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Epstein-Barr Virus Transcription in Nasopharyngeal Carcinoma

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Sequences which encode Epstein-Barr virus (EBV) RNA in nasopharyngeal carcinoma (NPC) tissue have been identified. We utilized human biopsy material directly as well as NPC grown in nude mice. Total RNA was extracted from the tumor material and separated into polyadenylated and nonpolyadenylated fractions by oligodeoxythymidylate-cellulose chromatography. This material was used as template to construct ^{32}P-labeled cDNA. The labeled cDNAs were hybridized to Southern blots of recombinant EBV DNA fragments. Three of the biopsies, F, 49, and 55, contained polyadenylated RNA homologous to the EBV *Bam*HI fragments V and K, and *Eco*RI-DIJhet. These same fragments encode the most abundant polyribosomal RNAs in latently infected lymphoblastoid cell lines. The sequences which encoded nonpolyadenylated RNA in NPC tumor 49 were more extensive and included *Bam*HI fragments C, V, B, E, and K, and *Eco*RI fragments DIJhet, E, F, and G1, a result that indicates selective polyadenylation in EBV RNA processing. A fourth biopsy, NPC tumor 18, contained polyadenylated RNA homologous to the *Bam*HI fragments H, B, K, Y, B1, I1, and A and *Eco*RI fragments F and G2. A similar pattern of transcription was identified in three tumor specimens from nude mice, 4, 5, and 8. Transformation of lymphocytes did not occur after cocultivation in vitro with explants from these nude mice tumors. This transcriptional pattern may represent an activated state of the EBV genome, formerly not detected in tumor tissue, which is analogous to the state of abortive infection identified in induced in vitro cell systems.

The Epstein-Barr virus (EBV) is the etiological agent of infectious mononucleosis, a benign lymphoproliferative disease (16), and is associated with African Burkitt's lymphoma, a monoclonal B lymphoblastoid malignancy (10), and nasopharyngeal carcinoma (NCP), a tumor arising from the squamous epithelium of the nasopharynx (19). Although EBV is thought to be lymphotrophic, in NPC tissue EBV DNA is detected in the malignant epithelial cells and not within the lymphocytes which heavily infiltrate the carcinomatous tissue (38). Biochemical analyses of the role of EBV in NPC have been hampered because the malignant epithelial cells cannot be cultivated in vitro, and pathological tissue can only be obtained by biopsy. However, these tumors can be grown in nude mice, which eliminates the infiltrating human lymphoid elements. These epithelial tumors when cultivated in the nude mice retain EBV DNA and the EBV nuclear antigen (25, 33).

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Each of the pathological processes associated with EBV has a distinct pattern of antibody response to viral antigens (17). Patients with NPC have elevated immunoglobulin G (IgG) and IgA titers (15, 17) to the viral capsid antigen (14) and early antigen (EA) (18) and to an EBV-associated DNase (2). These titers increase with tumor burden and can be used as indicators for prognosis (19). In addition, rises in EA titers precede the onset of NPC by 1 to 2 years (8). Although EA and viral capsid antigen cannot be detected in latently infected, growth-transformed lymphocytes and are only detected in cells which are abortively or productively infected, the serological profiles of patients with NPC and Burkitt's lymphoma suggest that an activated state of viral expression, perhaps analogous to an abortive infection, may be an important part of the malignant process. Moreover, in NPC, expression of EA and viral capsid antigen most likely occurs in tissues which stimulate IgA production.

Analyses of EBV transcription have been conducted in latently, abortively, and produc-

tively infected lymphoblastoid cell lines. In latently infected lymphocytes, three relatively abundant polyadenylated RNAs encoded by *Bam*HI V, X, and H, *Bam*HI-K, and *Eco*RI-Dhet are transcribed (23, 35). Several lines of evidence indicate that the transcript from *Bam*HI-K encodes a component of EBV nuclear antigen (20, 32).

