected cell population (47). Burkitt's lymphoma cells infected with transforming EBV, such as B95-8, express high-level CD23, while infection with nontransforming P3HR-1 EBV does not induce CD23 (2). EBV-1-infected cells have an enhanced growth phenotype (38) and higher CD23 levels compared with EBV-2-infected cells. Burkitt's lymphoma cells infected with Ag876 EBV-2 express 20% of the CD23 expressed on B95-8-infected Burkitt's lymphoma cells (2). B lymphocytes immortalized with recombinant EBV-containing EB1NA-2 show an enhanced growth phenotype and higher CD23 levels compared with cells immortalized with isogenic recombinant EBV containing EB2NA-2, which grow more slowly and express a heterogeneous CD23 profile (5). Thus, CD23, or a B-lymphocyte gene whose expression is coordinately regulated with CD23, is likely to be integral to EBV-induced B-lymphocyte proliferation, and further analysis of EBNA-2 and LMP1 effects on CD23 regulation is likely to be pertinent to the mechanism by which EBV transforms normal B-lymphocyte growth.

Our observation that EBNA-3C induces CD21 provides the first function for a member of the EBNA-3 gene family. This family of three tandemly placed genes encodes three distantly homologous high-molecular-weight nuclear proteins expressed in all latently EBV-infected cells (17, 34, 35, 39). In retrospect, data from a previous study provided evidence that EBNA-3C might be essential for EBV-induced growth transformation (45). The Raji EBV strain is deleted for a portion of the EBNA-3C gene (13, 36) and is unable to express EBNA-3C (35). P3HR-1 superinfection of Raji cells permits the endogenous latent Raji EBV DNA to replicate, but the transforming viruses obtained were all recombinants between P3HR-1 and Raji, suggesting that the Raji EBV is nontransforming (45). In addition, the transforming EBV recombinants analyzed had all incorporated the P3HR-1 EBNA-3C gene (45).

While these assays in EBV-negative B-lymphoma cells reveal effects of specific EBV genes and thereby provide an opportunity to dissect the mechanisms by which EBV proteins affect B-lymphocyte growth, the assay cells are malignant lymphocytes. Thus, some important EBV gene effects on cell proliferation are likely to be less evident or undetectable in these assays. In addition, proof that the effects observed in these assays are pertinent requires demonstration that similar effects are necessary for EBV-induced growth transformation of normal B lymphocytes.

