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Expression of the Epstein-Barr Virus *Bam*HI A Fragment in Nasopharyngeal Carcinoma: Evidence for a Viral Protein Expressed In Vivo

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A family of mRNAs that are transcribed rightward through the *Bam*HI A fragment have been detected in C15, a nasopharyngeal carcinoma (NPC) which has been passaged in nude mice. Northern (RNA) blot hybridizations indicate that these RNAs are also expressed in three other NPCs which have been established in nude mice and in an NPC obtained at biopsy. Moreover, hybridization in situ detected transcription from *Bam*HI A in 12 NPCs and 1 Epstein-Barr virus (EBV)-containing carcinoma of the parotid gland. In each case, transcription was detected in all of the malignant epithelial cells. Transcription was not detected in two cases of EBV-positive lymphoma biopsies by in situ hybridization nor in latently infected EBV-positive lymphoblastoid cell lines by Northern blot hybridization. The consistent transcription of these sequences in latently infected epithelial malignancy but not in lymphoid cells suggests that this viral function is associated with latent EBV infection of epithelial cells. Sequence analysis of a cDNA synthesized from the C15 tumor, representing the 3′ end of *Bam*HI A messenger RNA, revealed an open reading frame (ORF). Translation of this ORF in vitro produced several peptides that were immunoprecipitated with antisera from patients with NPC. The detection of antibodies to the protein encoded by the ORF present in the *Bam*HI A cDNA indicates that *Bam*HI A encodes a protein which is expressed in vivo and is antigenic.

In addition to the ability of the Epstein-Barr virus (EBV) to efficiently immortalize human B cells in vitro, the virus is consistently detected in two human malignancies, the endemic form of Burkitt's lymphoma and nasopharyngeal carcinoma (NPC) (24, 26, 33). It is likely that EBV is a contributing factor in the development of human malignancy. Therefore, it is important to identify the viral genes which are expressed in the EBV-associated cancers.

EBV transcription has primarily been characterized in lymphoid cell lines. In nonpermissively infected lymphoid cell lines, 11 transcripts and 9 distinct antigens have been identified (17, 28–30, 34). The proteins expressed during latency include six nuclear antigens (17), the latent membrane protein (LMP1) (9, 15), and the membrane proteins encoded by the two terminal mRNAs (LMP2), which are also called the terminal proteins (TP1 and TP2) (21, 31).

In NPC, EBV infection is predominantly latent (25, 27, 36). Examination of NPC tissue for specific viral antigens known to be expressed in lymphoid cell lines revealed that EBNA 1 was detected in all specimens, in the absence of the other EBNAs, and LMP1 was detected in approximately 65% of the specimens on immunoblots (8, 36).

It is possible that maintenance of latent infection in epithelial cells and alteration of expression of cellular genes may require expression of viral functions which are distinct from those expressed in lymphoid cells. To identify those genes which may be uniquely expressed in epithelial cells, viral transcription has been characterized in NPC tissue (27, 33). Initial studies of EBV transcription in C15, an NPC which has been successfully established in nude mice (6), identified several mRNAs that differ from those detected in lymphoid cell lines. The differences include the presence of an additional mRNA which can encode LMP1 and several RNAs, including a predominant 4.8-kb mRNA, which are transcribed rightward through the *Bam*HI A fragment (11, 14). The *Bam*HI A sequences are not transcribed in latently infected lymphoid cells (11). Sequence analysis of partial cDNAs revealed that the transcript was spliced and terminated at a polyadenylation signal at bp 160,986 (11). The cDNA contained a previously unidentified open reading frame (ORF), now termed *Bam*HI A rightward frame 0 (BARF0), which was located on the opposite strand to a major ORF, BALF3 (3). Hybridization in situ revealed that *Bam*HI A was transcribed in all cells in the C15 tumor (11). The C15 tumor does not contain linear forms of EBV DNA, nor are replicative antigens detected (11, 14, 36). These data and the detection of *Bam*HI A transcription in all of the cells suggest that the *Bam*HI A mRNAs are transcribed in latently infected epithelial cells in C15. In addition, the identification of a transcript in antisense orientation to known replicative functions suggested a potential regulatory role for the RNA (14).

To determine whether these sequences are consistently expressed in NPC, additional NPC specimens have been screened for expression by Northern (RNA) blot analysis and in situ hybridization. Rightward transcription through BARF0 was detected in all NPC established in nude mice, as well as in all biopsy samples obtained from patients with NPC. A synthetic mRNA prepared from a cDNA representing the 3′ end of the mRNA and the potential ORF was

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translated in vitro. Sera from NPC patients precipitated the in vitro translation products.

These data indicate that rightward transcription from *Bam*HI A is consistently detected in all cells in NPC tissue. The mRNA encodes proteins in vitro, 32 and 26 kDa in size, to which patients with NPC have antibody, revealing that the ORF is expressed in vivo. The identification of in vitro protein products suggests that in addition to a potential regulatory role, the RNA also encodes a protein. The RNA itself or the protein product may contribute to the maintenance of latent infection in epithelial cells or affect epithelial cell growth.