EBV transcription in abortive infection has been studied in latently infected Raji cells which can be induced to a more permissive infection with halogenated nucleosides, sodium butyrate, or phorbol esters (24). EA can be detected in these cells. After induction, transcription from *Bam*HI fragment H greatly increases, and additional sequences encode polyadenylated RNA, including *Bam*HI fragments A, B, E, and M.

In permissively infected cells, the complexity of polyadenylated RNA is approximately that of the single-stranded EBV genome. More than 50 EBV RNAs have been identified in B95-8 cells (21) and in HR-1 cells (37), which have been induced to a high level of permissive infection by treatment with the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate. Some of these RNAs are classified as persistent early RNAs based on their continued synthesis in the presence of phosphonoacetic acid (21). These RNAs have been shown to encode components of EA in in vitro translations (22). Many of these persistent early RNAs are encoded by the additional sequences that are transcribed in Raji cells after induction. From these various in vitro observations, it appears that abortive infection may be characterized by the synthesis of a subset of early viral replicative functions.

Based on such in vitro data, it should then be possible to ascertain the state of viral expression at the level of RNA by determining sequence complexity and transcriptional patterns directly in NPC—an important step forward toward understanding the virus-cell relations and the different antibody responses in the EBV-associated diseases. In the course of this work, we uncovered an EBV transcriptional pattern in NPC tissues, which we term "activated," that up to now had only been seen in vitro with the experimental manipulations mentioned earlier. We also analyzed EBV transcription in NPC cultivated in nude mice.

MATERIALS AND METHODS

Tumor material. NPC tissue documented histopathologically by G. Pearson and colleagues, Mayo Clinic, Rochester, Minn., and A. Huang and colleagues, Duke University, Durham, N.C., was used for this work. Specimens, NPC F, C, 49, and 55 were designated as World Health Organization (WHO) NPC class WHO 3, and specimen 18 was WHO class 1. The NPC specimens passaged in nude mice were all WHO class 3.

Isolation of RNA. All glassware used in the isolation and selection of RNA was treated with 0.2% diethyl pyrocarbonate for 2 h and then autoclaved or baked overnight at 250°C. The tumor samples were homogenized in a Tissumizer (Tekmar Inc., Cincinnati, Ohio) in a solution consisting of 4 M guanidine thiocyanate (Tridom, Inc., Hauppage, N.Y.), 0.5% Sarkosyl, 0.1 M 2-mercaptoethanol, 0.1% antifoam A (Sigma Chemical Co., St. Louis, Mo.), and 25 mM sodium citrate, pH 7.0 (3). The RNA was purified through a cesium chloride step gradient (30).

Polyadenylated and nonpolyadenylated RNAs were separated by oligodeoxythymidylate-cellulose chromatography (1) (type 3; Collaborative Research, Waltham, Mass.).

Identification of viral DNA which encodes RNA in nasopharyngeal tumor tissue. To identify the EBV sequences which are transcribed in the tumor tissue, cDNA to polyadenylated and nonpolyadenylated RNA was synthesized with avian myeloblastosis virus reverse transcriptase (J. Beard, Life Sciences, St. Petersburg, Fla.). The reaction mixture contained: 2 to 30 μg of RNA; 200 μCi of [^{32}P]dCTP (New England Nuclear Corp., Boston, Mass.); 500 μM dATP, dGTP, and dTTP (P-L Biochemicals, Milwaukee, Wis.); 30 mM 2-mercaptoethanol; 10 mM $MgCl_2$; 0.01% Nonidet P-40; 6 μg of actinomycin D per ml; and 30 U of reverse transcriptase in a 100-μl reaction volume. The reaction was primed with 0.1 absorbance units at 260 nm of oligodeoxythymidine (P-L Biochemicals) to provide a further selection for polyadenylated RNA. Regions of the EBV genome which may be transcribed but not polyadenylated were identified by synthesis of cDNA, primed with 200 μg of random oligodeoxynucleotides per ml (33), to the RNA obtained from the effluent of the oligodeoxythymidine-cellulose column.