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LITERATURE CITED

1. **Birkenbach, M., D. Liebowitz, F. Wang, J. Sample, and E. Kieff.** 1989. Epstein-Barr virus latent infection membrane protein increases vimentin expression in human B-cell lines. J. Virol. **63**:4079–4084.
2. **Calender, A., M. Billaud, J. P. Aubry, J. Banchereau, M. Vuillame, and G. M. Lenoir.** 1987. Epstein-Barr virus (EBV) induces expression of B-cell activation markers on in vitro infection of EBV-negative B-lymphoma cells. Proc. Natl. Acad. Sci. USA **84**:8060–8064.
3. **Clark, E. A., and J. A. Ledbetter.** 1986. Activation of human B cells mediated through two distinct surface differentiation antigens, Bp35 and Bp50. Proc. Natl. Acad. Sci. USA **83**:4494–4498.
4. **Cleary, M. L., R. F. Dorfman, and J. Sklar.** 1986. Failure in immunological control of the virus infection: post-transplant lymphomas, p. 163–181. *In* M. A. Epstein and B. C. Achong (ed.), The Epstein-Barr virus: recent advances. William Heinemann, London.
5. **Cohen, J. I., F. Wang, J. Mannick, and E. Kieff.** 1989. Epstein-Barr virus nuclear protein 2 is a key determinant of lymphocyte transformation. Proc. Natl. Acad. Sci. USA **86**:9558–9562.
6. **Dambaugh, T., F. Wang, K. Hennessy, E. Woodland, A. Rickinson, and E. Kieff.** 1986. Expression of the Epstein-Barr virus nuclear protein 2 in rodent cells. J. Virol. **59**:453–462.
7. **Defrance, T., J. P. Aubry, F. Rousset, B. Vanbervliet, J. Y. Bonnefoy, N. Arai, Y. Takebe, T. Yokota, F. Lee, K. Arai, J. deVries, and J. Banchereau.** 1987. Human recombinant interleukin 4 induces Fcε receptors (CD23) on normal human B lymphocytes. J. Exp. Med. **165**:1459–1467.
8. **Dustin, M. L., and T. A. Springer.** 1989. T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. Nature (London) **341**:619–624.
9. **Ernberg, E., G. Klein, B. C. Giovanella, J. Stehlin, K. J. McCormick, M. Andersson-Anvret, P. Aman, and D. Killander.** 1983. Relationship between the amounts of EBV-DNA and EBNA per cell, clonability and tumorgenicity in two EBV-negative lymphoma lines and their EBV-converted sublines. Int. J. Cancer **31**:163–169.
10. **Fennewald, S., V. van Santen, and E. Kieff.** 1984. Nucleotide sequence of an mRNA transcribed in latent growth-transforming virus infection indicates that it may encode a membrane protein. J. Virol. **51**:411–419.
11. **Gregory, C. D., T. Tursz, C. F. Edwards, C. Tetaud, M. Talbot, B. Caillou, A. B. Rickinson, and M. Lipinski.** 1987. Identification of a subset of normal B cells with a Burkitt's lymphoma (BL)-like phenotype. J. Immunol. **139**:313–316.
12. **Hammerschmidt, W., and B. Sugden.** 1989. Genetic analysis of immortalizing functions of Epstein-Barr virus in human B lymphocytes. Nature (London) **340**:393–397.
13. **Hatful, G., A. T. Bankier, B. G. Barrell, and P. J. Farrell.** 1988. Sequence analysis of Raji Epstein-Barr virus DNA. Virology **164**:334–340.
14. **Hatzubai, A., M. Anafi, M. G. Masucci, J. Dillner, R. A. Lerner, G. Klein, and D. Sulitzeanu.** 1987. Down-regulation of the EBV-encoded membrane protein (LMP) in Burkitt lymphomas. Int. J. Cancer. **40**:358–364.
15. **Henle, W., V. Diehl, G. Kohn, H. zur Hausen, and G. Henle.** 1967. Herpes-type virus and chromosome marker in normal leukocytes after growth with irradiated Burkitt cells. Science **157**:1064–1065.
16. **Hennessy, K., S. Fennewald, M. Hummel, T. Cole, and E. Kieff.** 1984. A membrane protein encoded by the Epstein-Barr virus. Proc. Natl. Acad. Sci. USA **81**:7207–7211.
17. **Hennessy, K., F. Wang, E. W. Bushman, and E. Kieff.** 1986. Definitive identification of a member of the Epstein-Barr virus nuclear protein 3 family. Proc. Natl. Acad. Sci. USA **83**: 5693–5697.
18. **Hildreth, J. E. K., F. M. Gotch, P. D. K. Hildreth, and A. J. McMichael.** 1983. A human lymphocyte-associated antigen involved in cell-mediated lympholysis. Eur. J. Immunol. **13**: 202–208.
19. **Jalkanen, S. T., R. F. Bargatz, L. R. Herron, and E. C. Butcher.** 1986. A lymphoid cell surface glycoprotein involved in endothelial cell recognition and lymphocyte homing in man. Eur. J. Immunol. **16**:1195–1202.