MATERIALS AND METHODS

Tissue specimens. Biochemical and in situ analyses of EBV transcription was performed on four NPC specimens, C15, C17, C18, and C19, established in nude mice (6) and in a metastatic NPC (CKT), which was surgically excised. Primary NPC specimens which were obtained at biopsy were characterized histopathologically and examined by in situ hybridization for EBV transcription. The clinical information concerning the carcinoma of the parotid gland has been published (20). The specimen analyzed here is specimen 5 from that study and represents an undifferentiated carcinoma of the parotid gland.

RNA preparation and nucleic acid blotting. To obtain RNA from frozen tissue samples, the specimens were pulverized in a microdismembrator (Braun Instruments, Fisher Scientific, Lexington, Mass.) before being dissolved in 4 M guanidine thiocyanate for CsCl step-gradient separation (7, 27).

Polyadenylated [poly(A)$^+$] RNA was separated from the total cellular RNA fraction by oligo-dT cellulose chromatography. Poly(A)$^+$ RNA (5 or 10 μg) was subjected to electrophoresis through a 0.8% agarose-formaldehyde gel and transferred to nitrocellulose (23).

Preparation of radiolabelled probes and hybridization. Radiolabelled probes were prepared from a *Sau*IIIA fragment encoding the EBER1 gene (coordinates 5418 to 6794), the 1.9-kb *Xho*I a fragment representing LMP, and the C25a cDNA spanning the BARF0 ORF cloned into the pGEM2 vector containing promoters for the SP6 and T7 bacteriophage RNA polymerases (Promega, Madison, Wis.) (2, 10, 11). Single-stranded RNA probes were synthesized in a reaction mixture consisting of 1 μg of linearized template in 50 μl of a solution containing 40 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 2 mM spermidine, 100 μg of bovine serum albumin per ml, 10 mM dithiothreitol (DTT), 0.5 mM unlabelled nucleotides, 120 μCi of ^{32}P- or ^{35}S-labelled UTP, and 10 U of T7 or SP6 polymerase. RNA probes were hybridized to Northern blots at 52°C in a hybridization solution consisting of 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 1× Denhardt's solution, 0.2% sodium dodecyl sulfate (SDS), 100 μg of calf thymus DNA per ml, and 50 mM PIPES (piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), pH 6.4, in 50% formamide (11).

In situ hybridization. In situ hybridization was performed on small tissue fragments obtained and quickly frozen at biopsy. The tissues were hybridized to EBER1 as a positive control for EBV infection (10, 11). The tissues were fixed for 15 min in 4% paraformaldehyde in phosphate-buffered saline (PBS) at 0°C, 15 min in 0.1% deoxycholate–0.1% Triton X-100 in PBS at room temperature, 10 min in PBS with $MgCl_2$ at room temperature, 10 min in 0.2 M Tris HCl (pH 7.5)–0.1 M glycine at room temperature.

The tissue was then hybridized overnight at 50°C in a solution containing 50% formamide, 0.6 M NaCl, 10 mM Tris (pH 7.5), 1 mM EDTA (pH 7.5), 1% SDS, 10 mM DTT, 0.25 μg of *Escherichia coli* tRNA, 1× Denhardt's, 10% polyethylene glycol 8000, and 2.0 × 10^6 to 2.5 × 10^6 cpm of ^{35}S-labelled riboprobe. Prior to hybridization, the riboprobe was hydrolyzed at 60°C for 2 h in 40 mM $NaHCO_3$–64 mM $NaCO_3$. After hybridization, unhybridized probe was removed by washing twice in PBS with $MgCl_2$ for 5 min at room temperature. Nonspecific probe was removed by incubation of the tissue sample for 30 min at 37°C in 20 μg of RNase A per ml in 0.5 M NaCl–10 mM Tris-HCl (pH 8.0).

The tissue was then washed twice in 2× SSC–50% formamide–10 mM DTT at 50°C for 30 min, in 2× SSC–50% formamide–10 mM DTT at 50°C for 30 min, and then in 1× SSC–50% formamide–10 mM DTT–0.05% Triton X-100 at 37°C for 30 min.

Tissue was then embedded and sectioned prior to autoradiography. The slides were dipped in a 1:1 solution of 0.06 M ammonium acetate and Kodak NTB2 emulsion warmed to 40°C, dried for 1 h, and sealed in a container of Drierite in darkness at 4°C. Exposure time varied from a few days to up to 2 weeks.

Exposed slides were then developed in Kodak D-19 developer for 4 min and then washed in distilled water and fixed in Kodak fixer for 4 min. After fixation, the tissue was stained with either Giemsa or hematoxylin and eosin (10, 11).

