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Definitive identification of a member of the Epstein–Barr virus nuclear protein 3 family

(herpesvirus/cancer/hybrid proteins/Burkitt lymphoma/anti-nuclear antibodies)

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ABSTRACT **Some Epstein–Barr virus (EBV) immune human antisera are known to react with a 142-kDa protein, EBV-encoded nuclear antigen 3 (EBNA3), which, like EBNA1 and EBNA2, is likely to be involved in the establishment of latent infection or growth transformation. We have now constructed gene fusions between *Escherichia coli lacZ* and an EBV DNA open reading frame (BERF1; *Bam*HI E fragment rightward open reading frame 1), which is transcribed into an mRNA in latently infected cells. Purified hybrid protein from one of these constructs, chosen because of its reactivity with EBNA3-positive human antisera, was used to affinity purify the specific antibody from human antiserum. This specific antibody was used to prove that EBNA3 is encoded, at least in part, by BERF1, and that EBNA3 is in the nucleus of each latently infected cell. In rodent cells, BERF1 encodes a 120- to 130-kDa protein, which translocates to the nucleus and is recognized by EBNA3-positive human antisera. Two other proteins similar in size to EBNA3 are detected in latently infected cells by EBV immune human antisera. Two EBV open reading frames related to BERF1 may encode these proteins.**

Epstein–Barr virus (EBV) may be an etiologic agent of human lymphoma (1). Latent EBV infection is prevalent in all human populations (for review, see ref. 2). The virus readily establishes latency in human B lymphocytes and persists as an episome or by integration into the host genome (3, 4). Infection of lymphocytes is not completely cryptic in that a highly restricted set of virus genes are regularly expressed and the cell is phenotypically converted to enhanced potential for growth *in vitro* or *in vivo* (for review, see ref. 5). The virus genes expressed in latent infection are presumed to play a role in the enhanced growth potential of latently infected cells or in the maintenance of latent infection.

Two virus-encoded nuclear proteins, EBV nuclear antigens 1 and 2 (EBNA1 and EBNA2), and a virus-encoded cell-membrane protein, the latent membrane protein (LMP), have been definitively identified in latently infected lymphocytes (5–16). The evidence that the virus encodes these proteins during latent infection includes the following: (*i*) Three proteins expressed in bacteria from exons of three latently infected cell mRNAs share epitopes with two nuclear proteins and one membrane protein found in all latently infected cells (7–9). (*ii*) Exons of each mRNA include nucleotide repeats whose length varies among EBV isolates and correlates with the length of the protein encoded by that isolate in latent infection (7, 10, 11). (*iii*) Expression of EBV DNA segments in mammalian cells from eukaryotic expression vectors, which provide for transcription but not translational initiation, results in expression of a full-sized EBNA1 (12–14), EBNA2 (15), or LMP (16).

Important for the relationship of EBNA1, EBNA2, and LMP with latent infection and growth transformation, has been the demonstration that the respective mRNAs and proteins are expressed in cell lines in which there is no evidence for expression of RNAs or proteins associated with virus replication (17–22). Another important criterion has been the demonstration by immunofluorescence that the putative latent protein is present in every latently infected cell (7, 25). Since genes involved in virus replication are typically expressed in high abundance in a few cells in cloned cell cultures in which most cells are latently infected (22), early data showed the importance of focusing on cell lines such as IB4 and Namalwa, which can be grown under conditions of tight restriction to latent infection, rather than cell lines such as Raji, which permit early replicative virus gene expression in 0.01–0.1% of the cells (17–21). This is particularly important, because early virus replicative cycle genes have previously been mistaken for latent viral genes (23).