The ^{32}P-labeled cDNAs were then hybridized to Southern blots (31) of recombinant DNA *Eco*RI fragments of EBV W91 DNA (27). In addition, blots of recombinant DNA of the *Bam*HI fragments V, K, R, B1, I1, W1, C, and H (4) were included in the hybridizations to verify possible hybridization to regions of particular interest. The *Bam*HI fragments V, K, and H are transcribed in latently infected lymphocyte cell lines (24). *Bam*HI-C encodes two polyadenylated RNAs of very low abundance and two nonpolyadenylated RNAs (35). The fragments B1, I1, and W1 contain the sequences deleted from the B95 strain (26, 27) and are thought to encode, in part, the most abundant RNAs in Burkitt tumor tissue (6).

Preparation of Southern blots and hybridization conditions. The recombinant DNAs of the three largest *Eco*RI fragments, A, B, and C, were digested with *Eco*RI and *Bam*HI, the recombinant DNAs of the *Eco*RI fragments DIJhet, E, F, G1, G2, and H were digested with *Eco*RI, and the recombinant DNAs of the *Bam*HI fragments were digested with *Bam*HI. The fragments were separated by electrophoresis through a 0.5% agarose gel and transferred to nitrocellulose (31). The blots of each fragment were cut into duplicate strips. One strip of each set was hybridized to EBV HR1 virion DNA labeled with [^{32}P]dCTP by nick translation (29). This step determined the position of the EBV fragment(s) on the blot and provided a comparison of the relative strength of each blot.

The nitrocellulose blots of the EBV fragments were pretreated for 4 h at 72°C in hybridization buffer consisting of 10× Denhardt reagent (7), 100 μg of single-stranded calf-thymus DNA per ml, 100 μg of polyadenylate (Sigma) per ml, 0.2% sodium dodecyl sulfate and 6× SSC (1× SSC is 0.15 M NaCl pus 0.015 M sodium citrate). The labeled cDNA was hybridized to the pretreated blots in fresh hybridization buffer at 72°C for 18 h. The blots were washed at 72°C in 0.2% sodium dodecyl sulfate in SSC in decreasing concentrations to 0.1× SSC.

RESULTS

Identification of the EBV fragments transcribed in NPC obtained by biopsy. (i) NPC specimens F and C. Biopsies F and C were each less than 0.05 g. Therefore, the ^{32}P-labeled cDNA was synthesized from the total RNA prepared from these biopsies and primed with oligodeoxythymidine as the only selection for polyadenylated sequences. The hybridizations with cDNA to RNA from specimen F, cDNA to RNA from specimen C, and ^{32}P-labeled HR1 DNA to the blots of the recombinant EBV DNA fragments are shown in Fig. 1. The hybridization with EBV HR1 DNA to each fragment is positioned on the left and is aligned with the cDNA hybridization. The strongest hybridization with the cDNAs prepared from specimens F and C was to the *Bam*HI V fragment, which is generated by a single cut within the large internal repeated sequence of the EBV genome. This hybridization was detected both on the blot of the *Eco*RI/*Bam*HI digestion of the *Eco*RI-A fragment and on the blot of the *Bam*HI V fragment. Hybridization to undigested *Bam*HI-V recombinant DNA was present on the blot of this fragment. Hybridization was also detected to the *Bam*HI fragments X and C, which are adjacent to *Bam*HI-V, each of which contains a part of the internal repeated sequence. The cDNA to RNA from specimen NPC F also hybridized to *Bam*HI-K within *Eco*RI-B and weakly to the clone of the fused-termini *Eco*RI fragment DIJhet. The regions of EBV that were transcribed in these two tumors are represented in the EBV genome map in Fig. 7a.