In vitro transcription and translation of *Bam*HI A cDNA. The 1.4-kb C25a cDNA was obtained from a lambda gt11 library prepared with *Eco*RI linkers. The cDNA was transferred into the $pGEM_2$ vector containing promoters for SP6 and T7 bacteriophage RNA polymerases. In addition, the 0.95-kb *Eco*RI (coordinates 159853 to 160986), 0.4-kb *Eco*RI (coordinates 159516 to 159853), and 0.78-kb *Pst*I (coordinates 160030 to 160986) subfragments of the C25a cDNA were also subcloned into the $pGEM_2$ vector (11). RNA synthesized in vitro was translated in rabbit reticulocyte lysates (Promega, Madison, Wis.). The in vitro translation (IVT) products were incubated with sera obtained from patients with NPC or EBV-negative antisera, immunoprecipitated with *Staphylococcus aureus* (Pansorbin; Calbiochem, San Diego, Calif.) and analyzed by electrophoresis through SDS-polyacrylamide gels. The ORF which encodes the EBV DNA polymerase was also translated in vitro as a positive control (22).

RESULTS

Identification of EBV mRNAs transcribed from *Bam*HI A. Initial studies of transcription in C15, an NPC passaged in nude mice, identified several RNAs, including a predominant 4.8-kb mRNA, transcribed rightward from the *Bam*HI A region (11, 14). In addition to the C15 tumor, three other NPC tumors, C17, C18, and C19, have been successfully established in nude mice (6). In each tumor, a single restriction enzyme fragment representing the fused termini of EBV was detected, without evidence of linear forms of EBV, indicating that the tumors were latently infected and that the EBV genome was clonally transmitted in these tumors (25).

To determine whether *Bam*HI A is consistently transcribed in NPC, Northern blots were prepared with RNA obtained from the additional NPC tumors passaged in nude mice and from a NPC sample obtained at biopsy. The filters were hybridized to a single-stranded RNA probe synthesized in antisense orientation from the C25a cDNA. This probe

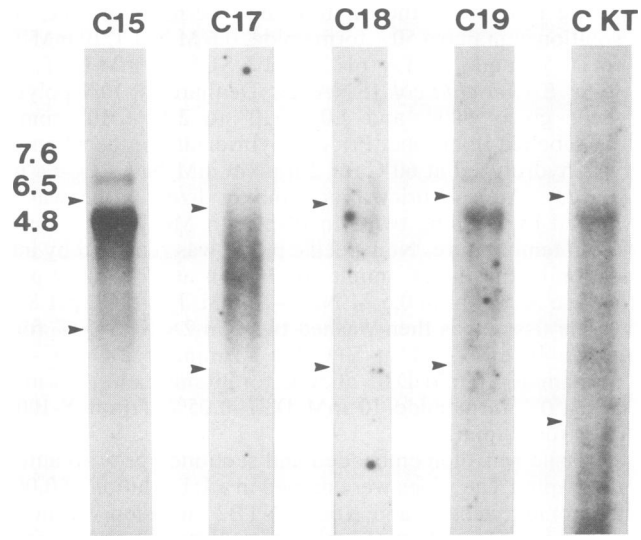

FIG. 1. Northern blot hybridization of *Bam*HI A to C15, C17, C18, C19, and CKT RNA. Blots prepared with poly(A)$^+$ RNA from the NPC specimens passaged in nude mice, C15, C17, C18, and C19 (10 μg) and total RNA (20 μg) from CKT, metastic NPC surgically excised, were hybridized to approximately 2×10^8 cpm of strand-specific riboprobe prepared from the C25a cDNA. Arrowheads indicate the position of the 5.2- and 2.0-kb rRNAs. Sizes of the detected bands are shown at left in kilobases.

represents sequence homologous to the 3′ end of the 4.8-kb message. In the C15 tumor, the predominant 4.8-kb mRNA as well as less abundant 6.5- and 7.6-kb mRNAs was identified (Fig. 1). It is at present unknown whether the larger RNAs are partially processed precursors of the 4.8-kb mRNA or are distinct mRNAs. The prominent 4.8-kb mRNA was also detected in C19 and CKT, an NPC specimen obtained at biopsy. Trace levels of hybridization were detected in the C17 and C18 tumors, which rapidly become necrotic during growth in nude mice, impeding preparation of RNA with good integrity.

The detection of the 4.8-kb RNA in all of the NPC specimens passaged in nude mice and in CKT, obtained at biopsy, suggests that this transcript is expressed in fresh NPC tissue and during passage in nude mice.