A 142-kDa protein was recently detected in latently infected B lymphocyte nuclei with EBV immune human antisera (24, 25). Evidence that the protein is a new latent infection viral gene product includes the following: (*i*) The presence of the protein in not only some (24) but all EBV-infected lymphocyte cell lines, including tightly latently infected Namalwa and IB4 cell lines (25); (*ii*) heterogeneity in the size of the protein in cells infected with different virus isolates (25); (*iii*) lack of reactivity of the protein with antisera against EBNA1, EBNA2, or LMP (25); (*iv*) presence of a nuclear antigen in all cells of the P3HR-1 cell line by using a serum specific for EBNA3 in these cells (25); and (*v*) the presence of an mRNA encoded by the EBV DNA *Bam*HI E fragment in tightly latently infected IB4 cells whose function is unknown, but which is sufficiently large to encode a 142-kDa protein (25). We now prove that this EBV DNA fragment encodes a 142-kDa protein, EBNA3, in all latently infected lymphocytes, and we provide evidence that there may be two additional proteins expressed during latent infection.

MATERIALS AND METHODS

Prokaryotic Expression. The 1.9-kilobase (kb) *Pvu* II fragment of the EBV DNA *Bam*HI E (26) first rightward long open reading frame (BERF1; see ref. 27) was isolated and partially digested with *Hin*cII (see Fig. 1). The termini were randomized with BAL 31 nuclease and repaired with T4 DNA polymerase. Fragments >300 base pairs (bp) long were ligated with *Sma* I-cut phosphatase-treated pMC1513 (28). After transformation of *Escherichia coli* MC1061, β-galactosidase-positive colonies were screened for EBV DNA and then for reactivity with an EBV immune human antisera,

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Abbreviations: EBV, Epstein–Barr virus; EBNA, EBV-encoded nuclear antigen; LMP, latent membrane protein; BERF1, *Bam*HI E fragment rightward open reading frame 1; kb, kilobase(s); bp, base pair(s).

WC, which has antibody to EBNA3 (25). The clones were first screened by immunodot blot and then by immunoblot (25). An *E. coli* clone containing plasmid pKH27, which expressed a stable 135-kDa immunoreactive fusion protein, was selected. The EBV insert in pKH27 was sequenced directly by the dideoxy method (29).

Affinity Purification of Antibodies. Late logarithmic-phase bacterial cultures were lysed and hybrid protein was purified by substrate–analog affinity chromatography (30). Intact fusion protein was further purified by gel exclusion column chromatography using Superose and a FPLC device (Pharmacia). Purified fusion protein (10 mg) was then coupled to trysl-activated Sepharose-4B as described by the manufacturer (Pharmacia). Human serum LP (100 ml) was passed through a column containing the pKH27 fusion protein linked to Sepharose 4B. β-Galactosidase antibody in the antiserum had been removed by passage through a β-galactosidase Sepharose 4B column. Antibodies bound to the fusion protein column were eluted with successive applications of 1 M propionic acid, 3 M $MgCl_2$, 10% dioxane, and, lastly, 8 M urea. After concentration and dialysis against phosphate-buffered saline, the antibody was further purified by a second round of fusion protein affinity chromatography prior to use.

Electrophoretic Blotting and Staining. Conditions for preparation of protein samples, electrophoresis, and immunoblotting have been described (25). Size markers were the high molecular mass set (Bio-Rad), which includes myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase B (97 kDa), bovine serum albumin (66 kDa), and ovalbumin (45 kDa). IB4 clone B and Namalwa cells used in these experiments were tightly latently infected, since blots of these cell proteins when reacted with EBV immune human antiserum WC containing high titers of antibody against replicative cycle proteins identified EBNA2 and EBNA3, but none of the early replicative cycle proteins.

Fluorescence Microscopy. Lymphocytes were dried onto a microwell slide and fibroblasts were grown on an eight-chamber microscope slide (Miles). The slides were fixed in absolute methanol and incubated with 10% goat serum, followed by human serum or purified antibodies. The biotin-strepavidin system was used for detection (Bethesda Research Laboratories) with fluorescein-conjugated strepavidin as the observable marker. IB4 clone B and Namalwa cells used in these experiments have <1 in 10^5 cells expressing replicative cycle antigens as detected by cytoplasmic fluorescence using WC antiserum.