20. **Jones, M., L. Foster, T. Sheedy, and B. Griffin.** 1984. The EB virus genome in Daudi Burkitt's lymphoma cells has a deletion similar to that observed in a non-transforming strain (P3HR-1) of the virus. EMBO J. **3**:813–821.
21. **Kieff, E., and D. Liebowitz.** 1989. The Epstein-Barr virus, p. 1889–1920. *In* B. Fields and D. Knipe (ed.), Virology. Raven Press, New York.
22. **Kikutani, H., S. Inui, R. Sato, E. L. Barsumian, H. Owaki, K. Yamasaki, T. Kaisho, N. Uchibayashi, R. R. Hardy, T. Hirano, S. Tsunasawa, F. Sakiyama, M. Suemura, and T. Kishimoto.** 1986. Molecular structure of human lymphocyte receptor for immunoglobulin E. Cell **47**:657–665.
23. **Kikutani, H., M. Suemura, H. Owaki, H. Nakamura, R. Sato, K. Yamasaki, E. L. Barsumian, R. R. Hardy, and T. Kishimoto.** 1986. Fcε receptor, a specific differentiation marker transiently expressed on mature B cells before isotype switching. J. Exp. Med. **164**:1455–1469.
24. **Kintner, C., and B. Sugden.** 1981. Identification of antigenic determinants unique to the surfaces of cells transformed by Epstein-Barr virus. Nature (London) **294**:458–460.
25. **Klein, G.** 1979. The relationship of the virus to nasopharyngeal carcinoma, p. 340–350. *In* M. Epstein and B. Achong (ed.), The Epstein-Barr virus. Springer-Verlag KG, Berlin.
26. **Klein, G., J. Zeuthen, P. Teraski, R. Billing, R. Honig, M. Jondal, A. Westman, and G. Clements.** 1976. Inducibility of the Epstein-Barr virus (EBV) cycle and surface marker properties of EBV-negative lymphoma lines and their in vitro EBV-converted sublines. Int. J. Cancer **18**:639–652.
27. **Lenoir, G. M.** 1986. Role of the virus, chromosomal translocations and cellular oncogenes in the aetiology of Burkitt's lymphoma, p. 183–205. *In* M. A. Epstein and B. C. Achong (ed.), The Epstein-Barr virus: recent advances. William Heinemann, London.
28. **Liebowitz, D., and E. Kieff.** 1989. Epstein-Barr virus latent membrane protein: induction of B-cell activation antigens and membrane patch formation does not require vimentin. J. Virol. **63**:4051–4054.
29. **Liebowitz, D., D. Wang, and E. Kieff.** 1986. Orientation and patching of the latent infection membrane protein encoded by Epstein-Barr virus. J. Virol. **58**:233–237.
30. **Mann, K., D. Staunton, and D. A. Thorley-Lawson.** 1985. An Epstein-Barr virus-encoded protein found in plasma membranes of transformed cells. J. Virol. **55**:233–237.
31. **Menezes, J., W. Liebold, G. Klein, and G. Clements.** 1975. Establishment and characterization of an Epstein-Barr virus (EBV)-negative lymphoblastoid B cell line (BJA-B) from an exceptional, EBV-genome-negative African Burkitt's lymphoma. Biomedicine **22**:276–284.
32. **Nilsson, K., and G. Klein.** 1982. Phenotypic and cytogenetic characteristics of human B lymphoid cell lines and their relevance for the etiology of Burkitt's lymphoma. Adv. Cancer Res. **37**:319–380.
33. **Patarroyo, M., P. G. Beatty, K. Nillson, and C. G. Gahmberg.** 1986. Identification of a cell-surface glycoprotein mediating cell adhesion in EBV-immortalized normal B cells. Int. J. Cancer **38**:539–547.
34. **Petti, L., and E. Kieff.** 1988. A sixth Epstein-Barr virus nuclear protein (EBNA3B) is expressed in latently infected growth-transformed lymphocytes. J. Virol. **62**:2173–2178.
35. **Petti, L., J. Sample, F. Wang, and E. Kieff.** 1988. A fifth Epstein-Barr virus nuclear protein (EBNA3C) is expressed in latently infected growth-transformed lymphocytes. J. Virol. **62**:1330–1338.
36. **Polack, A., H. Delius, U. Zimber, and G. W. Bornkamm.** 1984. Two deletions in the Epstein-Barr virus genome of the Burkitt lymphoma nonproducer line, Raji. Virology **133**:146–157.
37. **Pope, J. H., M. K. Horne, and W. Scott.** 1968. Transformation of fetal human leukocytes *in vitro* by filtrates of a human leukaemic cell line containing herpes-like virus. Int. J. Cancer **3**:857–866.
38. **Rickinson, A. B., L. S. Young, and M. Rowe.** 1987. Influence of