Analysis of *Bam*HI A transcription in situ. It has been shown by in situ hybridization that *Bam*HI A is transcribed in all the tumor cells of C15 (11). To determine whether the *Bam*HI A transcript was expressed in all cells or was transcribed focally within the additional NPC specimens which contained detectable mRNA by Northern analysis, single-stranded RNA probes labelled with ^{35}S were synthesized from C25a in both sense and antisense orientation and hybridized to tumor material. In hybridizations with the antisense RNA probes, grains indicating positive hybridization were throughout the C18 and C19 tumors passaged in nude mice (Fig. 2A and C). In the C18 tumor, hybridization to a region of stroma in the upper left portion of the section was not detected (Fig. 2A). Hybridization with the sense RNA probes was also not detected (Fig. 2B and D). In addition, hybridization of the antisense probe was detected in NPC specimens obtained at biopsy. In the CKT specimen, strong hybridization to islands of tumor was detected, whereas adjacent normal tissue and infiltrating lymphoid stroma did not hybridize to the *Bam*HI A probe (Fig. 2E). Hybridization with the *Bam*HI A antisense RNA is also shown in an additional primary NPC specimen (Fig. 2F).

To compare EBV expression in NPC with EBV-positive lymphoma, probes representing EBER1, LMP, and the *Bam*HI A cDNA were hybridized to the CKT specimen and to EBV-positive lymphoma, L93 (Fig. 3). Hybridization was detected with the EBER1 and LMP probes in all cells in both the NPC and lymphoma specimens; however, *Bam*HI A transcription was only detected in the NPC specimen but was not detected in the lymphoma sample.

To determine whether *Bam*HI A was consistently transcribed in vivo, NPC specimens obtained at biopsy were screened for expression of *Bam*HI A. Hybridization for the EBER1 RNA was used as a positive control. As presented in Table 1, transcription from *Bam*HI A and EBER1 was detected in the four tumors passaged in nude mice, in eight specimens of primary NPC, and in one specimen of carcinoma of the parotid gland. In contrast EBER1 but not *Bam*HI A transcription was detected in two specimens of EBV-positive B-cell lymphoma.

The hybridization in situ results reveal that detection of transcription from *Bam*HI A does not reflect expression in a few cells within a tumor but rather that *Bam*HI A is specifically transcribed in all of the malignant epithelial cells. In addition, the consistent detection of *Bam*HI A transcription in all NPC and in an EBV-positive carcinoma of the parotid gland but not in EBV-positive lymphoma suggests that *Bam*HI A is preferentially expressed in latently infected epithelial tissues.

In vitro translation of the *Bam*HI A cDNA. A schematic diagram of the C25a cDNA with potential translation initiation codons is shown in Fig. 4. The sequence revealed a previously unidentified open reading frame, BARF0, located at the 3′ end of the cDNA, from bp 159576 through 160986. A single splice of 171 bp which retained the reading frame was detected. However, potential ATG initiation codons at bp 160470 and 160509 are 3′ to the in-frame splice and would encode for proteins of approximately 20 and 18 kDa.

To determine whether the BARF0 ORF encodes protein which is expressed in vivo, RNA was synthesized in vitro from the entire 1.4-kb cDNA and from the two *Eco*RI subclones, 0.95 and 0.4 kb, and a 0.78-kb *Pst*I-*Eco*RI subclone. The RNAs were translated with [^{35}S]methionine in rabbit reticulocyte lysate, and the IVT products were precipitated with serum from patients with NPC and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE). As a positive control for a known EBV protein, the BALF5 ORF which encodes the EBV polymerase was also translated in vitro and reacted with NPC sera (22).

The 0.95-kb subclone, which encompasses the BARF0 ORF, produced several IVT products which could be immunoprecipitated with sera from NPC patients. Predominant polypeptides migrated with apparent molecular masses of approximately 32 and 26 kDa (Fig. 5). In addition, smaller polypeptides of 17, 15, and 11 kDa were recognized in the IVT reactions. The polypeptides could be precipitated with sera from four different NPC patients (data not shown). To obtain the broadest reactivity, the sera were pooled to screen the IVT products of the three subclones (Fig. 5). The products did not react with EBV-negative antisera (Fig. 5). Immunoprecipitable products were not produced from reticulocyte lysate incubated without RNA nor with RNA synthesized from the 0.4-kb subclone (coordinates 159516 to 159853), which lacks an ATG (data not shown). Small amounts of the 32- and 26-kDa polypeptides were produced in vitro from the 1.4-kb cDNA and were only detected on

