Epstein–Barr virus nuclear antigen 2 specifically induces expression of the B-cell activation antigen CD23

(trans-activation/immunoglobulin E Fc receptor/recombinant retrovirus)

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ABSTRACT Epstein–Barr virus (EBV) infection of EBV-negative Burkitt lymphoma (BL) cells induces some changes similar to those seen in normal B lymphocytes that have been growth transformed by EBV. The role of individual EBV genes in this process was evaluated by introducing each of the viral genes that are normally expressed in EBV growth-transformed and latently infected lymphoblasts into an EBV-negative BL cell line, using recombinant retrovirus-mediated transfer. Clones of cells were derived that stably express the EBV nuclear antigen 1 (EBNA-1), EBNA-2, EBNA-3, EBNA-leader protein, or EBV latent membrane protein (LMP). These were compared with control clones infected with the retrovirus vector. All 10 clones converted to EBNA-2 expression differed from control clones or clones expressing other EBV proteins by growth in tight clumps and by markedly increased expression of one particular surface marker of B-cell activation, CD23. Other activation antigens were unaffected by EBNA-2 expression, as were markers already expressed on the parent BL cell line, including BL markers (cALLA and BLA), proliferation markers (transferrin receptor and BK19.9), and cell adhesion-related molecules (LFA-1 and LFA-3). Increased CD23 expression in cells expressing EBNA-2 was apparent from monoclonal anti-CD23 antibody binding to the cell surface, from immunoprecipitation of the 45-kDa and 90-kDa CD23 proteins with monoclonal antibody, and from RNA blots probed with labeled CD23 DNA. The results indicate that EBNA-2 is a specific direct or indirect trans-activator of CD23. This establishes a link between an EBV gene and cell gene expression. Since CD23 has been implicated in the transduction of B-cell growth signals, its specific induction by EBNA-2 could be important in EBV induction of B-lymphocyte transformation.

Epstein–Barr virus (EBV) is an etiologic agent of some B lymphomas and of nasopharyngeal carcinoma (for review see ref. 1). Upon EBV infection *in vitro*, B lymphocytes are induced to secrete immunoglobulin and to proliferate indefinitely (2–6). The virus-infected cells grow in tight clumps and express on their surface high levels of cellular activation antigens similar to those expressed transiently on antigen- or mitogen-activated B lymphocytes (6–8). Several aspects of this virus-induced activation of normal resting B cells are mimicked by EBV infection of already established EBV-negative Burkitt lymphoma (BL) cell lines. The EBV-converted BL cells grow in tight clumps, express B-cell activation antigens, and show both a reduced serum dependence and an increased survival at saturation cell densities (6, 9, 10).

The EBV genome persists in cells either as an episome or by integrating into cell DNA (for review see ref. 11). A limited repertoire of EBV genes is characteristically expressed in

latently infected growth-transformed lymphocytes or in EBV-infected Burkitt tumor cells (11). The repertoire includes the gene encoding a latent infection membrane protein (LMP) and four genes encoding distinctly different EBV nuclear antigens, EBNA-1, EBNA-2, EBNA-3, and EBNA-LP. [EBNA-LP is encoded in the leader of the EBNA-2 mRNA (11–14).] EBNA-3 is probably a family of three related tandem genes (13). Since the EBNA and LMP genes are expressed in all latently infected growth-transformed lymphocytes, they are likely to have a role in maintaining cell growth transformation or latent infection. In fact, EBNA-1 is necessary for EBV episome maintenance (15). EBNA-2 or EBNA-LP is likely to be necessary for cell growth transformation, since virus strains that have deletions in part of the EBNA-LP and EBNA-2 mRNA coding domains are unable to transform normal lymphocytes, and transformation-competent recombinants between these strains and other EBV isolates have restored the wild-type EBNA-LP and EBNA-2 domain (11, 16). Further, expression of EBNA-2 in Rat-1 cells lessens their serum dependence (17). Moreover, preliminary data suggested that EBNA-2 stimulates lymphocyte DNA synthesis (18). LMP is also likely to be important in cell growth transformation, since expression of this gene in Rat-1 cells lessens serum dependence, contact inhibition, and anchorage dependence and increases tumorigenicity (19).

In the present work, we sought to examine the role of individual EBV latent genes in the process of B-cell growth transformation by introducing these genes into EBV-negative BL cells. Using recombinant retrovirus-mediated gene transfer and stringent positive selection, we have succeeded in achieving stable expression of these EBV genes in BL cells, and we have begun to characterize the phenotypic effects of each of the EBV genes in these cells.