Rodent Cell Expression. The EBV DNA *Bam*HI E *Bam*HI/*Bgl* I fragment (25–27) was cloned into pZip-Neo-SV(X)1 (31) after T4 DNA polymerase treatment of both the vector and the insert (see Fig. 1). The orientation of the insert was determined by restriction endonuclease digestion. The resultant recombinant plasmid, pZip-E3 (see Fig. 1), was linearized with *Sal* I before transfection into NIH 3T3, psi-am22b (32), or Rat-1 cells by calcium phosphate precipitation (33). Forty-eight hours after transfection, cells were split 1:10 into selective medium containing 500 μg of G418 per ml. Resistant foci appeared within 14 days and were isolated by using glass cylinders. Clones were screened for expression by immunoblot analysis.

RESULTS

EBNA3 Is Encoded by an EBV DNA Fragment. Since the proteins translated from each of the previously well-characterized EBV latent infection mRNAs are known, our working hypothesis was that EBNA3 is encoded by the recently identified 4.5-kb mRNA (25). This RNA is at least partly encoded by the *Bam*HI/*Bgl* I subfragment of the EBV DNA *Bam*HI E fragment (ref. 25; Fig. 1). The important characteristics of this DNA segment (27) are as follows: (*i*) A

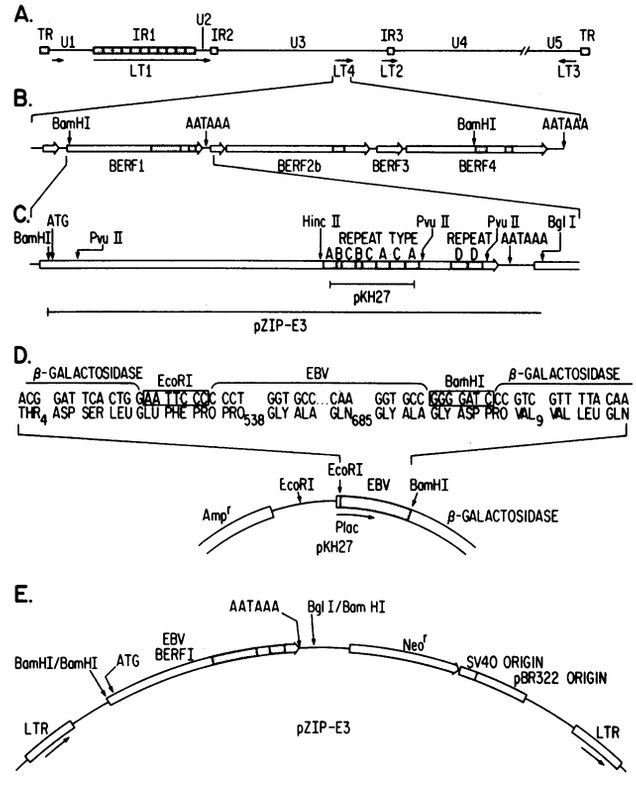

FIG. 1. Cloning of the BERF1. (*A*) Scheme of the B95-8 and IB4 EBV genomes showing major internal (IR) and terminal (TR) repeat domains (shaded boxes) and largely unique (U) DNA regions (5, 26, 27). Viral transcripts detected during latent infection (LT) are also indicated. Two rare polyadenylylated and two nonpolyadenylylated transcripts have been mapped to the U1 domain. Their role in latent infection is uncertain (for review, see ref. 5). (*B*) The *Bam*HI E fragment showing the location of reading frames, minor repeats (shaded boxes), and polyadenylylation signals. (*C*) Detailed scheme of BERF1. ATG indicates three consensus in-frame translational start sites in BERF1. The location of four classes of DNA repeats (shaded boxes A–D) and the segments that have been cloned into prokaryotic (28) or eukaryotic expression vectors (31) to yield recombinant expression plasmids pKH27 and pZip-E3 are also shown. (*D*) Structure of the recombinant prokaryotic expression vector pKH27. The sequence of the BERF1–*lacZ* gene fusion recombination sites was determined as described. Subscripts on the protein sequence indicate the codon number in either β-galactosidase (34) or BERF1 (26). Cloning sites of pMC1513 are boxed. The ampicillin-resistance gene (Amp^r) and the *lac* promotor (Plac) are also indicated. (*E*) Structure of the recombinant eukaryotic expression vector pZip-E3. The location of the insert relative to the long terminal repeat (LTR) promotors, neomycin-resistance (Neo^r) gene, and simian virus 40 (SV40) origin of the parent expression vector, pZip-Neo-SV(X)1 (31), is shown. Since the EBV DNA and pZip-Neo-SV(X)1 were treated with T4 DNA polymerase before ligation, the *Bam*HI/*Bam*HI site cannot be cut with *Bam*HI, but the hybrid *Bgl* I/*Bam*HI site is a functional *Bam*HI site. The unshown portion of the plasmid is derived from pBR322.