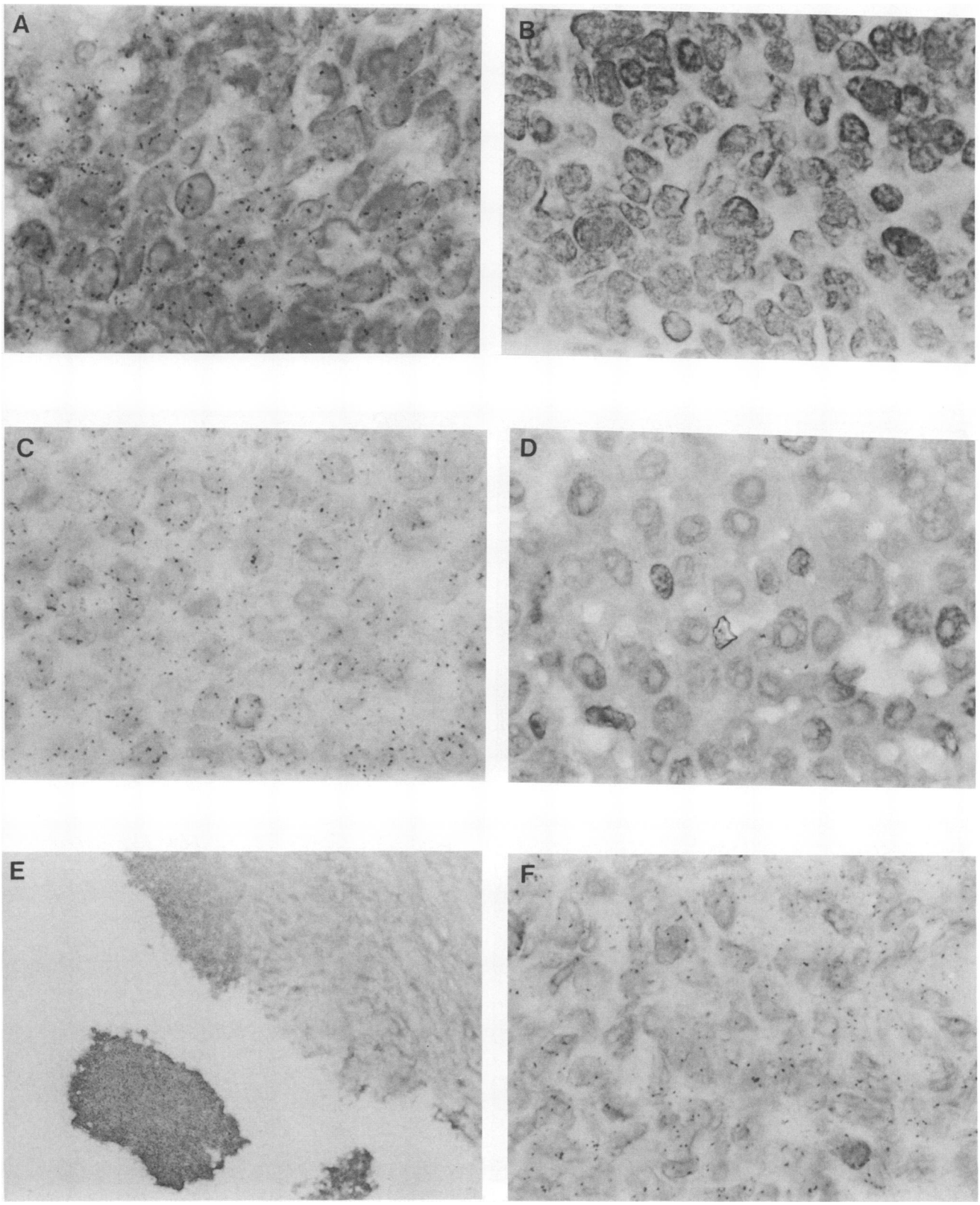

FIG. 2. In situ hybridization of *Bam*HI A to NPC tissue. Sense and antisense ^{35}S-labelled riboprobes synthesized from the C25a cDNA were hybridized to NPC tissue and exposed to emulsion for 1 week as follows: antisense (A) and sense (B) to C18; antisense (C) and sense (D) to C19; antisense to CKT (E); antisense to NPC 11, an Egyptian NPC (F). Magnification, ×1,000 (A, B, C, D, and F) and ×100 (E).