MATERIALS AND METHODS

Insertion of EBV DNA into a Recombinant Retrovirus. The expression vector pZip-Neo-SV(X)1 (20) was opened at a unique cloning site by digestion with *Bam*HI. The ends were repaired with T4 DNA polymerase. Fragments of EBV DNA encoding EBNA-1 [nucleotides 107930–110176 of the EBV genome (11, 21)], EBNA-2 [nucleotides 48475–50306 (11, 21)], EBNA-3 [nucleotides 92705–95398 (13, 21)], EBNA-LP [cDNA clone T65 (12)], or LMP [nucleotides 169581–166614 (11, 21)] were repaired with T4 DNA polymerase, gel puri-

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Abbreviations: LMP, latent infection membrane protein; EBV, Epstein–Barr virus; EBNA, EBV nuclear (protein) antigen; MuLV, murine leukemia virus; mAb, monoclonal antibody; BL, Burkitt lymphoma; LTR, long terminal repeat; Amp^r, ampicillin resistance (resistant); Neo^r, neomycin (G418) resistance (resistant).

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fied, and then separately ligated into the repaired *Bam*HI site of pZip-Neo-SV(X)1 with T4 DNA ligase.

Recombinant pZip-Neo-SV(X)1/EBV DNA plasmids were transfected into PA12 mouse cells (22) by calcium precipitation (17). Successfully transfected cells were selected in Dulbecco's modified Eagle's medium with 10% fetal calf serum and the neomycin analogue G418 (GIBCO) at 500 μ g/ml. Clones were picked with the aid of glass cloning cylinders and verified to be expressing the appropriate EBV protein by immunoblotting (14). Recombinant retroviruses were obtained by allowing the cloned cell lines to grow to near confluence in selective media in a 75-cm² flask, replacing the medium with 10 ml of fresh medium without G418 for 2 days. The medium was filtered through a 0.45- μ m-pore filter and stored at -80°C until use. Virus titer was determined by incubating 1 ml of supernatant in a 50–75% confluent 25-cm² flask of Rat-1 or 3T3 mouse cells in Polybrene at 8 μ g/ml at 37°C for 4 hr. The next day, cells were split 1:6 into medium with G418 at 500 μ g/ml. The number of G418-resistant colonies was determined after 2 weeks.

Retroviral Infection and Selection of Transfected Human Lymphocytes. Louckes cells (13) grown in logarithmic phase in RPMI medium with 10% fetal calf serum were infected with 2–5 ml of retrovirus-containing supernatant with Polybrene at 8 μ g/ml at 37°C for 4 hr. After 24 hr, cells were plated in flat-bottom 96-well microtiter plates at 10,000, 5000, and 2500 cells per well in medium containing G418 at 3 mg/ml. Cells were fed once a week with medium containing G418, and clones of resistant cells appeared after 4–6 weeks. No cells survived without infection. Resistant cells were grown and passaged 1:10 once a week in medium containing G418 at 3 mg/ml. Expression of EBV proteins was tested by immunoblot (14) as soon as sufficient cells were available and, thereafter, at least once every 3 weeks.

Immunoblotting and Immunoprecipitation. EBNA-1, EBNA-2, EBNA-3, and EBNA-LP were detected by using a pool of sera from EBNA-positive EBV-immune individuals (13), followed by binding of ¹²⁵I-labeled staphylococcal protein A and autoradiography. LMP was detected by using the mouse monoclonal antibody (mAb) S12 (23), followed by rabbit anti-mouse immunoglobulin and ¹²⁵I-protein A.

Cell surface proteins were iodinated with carrier-free Na¹²⁵I by using lactoperoxidase. Precleared lysates from 3 × 10⁶ iodinated cells were mixed with 2 μ l of mAb MHM6 (7, 10) ascitic fluid. The immunoprecipitates were isolated on protein A-Sepharose, dissolved by boiling in NaDodSO₄/gel sample buffer, and separated on a 7.5% acrylamide gel (24).