2517-bp open reading frame, BERF1; (*ii*) three in-frame $\begin{matrix}A\\G\end{matrix}$XXATG sequences near the beginning of the open reading frame, which could initiate translation (35); (*iii*) an AATAAA sequence 105 bp downstream of the stop codon, which is likely to act as a site for addition of poly(A); and (*iv*) several DNA repeat segments within the open reading frame, which could account for the observed polymorphism in EBNA3 size (25). Although the protein encoded by the open reading frame would be only 812 amino acids, considerably smaller than the 142-kDa EBNA3 size indicated by denaturing polyacrylamide gels, EBNA1 and EBNA2 are also considerably

smaller than their indicated size on denaturing polyacrylamide gels (5).

To determine whether the BERF1 open reading frame encodes part of EBNA3, fusions were made between BERF1 and the *lacZ* gene of a prokaryotic expression vector (Fig. 1 *A–C*). Three hundred *E. coli* with various EBV BERF1–*lacZ* fusions were screened by dot blot for expression of antigens reactive with WC antiserum, an EBNA3 reactive human antiserum. Twelve positive clones were selected and examined by immunoblot analysis. Four clones produced stable immunoreactive fusion proteins of 130–135 kDa. One clone, pKH27, was determined to express a stable 135-kDa fusion protein as at least 1% of total cell protein. The nucleotide sequences of the sites of recombination between EBV DNA and the *lacZ* gene in pKH27 were determined (Fig. 1*D*). The fusion was found to consist, in-frame, of the first seven codons of *lacZ*, three codons of linker, codons 538–687 of the EBV open reading frame (26), two codons of linker, and the rest of the *lacZ* gene.

After removal of β-galactosidase reactivity, EBNA3-reactive EBV immune human antisera react with the fusion protein; antisera that react only with EBNA1 or EBNA2 do not react with the fusion protein (Fig. 2). These data are consistent with the working hypothesis that the EBV DNA segment encodes a protein that has EBNA3 epitopes. As a more definitive test, the fusion protein was purified to homogeneity and used in affinity chromatography to determine whether it would specifically select for EBNA3 antibody. Chromatography of EBV immune human antisera on columns containing the fusion protein removed all other reactivity from the human antisera and significantly enriched for antibody against IB4, Namalwa, and Raji EBNA3 (see Figs. 3 and 5). EBNA3, thus defined, was 142 kDa in IB4 cells, 145 kDa in Namalwa cells, and 137 kDa in Raji cells (Figs. 2 and 3). No reactivity was detected to non-EBV-infected B lymphoblast (Louckes) proteins (Figs. 2 and 3). These data therefore prove that BERF1 encodes epitope(s) of EBNA3. Furthermore, only a 142-kDa EBNA3 was detected in immunoblots of B95-8 cell proteins even though some of these cells are permissive of virus replication and produce many of the replicative cycle viral proteins in high abundance (data not shown). Thus, no early or late virus replicative