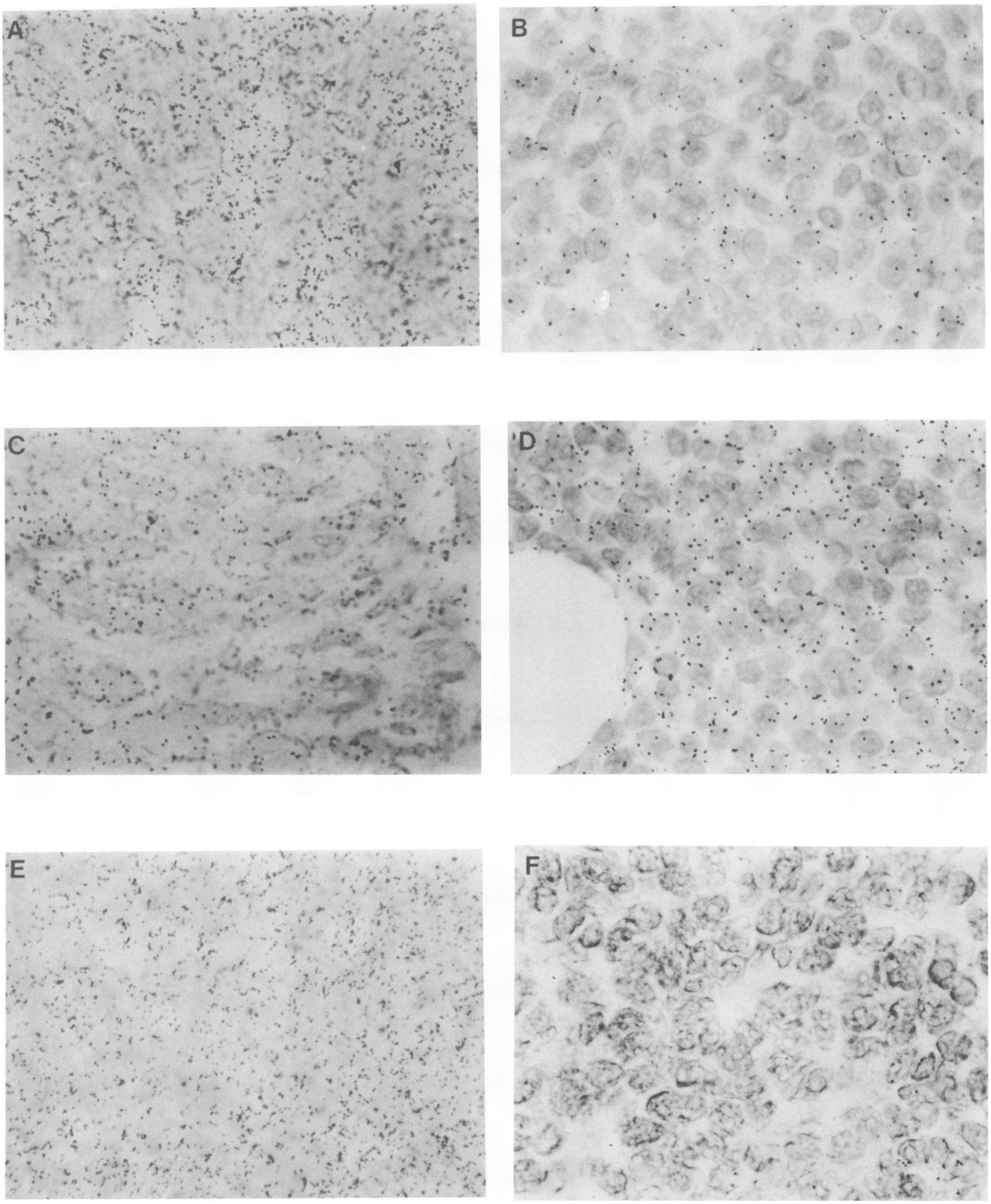

FIG. 3. In situ hybridization of EBER1, LMP, and *Bam*HI A to NPC and non-Hodgkins lymphoma. Antisense ^{35}S-labelled riboprobes synthesized from a 0.5-kb *Sau*IIIA fragment homologous to EBER1, a 1.9-kb *Xho*I fragment homologous to LMP or the C25a cDNA were hybridized to thin sections of NPC (CKT) (A, C, and E) and non-Hodgkin lymphoma (L93) (B, D, and F) tissue and exposed to emulsion for 1 week as follows: (A) EBER1 to CKT, (B) EBER1 to L93, (C) LMP to CKT, (D) LMP to L93, (E) C25a to CKT, and (F) C25a to L93. Magnification, ×1,000.

TABLE 1. Summary of EBV transcription in related malignancies

Tumor specimen	No. transcribed/no. tested	
	EBER	*Bam*HI A
NPC		
Nude mouse passaged	4/4	4/4
Fresh biopsies	8/8	8/8
Parotid carcinoma	1/1	1/1
Non-Hodgkin lymphoma	2/2	0/2

long exposure after immunoprecipitation. The low level of IVT products produced from the 1.4-kb cDNA may be due to the TGA stop codon at bp 159576 or may reflect the longer distance before the potential ATG.

The methionine initiation codons at bp 160470 and 160509 predict polypeptides of approximately 20 and 18 kDa. Alternative initiation codons with good Kozak consensus sequence are indicated in Fig. 4. However, the 0.4-kb *Eco*RI subfragment representing bp 159516 through 159853 did not produce any precipitable protein, whereas the 0.95-kb *Eco*RI subfragment from bp 159853 through 160986 and the 0.78-kb *Pst*I subfragment from bp 160030 through 160986 both resulted in the synthesis of the 32- and 26-kDa polypeptides. This result suggests that the 32-kDa polypeptide, which is synthesized with somewhat lower efficiency than the 26-kDa polypeptide, results from in vitro translation initiated at the CTG at bp 160252, which has a favorable Kozak context with a purine present at the −3 position and a G in the +4 position (18, 19). The 26-kDa polypeptide which is synthesized with somewhat better efficiency most likely represents initiation at the first ATG at bp 160470. The putative protein is approximately 15% arginine, which may contribute to its somewhat anomalous migration. Proteins such as histones which have a high concentration of basic amino acids migrate slower in SDS-PAGE, resulting in an overestimation of molecular weight (12). In comparison, IVT of the BALF5 ORF also produced multiple polypeptides, although only the predicted 110-kDa polypeptide representing the entire ORF was precipitable (22).