(ii) NPC specimens 55 and 49. Hybridization with cDNA to the polyadenylated RNA from NPC specimens 55 and 49 was detected to the *Bam*HI fragments V and K and *Eco*RI-DIJhet (Fig. 2a and b). The intensity of hybridization to the *Eco*RI DIJhet fragment was much stronger in these specimens compared to the hybridizations with the cDNAs prepared from NPC specimens F and C, which might reflect differences in the relative abundance of transcription from this fragment.

The oligodeoxynucleotide-primed cDNA to the nonpolyadenylated RNA from biopsy 49 hybridized to the *Bam*HI fragments C, H, and V within *Eco*RI-A; to *Bam*HI fragments B, E, and K within *Eco*RI-B; and to *Eco*RI fragments D, E, F, and G1 (Fig. 2c). These results indicate that there was good selection for polyadenylated sequences in the previous hybridization and that a subset of the total EBV RNAs which are transcribed in NPC 49 is polyadenylated. Similar results with RNA from EBV-infected lymphoblastoid cell cultures suggests selective processing of EBV viral RNA (23, 24).

(iii) NPC specimen 18. Hybridization with cDNA to the polyadenylated RNA from specimen 18 was detected to *Bam*HI-H within *Eco*RI-A; to *Bam*HI fragments B and K and faintly to fragments E and Y within *Eco*RI-B; to *Bam*HI fragments B1, I1, V1, and A within *Eco*RI-C; to *Eco*RI-DIJhet; and weakly to *Eco*RI fragments F and G2 (Fig. 3a). The *Bam*HI fragments H and B1 contain homologous duplicated sequences (5, 27). Therefore, cDNA from RNA transcribed from either of the duplicated sequences will hybridize to B1 and H. Hybridization with the cDNA to the nonpolyadenylated RNA was detected to *Bam*HI fragments C, H, B1, and V and to *Eco*RI fragments DIJhet, F, and E (Fig. 3b). In summary, although sequences from *Bam*HI-V can be detected in the nonpolyadenylated RNA, no hybridization was detected to *Bam*HI-V with the cDNA from the polyadenylated RNA from NPC 18. Moreover, hybridization was readily detected to the *Bam*HI-H fragment from which transcription greatly increases in iododeoxyuridine (IUdR)-induced Raji cells (24) and which encodes an abundant 2.5-kilobase persistent early RNA in a productive infection (21). Hybridization was also detected to additional sequences, many of which were transcribed after induction of Raji cells with IUdR.

Identification of the EBV sequences transcribed in NPC grown in nude mice. NPC, like other tissues of epithelial origin, can be cultivated only with difficulty, if at all, in vitro. Therefore, we turned to specimens of NPC that had been cultivated in nude mice. The three original NPC specimens were from three Taiwanese patients. Five tumors derived by cultivation of these three specimens in nude mice were analyzed. Three of the tumors are different passages of the same original NPC. One of the tumors is a lymphoblastoid tumor which resulted from the inoculation of a lymphoblastoid cell line established during attempts to cultivate NPC tissue, but it is not epithelial tissue.

(i) NM 4, 5, and 8. Nude mouse tumors (NMs) 4, 5, and 8 are different passages of the same original NPC specimen. NM 4 had been passaged in nude mice 38 times, NM 5 had been passaged 39 times, and NM 8 had been passaged 13 times, cultivated in vitro once, and then passaged an additional 20 times. Cocultivation