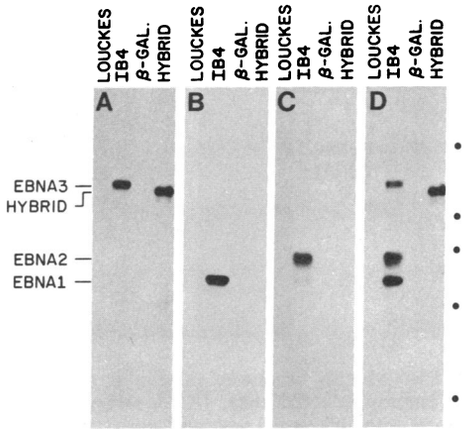

FIG. 2. Expression of the *Bam*HI E BERF1 open reading frame in *E. coli* yields epitopes of EBNA3. Immunoblots of Louckes (an EBV-negative lymphocyte cell line); IB4, (latently EBV-infected lymphocytes), 1 μg of β-galactosidase (β-gal) or 0.1 μg of purified hybrid protein underwent reaction with affinity-purified antibodies to the pKH27 fusion protein (*A*); 457, a human anti-EBNA1 antiserum (*B*); 125, a human anti-EBNA2 antiserum (*C*); or a pool of human EBNA reactive antisera (*D*). The migration of molecular-size markers is indicated by dots.

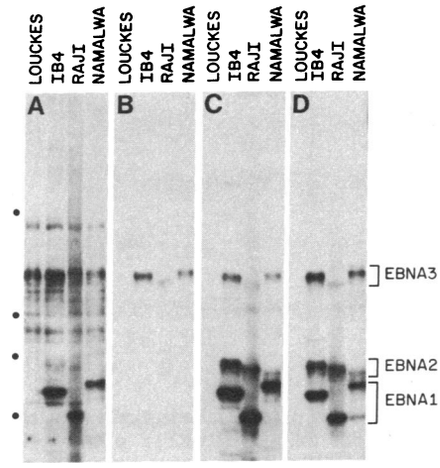

FIG. 3. Affinity-purified antibodies to the pKH27 fusion protein specifically react with a protein that varies in size like EBNA3. Unpurified human (LP) antiserum (*A*), pKH27 fusion protein affinity-purified antibodies from LP antiserum (*B*), MC antiserum (*C*), or pooled EBNA antisera (*D*) underwent reaction with immunoblots of nuclear proteins from the indicated latent infection (IB4, Namalwa), semilatent infection (Raji), or noninfected (Louckes) cell lines. Size markers are indicated by dots.

cycle proteins include the EBNA3 epitopes expressed from pKH27.

Expression of the EBNA3 Open Reading Frame in Rodent Cells. Since prokaryotic expression indicated that the BERF1 open reading frame encodes at least part of EBNA3 and the other EBNA proteins are encoded by an open reading frame within a single exon (5), we tested whether BERF1 encodes all of EBNA3. Accordingly, a fragment that begins 23 bases 5′ to the first ATG of BERF1 was inserted into the eukaryotic expression vector pZip-NEO-SV(X)1. This vector provides for transcriptional initiation, an mRNA cap site, a 5′ untranslated leader, and a separate exon encoding neomycin phosphotransferase, which enables cells to survive in the presence of G418. Other important features of this recombinant construction (Fig. 1*E*) are that (*i*) translation is not known to initiate in the murine leukemia virus leader sequence (36), (*ii*) there is no ATG in the 23 bases 5′ to the three in-frame EBV ATGs, (*iii*) there is no other long open reading frame in BERF1, and (*iv*) the EBV DNA open reading frames for EBNA1 and EBNA2 recombined into pZip-NEO-SV(X)1 are regularly expressed in rodent cells as full-sized proteins (ref. 15; Fig. 4). The recombinant EBV DNA pZip-NEO-SV(X)1 plasmid, pZip-E3, was transfected into NIH 3T3, psi-am22b, or Rat-1 cells, and G418-resistant clones were derived. Most clones transfected with pZip-E3 expressed a protein reactive in immunoblot with anti-EBNA3 antisera (Fig. 4). The proteins expressed in rodent cells were 120–130 kDa, however, while EBNA3 expressed in lymphocytes from the same EBV genome from which the recombinant EBV DNA fragment was derived is 142 kDa. The smaller size of EBNA3 in rodent cells transfected with pZip-E3 is probably not an artifact of protease cleavage during sample preparation, since Rat-1 cells expressing EBNA1 or EBNA2 contained full-sized EBNA1 or EBNA2 (Fig. 4; data not shown). pZip-E3-transfected psi-am22b cells produced a recombinant murine leukemia virus, designated MuLV-E3, which induced expression of the E3 protein in Rat-1 or 3T3 cells (data not shown).