DISCUSSION

The data presented in this report indicate that a 4.8-kb mRNA is transcribed from the *Bam*HI A fragment in all examined NPCs. This message has not been detected in the latently infected Raji, IB4, or CB4 cell lines, nor was transcription from *Bam*HI A detected by in situ hybridization in specimens of EBV-positive non-Hodgkin's lymphoma (11, 34). A related RNA is transcribed from the same sequences early after induction of replication in P3HR1, B95-8, and Akata cells and in superinfected Raji cells (4, 16, 32). The transcript identified in the lymphoid cell lines differs in size from the mRNA identified in NPC in that it is 4.2 rather than 4.8 kb. Sequence analysis of a *Bam*HI A cDNA cloned from B95-8 poly(A)$^+$ RNA indicates that the 4.8-kb mRNA expressed in latently infected epithelial cells in NPC and the 4.2-kb early replicative RNA expressed in lymphoid cell lines are 3′ coterminal (data not shown).

The EBV transcripts in latent infection are spliced (5).

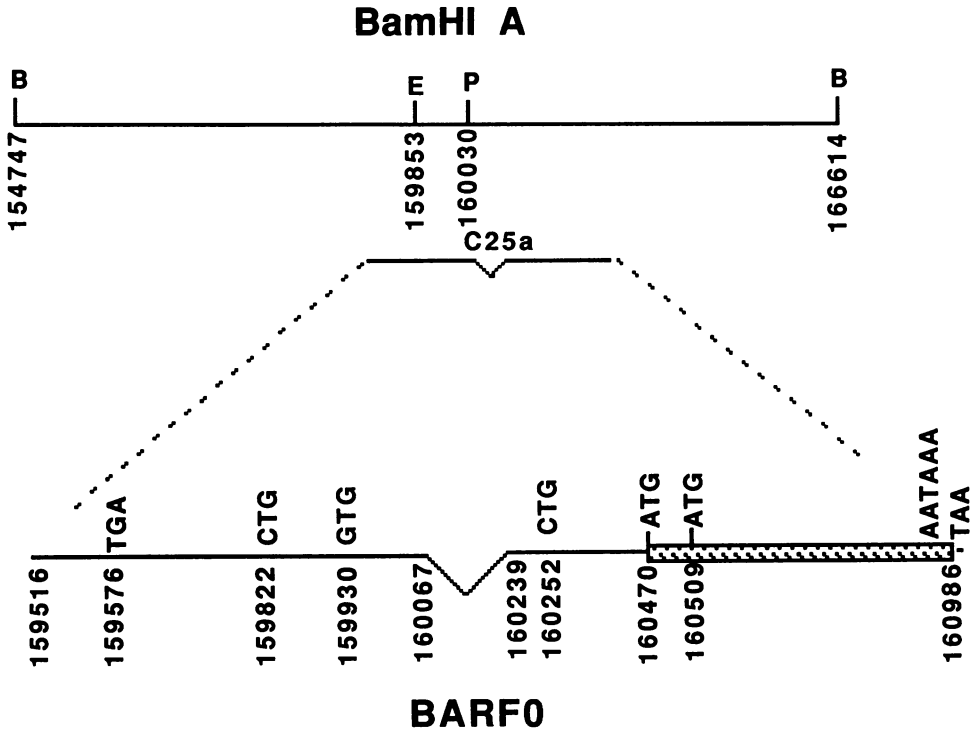

FIG. 4. Diagram of BARF0. The C25a cDNA in *Bam*HI A containing the BARF0 ORF is shown with map coordinates. Genomic restriction endonuclease sites are indicated above as follows: B, *Bam*HI; E, *Eco*RI; and P, *Pst*I. ATG initiation codons are denoted as are possible CTG and GTG initiation codons. TGA and TAA stop codons defining BARF0 are indicated with map coordinates. The in-frame splice in C25a is depicted by hashed lines in center. The cross-hatched bar represents the portion of the ORF which follows the ATG initiation codon.