Cell Surface Phenotyping. Viable cells were incubated for 1 hr at 4°C with MHM6 ascitic fluid diluted 1:100 in phosphate-buffered saline (138 mM NaCl/9.5 mM potassium phosphate, pH 7.4) containing 20% normal goat serum (PBS-NGS), washed twice in PBS, and incubated for a further 1 hr at 4°C with fluorescein-conjugated goat anti-mouse immunoglobulin (Sigma) diluted 1:100 in PBS-NGS containing 10% normal human serum and 0.02% sodium azide. After washes the labeled cells were fixed in PBS containing 1% formaldehyde and 2% serum, and analyzed for fluorescence (525–545 nm) by using a Becton Dickinson FACS IV flow cytometer.

RNA Isolation and Hybridization. Cytoplasmic RNA was prepared from 10⁹ cells by using the guanidinium isothiocyanate/cesium chloride centrifugation method (25). Approximately 20 μ g of each RNA was size fractionated on a 1% agarose gel containing 2.2 M formaldehyde and transferred to nitrocellulose membranes (26) for hybridization (27). CD23 and γ -actin probes were prepared by nick-translation of the 1.7-kilobase (kb) *Eco*RI cDNA fragment from plasmid pFceR-1 (27) or by nick-translation of the 284-base-pair (bp) fragment of the γ -actin gene [kindly provided by Keith Robbins (28)].

RESULTS

Previous experiments indicated that the Moloney murine leukemia virus (MuLV) long terminal repeat (LTR) would promote transcription of heterologous genes in some murine (20, 29) or human (30) hematopoietic or lymphoid cells. We therefore selected a MuLV-based vector, pZip-NeoSV-(X)1 (20) to express EBV genes in human lymphocytes. Important features of pZip-Neo-SV(X)1 are as follows: (i) a bacterial plasmid replication origin; (ii) a penicillinase gene conferring ampicillin resistance (*Amp*^r), which permits positive selection of transfected bacteria; (iii) two MuLV LTRs and part of the MuLV gag protein leader, which provide for transcription of heterologous DNA inserted at a unique *Bam*HI site in the gag leader and for polyadenylation of the transcript; (iv) a cis-packaging signal allowing for incorporation of the vector into infectious MuLV by cell lines that provide packaging proteins; and (v) the neomycin phosphotransferase gene, which permits selection for successfully transfected eukaryotic cells by virtue of their resistance to the neomycin analogue G418 (*Neo*^r). EBV DNA segments encoding EBNA-1, EBNA-2, most of EBNA-3, EBNA-LP, or LMP were inserted into the *Bam*HI cloning site of pZip-Neo-SV(X)1 (see Fig. 1). The recombinant EBV/pZip-Neo-SV(X)1 plasmids (designated pZip-E1, -E2, -E3, -ELP, or LMP) were transfected into PA12, a NIH3T3 cell line that expresses amphotropic MuLV trans-packaging functions (22). G418-resistant (*Neo*^r) PA12 cell clones were derived and were demonstrated by immunoblotting to express the appropriate EBV protein. pZip-E2-transfected PA12 clones produced 10² MuLV(*Neo*^r) per ml of culture supernatant. pZip-Neo-SV(X)1-transfected PA12 clones produced 10⁵ MuLV(*Neo*^r) per ml of supernatant. PA12 clones transfected with EBV/pZip-Neo-SV(X)1 recombinants other than pZip-E2 produced MuLV(*Neo*^r) at titers intermediate between those of pZip-Neo-SV(X)1 and pZip-E2. Almost all of the *Neo*^r Rat-1 or 3T3 cells infected with recombinant MuLV expressed the viral protein encoded by the respective EBV DNA segment (data not shown).

EBV-negative BL cell lines were relatively resistant to G418. Their resistance was dependent on cell density. Selection for MuLV(*Neo*^r)-infected clones required plating cells at less than 5 × 10⁴ cells per ml in G418 at 3 mg/ml. Under these conditions, *Neo*^r clones of Louckes cells infected by MuLV-VEC (vector alone), MuLV-E1, MuLV-E2, MuLV-E3, MuLV-ELP, or MuLV-LMP were obtained. The resultant MuLV-E1-infected Louckes clones were designated E1.1, E1.2, etc. Other infected Louckes clones were similarly designated. Each clone expressed the appropriate EBV

FIG. 1. (A) Schematic representation of the EBV genome and viral transcripts characteristic of transformed cells (depicted by arrows). Exons used for expression of viral proteins in Louckes cells are indicated. TR, terminal repeat; IR, internal repeat; U, unique DNA. (B) Major features of the eukaryotic expression vector pZip-Neo-SV(X)1 include LTRs; the neomycin phosphotransferase gene (*Neo*^r), the simian virus 40 origin of replication (SV40 ori), and the pBR322 origin of replication (pBR ori).