The pZip-E3-expressed protein translocated to the rodent cell nucleus where the fluorescence observed with affinity-purified anti-EBNA3 antibody was similar to that observed with this antibody in EBV-infected lymphocytes (ref. 25; Fig. 5). In pZip-E3-transfected rodent cells and EBV-infected

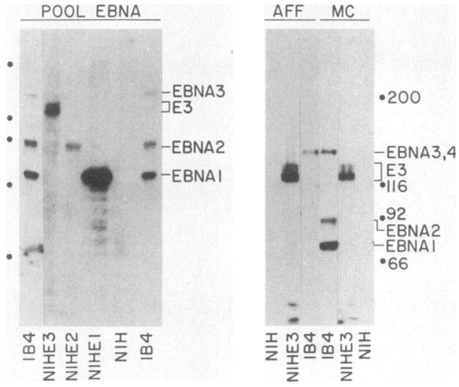

FIG. 4. Expression of pZip-E3 in rodent cells. A NIH 3T3 cell line was transfected with pZip-E3 (BERF1), pZip-E2 (EBNA2), or pZip-E1 (EBNA1). G418-resistant clones were derived and designated NIH E3, NIH E2, or NIH E1, respectively. The EBV-encoded proteins were identified on an immunoblot by using pooled EBNA antiserum, MC antiserum, or pKH27 affinity-purified LP antiserum (AFF). IB4 nuclear proteins are a positive control. NIH 3T3 cells transfected with pZip-NEO-SV(X)1 are a negative control.

lymphocytes, there was intense fluorescence over most of the nucleus except for discrete patches, which appear to result from nucleolar sparing (Fig. 5). As expected, the affinity-purified anti-EBNA3 antisera reacted with nuclei of EBNA3-expressing rodent cells, but not with nuclei of EBNA1- or EBNA2-expressing cells (Fig. 5). The pKH27 affinity-purified and EBNA3-specific antibody detects EBNA3 in all IB4 cells, extending the evidence that EBNA3 is consistently expressed in latent infection (Fig. 5; data not shown).

Other Possible EBNA Proteins. Some human antisera identified at least two proteins in IB4 and Namalwa cell extracts in the size range 137–145 kDa, while other human antisera reproducibly recognized only a single protein in immunoblots of the same sample run on the same polyacrylamide gel (25). For example, WC antiserum consistently recognizes only the EBNA3 protein. This suggests that there is more than one 140-kDa protein in nuclear extracts of IB4 and Namalwa cells. Three EBNA reactive human antisera have been found that react with at least two and probably three different proteins of 142–145 kDa in IB4 cells (Fig. 6*A*). The smallest protein in this size range reacts with the pKH27 affinity-purified anti-EBNA3 antiserum (Fig. 6*B*), while RY antiserum reacts with EBNA3 as well as a slightly larger 143-kDa protein, tentatively designated EBNA4 (Fig. 6*C*). RB antiserum reacts rather specifically with this larger 143-kDa protein, while RS antiserum reacts with an even larger 145-kDa protein, tentatively designated EBNA5 (Fig. 6*A*); MC antiserum reacts with EBNA3 and EBNA4 (Fig. 6*B*). The sizes of the proteins recognized by these antisera in Namalwa cells were 145, 146, and 147 kDa (Fig. 6*C*). Only a 137- and possibly a 138-kDa protein were detected in Raji cells (data not shown).