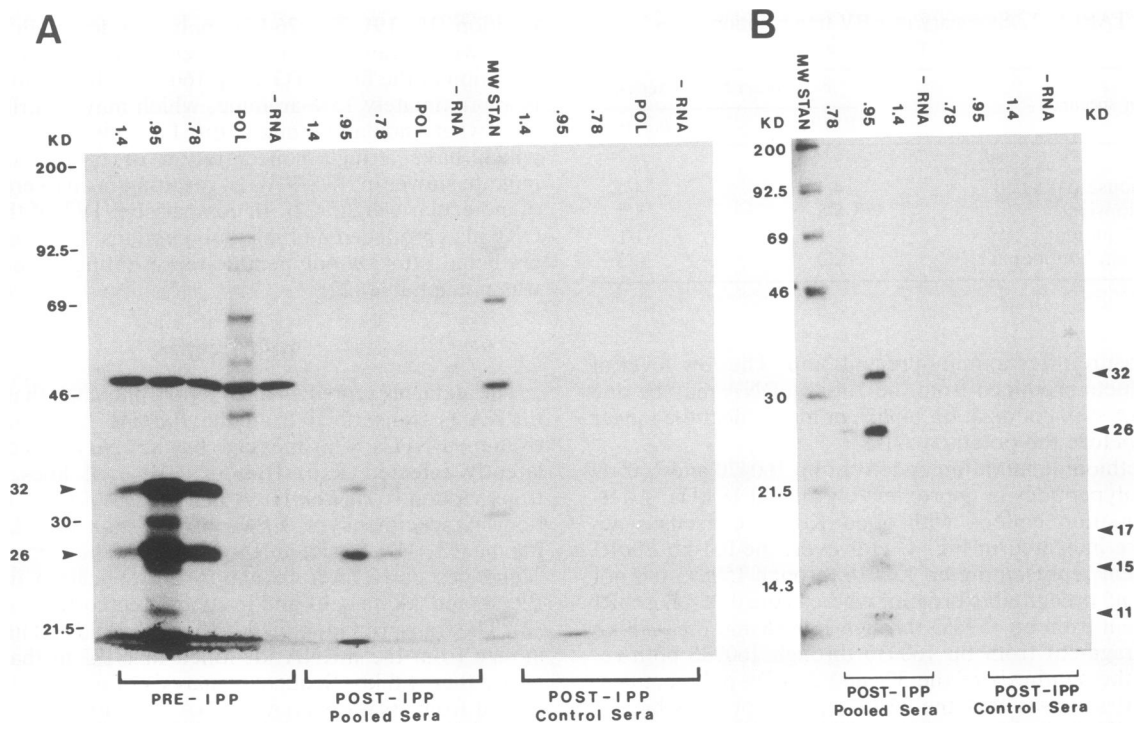

FIG. 5. In vitro translation of cDNA containing BARF0. RNA synthesized in vitro from the 1.4-kb cDNA, the 0.95-kb *Eco*RI-*Eco*RI, and the 0.78-kb *Pst*I-*Eco*RI subfragments of the C25a cDNA containing BARF0 were translated in vitro in rabbit reticulocyte lysate with ^{35}S-labeled methionine and immunoprecipitated (IPP) with pooled high-titer NPC sera or EBV-negative sera. The BALF5 ORF which encodes the EBV DNA polymerase (POL) was also translated in vitro (27). Immunoprecipitated rabbit reticulocyte lysate without input RNA (−RNA) was included as a negative control. IVT products are denoted by arrowheads. Molecular masses are indicated in kilodaltons. Molecular mass protein standards (MW STAN) are indicated on the right. (A) 10% polyacrylamide gel; (B) 12.5% gel used to identify smaller polypeptides.

Comparison of the latent and lytic forms of the LMP1 mRNAs revealed that these mRNAs are 3′ coterminal, but the lytic form of LMP1 is not spliced and encodes a protein which lacks the amino terminus of LMP1 (15). In the *Bam*HI A cDNA the splice is 5′ to the potential ATG methionine start codons and should not affect the encoded protein unless an alternate codon is used to initiate translation. The existence of two major IVT products of apparent molecular masses of 32 and 26 kDa suggests that the translation initiates from the CTG at bp 160252 in vitro (19). Initiation at CTG has been identified in alternative forms of two oncogenes, c-*myc* and *int-2*, a growth factor related to fibroblast growth factor (1, 13).

It has been suggested that the *Bam*HI A transcripts negatively regulate transcription of the replicative functions, including the viral polymerase encoded by BALF5, which are encoded by the opposite strand (14). The identification of a potential protein product which is recognized by sera obtained from patients with NPC suggests that in addition to the potential antisense regulatory role, the RNA encodes a protein which is expressed in vivo.

The consistent detection of the 4.8-kb mRNA transcribed through BARF0 in NPC and the lack of expression in latently infected lymphoid cell lines and lymphoid tissues suggests that this RNA is preferentially expressed in epithelial cells. Similarly, some of the EBNA genes may be uniquely expressed in lymphoid cells, since it has previously been shown that the EBNA 2, 3A, 3B, and 3C genes are not expressed in NPC (8, 36). Recent studies indicate that EBNA 2 transactivates expression of the LMP1 promoter in lymphoid cells (35). However in NPC, LMP1 is expressed in the absence of EBNA 2 expression (8, 11, 14, 36). Therefore, cellular transcription factors may be sufficient for expression of LMP1 in NPC, or other uncharacterized viral factors may regulate LMP expression.

The lack of expression in NPC of viral functions which are expressed in latently infected lymphoid cells and the detection of transcription which is specifically expressed in epithelial cells support the hypothesis that different viral functions are required to maintain latent infection or alter the growth properties of lymphoid or epithelial cells. The expression of distinct viral genes in the two cell types may underlie the ability of the virus to establish latent infection in different cellular environments.

ACKNOWLEDGMENTS

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