protein as determined by immunoblot (Fig. 2). IB4, an EBV-transformed and latently infected cell line, was a positive control for a lymphoblast that expresses EBNA-1 (72 kDa), EBNA-2 (88 kDa), EBNA-3 (142 kDa), and EBNA-LP (40 kDa). These proteins were detected in IB4 cells by EBV-immune human sera as shown in Fig. 2 *A* and *B*. LMP was detected by the monoclonal antibody S12 (Fig. 2*C*). As can be seen from Fig. 2, the only difference in EBV latent infection cycle protein expression in recombinant MuLV-infected Louckes cell lines from the IB4 latently infected growth-transformed control cell lines was in the sizes of EBNA-1 and EBNA-3. Thus, MuLV-E1-infected clones (Fig. 2*A*, E1) expressed a smaller EBNA-1 protein, presumably as a consequence of deletion of part of the GG-GGCAGGA repeat sequence encoding the glycine-alanine copolymer domain of EBNA-1 (11). MuLV-E3-infected clones (Fig. 2*A*, E3.1, E3.2) expressed a 130-kDa as opposed to a 142-kDa EBNA-3 protein, presumably because the construct lacks the 5' exon of EBNA-3 that contributes a small part of the coding sequence (13, 31).

As Neo^r Louckes clones arose after infection with recombinant MuLV, it was immediately apparent that those infected with MuLV-E2 could be distinguished from all others by virtue of their growth in clumps (Fig. 3). Such clumping, which is a characteristic feature of the growth of all EBV-transformed lymphoblastoid cell lines, was apparent on microscopic inspection of all 10 EBNA-2-positive clones. By contrast, three clones of MuLV-VEC-infected, seven clones of MuLV-E1-infected, two clones of MuLV-E3-infected, four clones of MuLV-ELP-infected, and five clones of MuLV-LMP-infected Louckes cells grew almost exclusively as single cells, just like the parental Louckes cell line.

The various clones were subsequently analyzed by mAb binding for any concomitant changes in surface expression of B-lymphocyte-associated markers. Several antigens, including two markers of the cells' malignant BL origin [cALLA and the glycolipid antigen BLA (10, 32)], two proliferation-associated markers [the transferrin receptor and the BK19.9 antigen (33)], and the pan B-cell C3d receptor molecule (CD21), were already expressed on the parental Louckes cells and were not expressed differently in any of the clones. Furthermore, the altered growth phenotype of the EBNA-2-positive clones was not associated with any quantitative change in the expression of the two cell adhesion-related molecules LFA-1 and LFA-3 (34).

One important difference was observed, however, when the analysis was extended to include those B-cell activation antigens associated with EBV-induced lymphocyte proliferation. EBNA-2-expressing clones showed a marked and specific increase in the expression of one particular antigen, the CD23 cell surface protein (7, 8, 27, 35). Fluorescence-

FIG. 3. Microscopic appearance of recombinant EBV/MuLV-infected Louckes cell clones. ($\times 40$.) Louckes clone E2.2 expressing EBNA-2 grew in large clumps (*C*), while Louckes clone E1 expressing EBNA-1 (*B*) or a Louckes clone infected with MuLV-(Neo^r) without EBV DNA (VEC) (*A*) grew dispersed as single cells or in very small clumps.

activated cell sorter (FACS) analysis of binding of the anti-CD23 mAb MHM6 (7, 10) was performed on all EBV recombinant- and control MuLV-infected Louckes cell clones. Representative FACS profiles are shown in Fig. 4*A*. CD23 expression in many cells of all 10 Louckes E2 clones (e.g., see E2.1 and 2.2 in Fig. 4) was much higher than that seen in Louckes parental cells, in MuLV-VEC-infected clones, or in recombinant EBV/MuLV-infected Louckes cell clones expressing EBNA-1, EBNA-3, EBNA-LP, or LMP (Fig. 4*A*). Virtually no cells in the latter clones had a fluorescence intensity greater than 4 (fluorescence intensity linear scale, Fig. 4*A*). Indeed, the CD23 staining profile for many cells in all Louckes E2 (EBNA-2) clones was now comparable to that seen in a typical EBV-transformed lymphoblastoid cell line (Fig. 4*A*). Binding assays with iodinated mAb MHM6 confirmed that CD23 expression was 10-fold greater in Louckes E2 clones than in any of the other Louckes cell clones or in the parent Louckes cell line (data not shown). Four other anti-CD23 mAbs (7), which define three distinct epitopes on the CD23 molecule, including that detected by MHM6, all showed the same pattern of increased binding. Moreover, using MHM6, we could readily detect CD23 by immunoprecipitation from the membrane of E2 clones (Fig. 4*B*), both in its usual 45-kDa form and as the higher molecular weight 90-kDa species that is present, though comparatively less prominent, in immunoprecipitates of EBV-transformed lymphoblastoid cell lines (8, 36). Both forms of CD23 were easily detected in the three different Louckes E2 clones tested, while little of either CD23 form was detected by immunoprecipitation from representative Louckes-VEC, EBNA-1, EBNA-3, EBNA-LP, or LMP clones (Fig. 4*B* and data not shown). In contrast to CD23, the expression of other