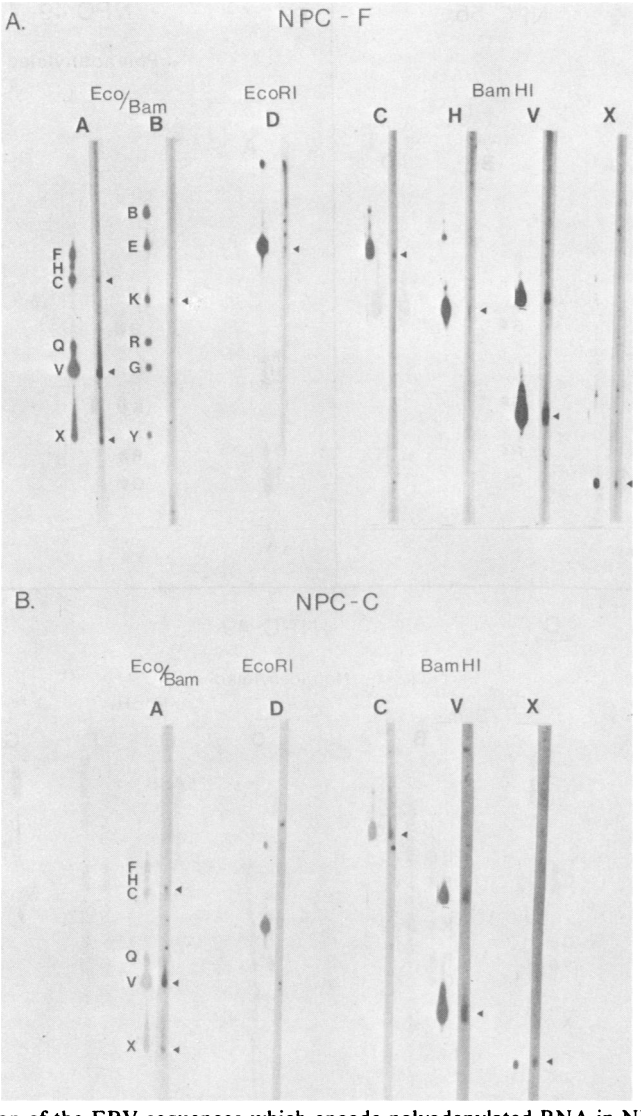

FIG. 1. Identification of the EBV sequences which encode polyadenylated RNA in NPC biopsy specimens F and C. ^{32}P-labeled cDNAs synthesized to the polyadenylated RNA from tumor F (panel A) and to the polyadenylated RNA from tumor C (panel B) were hybridized to duplicate strips of blots of restriction enzyme fragments of EBV. The *Eco*RI fragments, A, B, and C were digested with *Eco*RI and *Bam*HI. The *Bam*HI fragments within each of the *Eco*RI fragments are indicated at the left. A duplicate strip of each blot, which was hybridized to ^{32}P-labeled HR1 viral DNA, is shown to the left of the cDNA hybridization. The arrows indicate hybridization with the cDNA probe.

of in vitro explants of these tumors with lymphocytes did not result in lymphocyte transformation.

The transcriptional patterns of these tumor passage levels were very similar. The cDNAs prepared from the polyadenylated RNA from NMs 4, 5, and 8 (Fig. 4a, b, and c) hybridized to *Bam*HI-H within *Eco*RI-A; to *Bam*HI fragments B, K, and Y within *Eco*RI-B; to *Bam*HI-B1 within *Eco*RI-C; and to *Eco*RI fragments D, E, F, G2, and H. The cDNA from NMs 4 and 5 also hybridized to *Bam*HI fragments I1, V1, and A within *Eco*RI-C. The bacterial clones containing the *Eco*RI fragments E, G1, G2, and H also contain *Eco*RI-C to which hybridization can be detected with the cDNA from NMs 4 and 5. The arrows denote the positions of E, G1, G2, and H.

The cDNA from the nonpolyadenylated RNA from NM 8 hybridized to *Bam*HI fragments F, H, and V within *Eco*RI-A; to *Bam*HI fragments B, E, and K within *Eco*RI-B; to *Bam*HI frag-