the Epstein-Barr virus nuclear antigen EBNA 2 on the growth phenotype of virus-transformed B cells. J. Virol. **61**:1310–1317.

39. **Ricksten, A., B. Kallin, H. Alexander, J. Dillner, R. Fahraeus, G. Klein, R. Lerner, and L. Rymo.** 1988. BamHI E region of the Epstein-Barr virus genome encodes three transformation-associated nuclear proteins. Proc. Natl. Acad. Sci. USA **85**:995–999.
40. **Rothlein, R., M. L. Dustin, S. D. Marlin, and T. A. Springer.** 1986. A human intracellular adhesion molecule (ICAM-1) distinct from LFA-1. J. Immunol. **137**:1270–1274.
41. **Rousset, F., M. Billaud, D. Blanchard, C. Figdor, G. M. Lenoir, H. Spits, and J. E. de Vries.** 1989. IL-4 induces LFA-1 and LFA-3 expression on Burkitt's lymphoma cell lines. J. Immunol. **143**:1490–1498.
42. **Rowe, M., J. E. K. Hildreth, A. B. Rickinson, and M. A. Epstein.** 1982. Monoclonal antibodies to Epstein-Barr virus-induced transformation-associated cell surface antigens: binding patterns and effects on virus-specific T cell cytotoxicity. Int. J. Cancer **29**:373–381.
43. **Sanchez-Madrid, F., A. M. Krensky, C. F. Ware, E. Robbins, J. L. Strominger, S. J. Burakoff, and T. A. Springer.** 1982. Three distinct antigens associated with human T-lymphocyte mediated cytolysis: LFA-1, LFA-2, and LFA-3. Proc. Natl. Acad. Sci. USA **79**:7489–7493.
44. **Schwab, U., H. Stein, J. Gerdes, H. Lemke, H. Kirchner, M. Schaadt, and V. Diehl.** 1982. Production of a monoclonal antibody specific for Hodgkin and Sternberg-Reed cells of Hodgkin's disease and a subset of normal lymphoid cells. Nature (London) **299**:65–67.
45. **Skare, J., J. Farley, Jack L. Strominger, K. O. Fresen, M. S. Cho, and H. zur Hausen.** 1985. Transformation by Epstein-Barr virus requires DNA sequences in the region of *Bam*HI fragments Y and H. J. Virol. **55**:286–297.

45a. **Suter, U., R. Bastos, and H. Hofstetter.** 1987. Molecular structure of the gene and the 5′-flanking region of the human lymphocyte immunoglobulin E receptor. Nucleic Acids Res. **15**:7295–7308.

46. **Tedder, T. F., L. T. Clement, and M. D. Cooper.** 1984. Expression of C3d receptors during human B cell differentiation: immunofluorescence analysis with the HB5 monoclonal antibody. J. Immunol. **133**:668–673.
47. **Thorley-Lawson, D. A., and K. P. Mann.** 1985. Early events in EB viral infection provide a model for B cell activation. J. Exp. Med. **162**:45–59.
48. **Thorley-Lawson, D. A., L. M. Nadler, A. K. Bhan, and R. T. Schooley.** 1985. BLAST-2 (EBVCS), an early cell surface marker of human B cell activation, is superinduced by Epstein-Barr virus. J. Immunol. **134**:3007–3012.
49. **Wang, D., D. Liebowitz, and E. Kieff.** 1985. An EBV membrane protein expressed in immortalized lymphocytes transforms established rodent cells. Cell **43**:831–840.
50. **Wang, D., D. Liebowitz, and E. Kieff.** 1988. The truncated form of the Epstein-Barr virus latent-infection membrane protein expressed in virus replication does not transform rodent fibroblasts. J. Virol. **62**:2337–2346.
51. **Wang, D., D. Liebowitz, F. Wang, C. Gregory, A. Rickinson, R. Larson, T. Springer, and E. Kieff.** 1988. Epstein-Barr virus latent infection membrane protein alters the human B-lymphocyte phenotype: deletion of the amino terminus abolishes activity. J. Virol. **62**:4173–4184.
52. **Wang, F., C. D. Gregory, M. Rowe, A. B. Rickinson, D. Wang, M. Birkenbach, H. Kikutani, T. Kishimoto, and E. Kieff.** 1987. Epstein-Barr virus protein 2 specifically induces expression of the B cell activation antigen, CD23. Proc. Natl. Acad. Sci. USA **84**:3452–3456.
53. **Yates, J. L., N. Warren, and B. Sugden.** 1985. Stable replication of plasmids derived from Epstein-Barr virus in various mammalian cells. Nature (London) **313**:812–815.
54. **Yokota, A., H. Kikutani, T. Tanaka, R. Sato, E. L. Barsumian, M. Suemura, and T. Kishimoto.** 1988. Two species of human Fcε receptor II (FcεRII/CD23): tissue specific and IL-4-specific regulation of gene expression. Cell **55**:611–618.
55. **Young, L., C. Alfieri, K. Hennessy, H. Evans, C. O'Hara, K. C. Anderson, J. Ritz, R. S. Shapiro, A. Rickinson, E. Kieff, and J. I. Cohen.** 1989. Epstein-Barr virus transformation associated genes are expressed in tissues from patients with EBV lymphoproliferative disease. N. Engl. J. Med. **321**:1080–1085.