FIG. 2. Immunoblots of clones of Louckes cells infected with recombinant Moloney MuLV(Neo^r) carrying the EBV EBNA-1, EBNA-2, EBNA-3, EBNA-LP, or LMP DNA segments indicated in Fig. 1. IB4 and Jijoye are EBV-positive control cell lines. Clones of Louckes cells were derived after infection by MuLV(Neo^r) (VEC), MuLV(Neo^r) with EBNA-1 (E1), EBNA-2 (E2.1, E2.2, E2.3, E2.4, E2.5), EBNA-3 (E3.1, E3.2), EBNA-LP (ELP.1, ELP.2), or LMP (LMP.1, LMP.2). The lower molecular weight band in some EBNA-2 transfectants most likely represents an EBNA-2 degradation product since other blots of protein preparations from these cells frequently showed only the larger (88-kDa) band. Numbers refer to the masses of marker proteins in kDa.

FIG. 4. Surface CD23 is induced by EBNA-2. (A) FACS profile of CD23 on the surface of clones of Louckes cells infected with MuLV(Neo^r) (VEC), or EBV recombinant MuLV(Neo^r) expressing EBNA-1 (E1), LMP (LMP1), or EBNA-2 (E2.1 and E2.2). A reference profile of an EBV latently infected growth-transformed lymphoblastoid cell line (EB-LCL) is also shown. The solid lines represent the immunofluorescence profile of cells incubated with anti-CD23 (MHM6) antibody and with a fluorescein-conjugated second antibody, and the broken lines represent the background immunofluorescence obtained by incubating cells with the fluorescein conjugate only. The percentage of cells exhibiting MHM6-specific fluorescence was 34% with Louckes VEC, 33% with Louckes E1, 22% with Louckes LMP, 61% with Louckes E2.2, 58% with Louckes E2.1, and 78% with the EBV-transformed lymphoblastoid cell line. (B) Autoradiograph of cell surface ¹²⁵I-labeled CD23, immunoprecipitated with MHM6 and separated by NaDodSO₄/polyacrylamide gel electrophoresis. Immunoprecipitates from Louckes VEC and E2.2 clones and from an EBV-transformed lymphoblastoid cell line are shown. Numbers on the right are masses of marker proteins in kDa.

activation antigens usually associated with EBV-induced transformation of B lymphocytes was not affected by EBV gene transfection. Thus, the p80 antigen defined by mAb AC2 (24) and those antigens defined by mAbs Ki-1 (32) and 11EF7 (33) were not expressed on the parental cells and were not induced in the Louckes E2 clones, nor indeed in any of the other transfectants. Expression of another activation antigen, defined by mAb Ki-24, known to be present on a number of EBV-negative BL lines, including Louckes (10), was also unchanged in all of the Louckes clones.

To determine whether surface CD23 expression was due to EBNA-2 induction of increased levels of CD23 mRNA or to differences in translation or post-translational processing of CD23, cytoplasmic RNA was isolated from clones of each type. The level of CD23 mRNA in cells of each clone was compared to γ -actin mRNA, which was assumed to be constant in cells of the various clones. As shown in Fig. 5, CD23 mRNA levels are greatly enhanced relative to γ -actin mRNA in two Louckes E2 clones compared to Louckes E1, E3, E-LP, or LMP clones. In Fig. 5A, CD23 mRNA appears to be increased in the Louckes E1.1 clone. When this blot was washed and probed with γ -actin [³²P]mRNA, however (Fig. 5B), it was apparent that the Louckes E1.1 RNA preparation is enriched for actin as well as CD23 mRNA and that CD23

mRNA is more abundant relative to actin mRNA only in Louckes E2.4 (and in Louckes E2.6 in Fig. 5A).