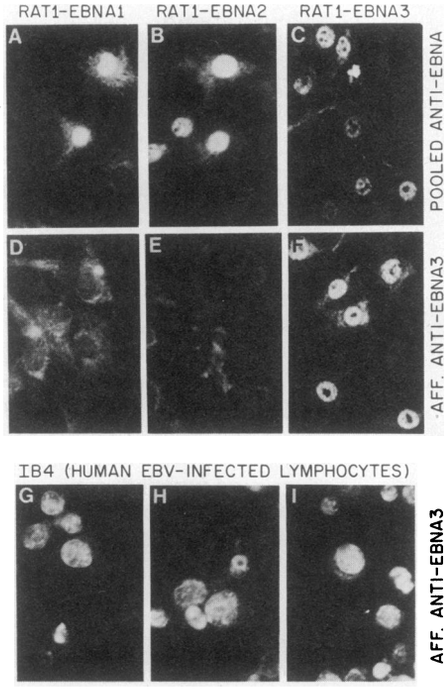

FIG. 5. EBNA3 is a nuclear protein made in all latently infected cells. Either a pool of human EBNA-reactive antisera (*A–C*) or affinity-purified antibodies to the pKH27 fusion protein (*D–F*) were used to stain Rat-1 EBNA1 (*A* and *D*), Rat-1 EBNA2 (*B* and *E*), or Rat-1 EBNA3 (*C* and *F*) cell lines. The cell lines were established by transfection with the recombinant vectors described in the legend to Fig. 4. The pKH27 affinity-purified antibody stains the nuclei of every IB4 cell (*G–I*). Louckes, an EBV-negative cell, shows no nuclear staining. The resultant blank frame is not displayed.

DISCUSSION

These data prove that at least part of the EBV BERF1 open reading frame is translated into EBNA3. Since this DNA segment encodes a single 4.5-kb RNA in latently infected cells (25), that RNA is identified as the EBNA3 mRNA. This is the fourth gene proven to be active in latent infection and growth transformation and is therefore designated *LT4*.

BERF1 contains three DNA repeat segments—A, B, and C—of 69, 24, and 66 bp, respectively, arrayed ABCBCACA in the EBV genome (27). Variation among viral isolates in the number of repeats is probably responsible for the variation in EBNA3 size on $NaDodSO_4$/PAGE. This type of variation is common in EBV proteins (7–11). The A, B, and C elements are not related to each other and copies of each element have diverged so that the A repeats encode peptides that are 87% conserved, B repeats encode peptides that are only 25%

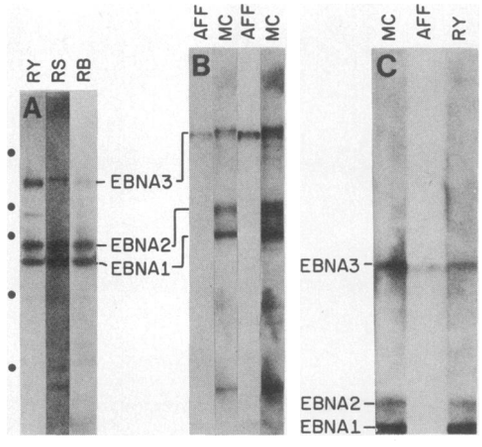

FIG. 6. EBNA3 may be one of the family of 140-kDa EBNA proteins in latently infected cells. (*A*) A series of EBV immune human antisera with differential reactivity with IB4 cell EBV nuclear proteins in the 140-kDa size range on immunoblots. (*B*) The pKH27 affinity-purified EBNA3-specific antibodies (AFF) react with the smallest protein in the 140-kDa size range. Two exposures of the same IB4 nuclear protein immunoblots beside each other show that a human antiserum, MC, reacts with a protein larger than the EBNA3 protein identified by affinity-purified antibody to the pKH27 fusion protein. (*C*) Adjacent strips from one immunoblot of Namalwa nuclear proteins show differential reactivity of MC antiserum or the affinity-purified antibody with 140-kDa proteins. Molecular size markers are indicated by dots in *A*.

conserved, and C repeats encode peptides that are 85% conserved. The peptides encoded by the A and C repeats include amino acid sequences that are likely to assume α-helical conformation based on algorithms derived for globular proteins of known structure (37). Perhaps significantly, constructs expressing fusion proteins that were reactive with human antisera contain this segment of BERF1. The repeated or structured features of those domains may account for their seemingly prominent antigenicity as evidenced by the repeated isolation of this segment from the pool of 300 fusion proteins screened with human antisera.