DISCUSSION

Using a Moloney MuLV-derived amphotropic recombinant retrovirus vector carrying the neomycin phosphotransferase gene, we have been able to make a series of EBV/MuLV recombinant retroviruses that infect human B lymphoblasts. With stringent selection, stable isogenic clones have been selected that express an EBV protein ordinarily expressed in latent, growth-transforming, EBV infection. These cloned cell lines, which differ in their expression of only a single EBV gene, are an important reagent for detailed studies of specific EBV gene function in BL cells, a cell type similar to the natural EBV host. Such cells should be useful for evaluating the role of these EBV genes in processes such as regulation of latent infection cycle EBV gene expression, maintenance of EBV episomes, regulation of cell gene expression, induction of transformation-associated changes in cell phenotype, or regulation of lytic infection cycle EBV gene expression, any of which may require B-lymphocyte-specific cofactors. They should also be useful in identifying which EBV genes contain important epitopes for specific recognition by (EBV-immune) human T lymphocytes.

In the experiments described in this report, EBNA-2 caused a striking change in the growth of BL cells. EBNA-2 caused growth in large clumps, resembling the most obvious *in vitro* effect of EBV infection. This effect correlated with the specific induction of a B-cell surface protein, CD23. CD23 has been of interest for several years because of its prominent early expression on the surface of antigen- or mitogen-activated normal B lymphocytes, its induction to high levels soon after EBV infection, and its activity as an IgE Fc receptor (7, 8, 27, 35). Curiously, two forms of CD23 are found in B lymphocytes and in the EBNA-2 expressing Louckes clones, a 45-kDa and a 90-kDa form. The larger molecule may be a dimer, since the predicted translation product of the CD23 mRNA is 321 amino acids long (27) and the protease cleavage products of the 45- and 90-kDa proteins are quite similar (36). The specific relationship between EBNA-2 expression, up-regulation of CD23, and clumped cell growth is particularly intriguing; it can now be directly investigated. CD23 may play a central role in EBV-induced B-cell proliferation. Recent evidence indicates a key role for CD23 in growth signal transduction at the B-cell surface. Ligation of CD23 by the mAb MHM6 dramatically enhanced the entry of phorbol ester-activated human tonsillar B lym-

FIG. 5. Blot of RNA from recombinant EBV/MuLV Neo^r Louckes cell clones probed with CD23 mRNA cDNA probe (27) or with γ -actin mRNA probe (28). (A) Blot that was probed with a mixture of labeled actin and CD23 DNAs. (B and C) Autoradiographs of a blot that was probed with labeled CD23 DNA and exposed to film (B), then probed with labeled γ -actin DNA and reexposed to film (C).

phocytes into S phase (37). Moreover, the capacity of EBV-transformed lymphoblastoid cell lines to adsorb a purified low molecular weight B-cell growth factor (38) could be specifically abrogated by mAb blockade of CD23 (39). CD23 may also be important in cell adhesion, since it has a high degree of homology to the asialoglycoprotein receptor (27).

Previous experiments cited in the introduction had suggested a role for EBNA-2 in B-cell growth transformation. The direct effect of EBNA-2 in inducing clumped cell growth and CD23 expression supports a central role for EBNA-2 in EBV induction of cell transformation. EBNA-2 probably induces CD23 transcription (or mRNA processing), since our data indicate that CD23 mRNA is sufficiently higher in EBNA-2-expressing cells to account for the increase in cell surface CD23. The increased CD23 mRNA in EBNA transfectants is likely to be due to a direct or indirect EBNA-2 trans-activation of CD23 gene expression. If CD23 indeed proves to be a growth factor receptor, its specific induction to high level expression by EBNA-2 provides a striking parallel to the recently reported trans-activation of the interleukin 2 receptor gene in T lymphocytes by the *tat* gene of human T-cell lymphotropic virus type II (40). If EBNA-2 induction of CD23 expression is central to its role in cell growth transformation, its influence may be fully demonstrable only in B lymphocytes, since CD23 is a B-lymphocyte-specific cell protein. This could explain numerous unsuccessful attempts in our laboratory to immortalize rat embryo fibroblasts with MuLV-E2 or other EBNA-2 recombinant expression vectors.

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