Our data suggest that IB4 and Namalwa cells, although tightly restricted to latent infection, are expressing not only EBNA1, EBNA2, EBNA3, and LMP, but also two other proteins similar in size to EBNA3. The larger size of these antigens excludes them from being degradation products of EBNA3. Since the affinity-purified EBNA3 antibody and antibody against EBNA1, EBNA2, or LMP do not react with these other proteins, they are distinct proteins and may be EBNA4 and EBNA5. Although each of the proteins previously identified in latently infected IB4 or Namalwa have been shown to be characteristic of latent infection, EBNA4 and EBNA5 are tentative designations, since it remains to be demonstrated that these proteins are consistently expressed from the EBV genome in every latently infected cell.

BERF1 is one of three EBV DNA *Bam*HI E fragment open reading frames, which are similar in size and have considerable homology at their 5′ ends (27). Outside of the 5′ end, there is little homology, consistent with our finding that antibody to the repeat domains of EBNA3, which are encoded by the 3′ end of BERF1 (and expressed from pKH27), reacts only with EBNA3. Based on the similar sizes of EBNA3 and the putative EBNA4 and EBNA5 proteins, our current working hypothesis is that the BERF1-related open reading frames, BERF2b and BERF4, encode EBNA4 and EBNA5. Some support for this hypothesis derives from the observations that part of the EBV DNA *Bam*HI E fragment is deleted from the EBV genome in Raji cells (38, 39), and, while EBNA3 is detected in Raji, one of the other two large EBNAs cannot be detected (ref. 25; Figs. 2 and 3). In addition, only a small fraction of the latent viral genome is transcribed into mRNA, making it likely that any unidentified messages come from one of those regions already identified as being transcribed into stable mRNA (25).

The other salient features of BERF1 are as follows: (*i*) There are only 12 lysine codons in the 811 codons after the first methionine and none in a run of three basic amino acids as characterizes the nuclear location signals of polyoma or simian virus 40 T proteins (40). Despite this, the "EBNA3" protein encoded by BERF1 in rodent cells localizes to the nucleus. (*ii*) There is no significant homology to nuclear proteins whose sequence was in the Genbank databank as of January 1985. (*iii*) There are not two neighboring predicted helical domains similar to the suggested consensus sequence for DNA binding proteins (41).

The role of EBNA3 in latent infection remains to be defined, as does that of EBNA4 and EBNA5 (should further investigation confirm that these are also encoded by the EBV genome in latent infection). EBNA2 affects the ability of cells to grow in a low level of serum (15). LMP transforms cells to anchorage independence and tumorigenicity in nude mice (16). EBNA1 is important in transactivating an EBV "ori"-like sequence for episome maintenance and possibly also for enhancer activation (42). More than one gene may be involved in the regulation of viral and cellular gene expression in latency. The expression of part or all of EBNA3 in rodent cells may make it possible to define EBNA3 functions in these cells.

At the 1985 International Herpes Virus Workshop, Dr. Bengt Kallin presented immunoblots with evidence for more than one high molecular weight EBNA (43) and pointed out that our data (25) were consistent with this possibility. L. Petti, G. Tosato, M. Hirsch, and H. zur Hausen provided sera for these studies. Terri Cole-Reid did the immune microscopy. This work was supported by Grants CA 17281 and CA 19264 from the Public Health Service National Institutes of Health and ACS MV32K from the American Cancer Society. K.H., F.W., and E.W.B. are postdoctoral trainees supported by Grants GM-07183 and AI-07099 from the Public Health Service, National Institutes of Health and PF-2617 from the American Cancer Society, respectively.

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