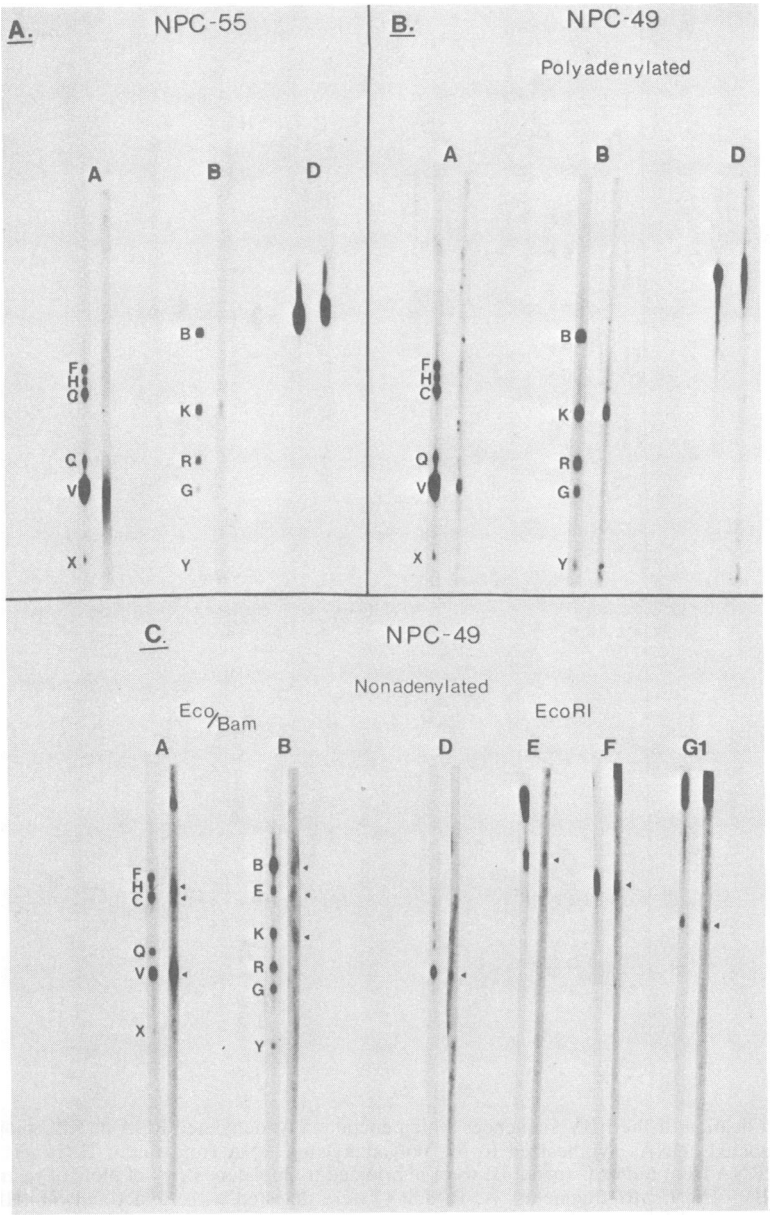

FIG. 2. EBV transcription in NPC biopsy specimens 55 and 49. The hybridization with ^{32}P-labeled cDNAs to polyadenylated RNA from NPC 55 (A) and polyadenylated (B) and nonpolyadenylated (C) RNA from NPC 49 are shown in the right-hand strip of each fragment and are indicated with arrows. The left strip was hybridized to ^{32}P-labeled HR1 viral DNA.

ments B1, I1, and A within *Eco*RI-C; and to *Eco*RI fragments D, E, and F (Fig. 4d). These results indicate that RNA processing through selective adenylation is retained in the NPC tissue grown in nude mice.

(ii) NM 1. NM 1 was passaged in nude mice 38 times. Unlike the other NMs transformation of lymphocytes occurred after cocultivation with cells from this tumor, which indicates the presence of infectious virus. The cDNA to the polyadenylated RNA hydridized to all of the *Bam*HI fragments within *Eco*RI-B; to *Bam*HI fragments B1, I1, V1, W, and A within *Eco*RI-C; and to *Eco*RI fragments D, E, F, G1, G2, and H (Fig. 5). Within *Eco*RI-A, abundant transcription from *Bam*HI fragments H and F was detect-

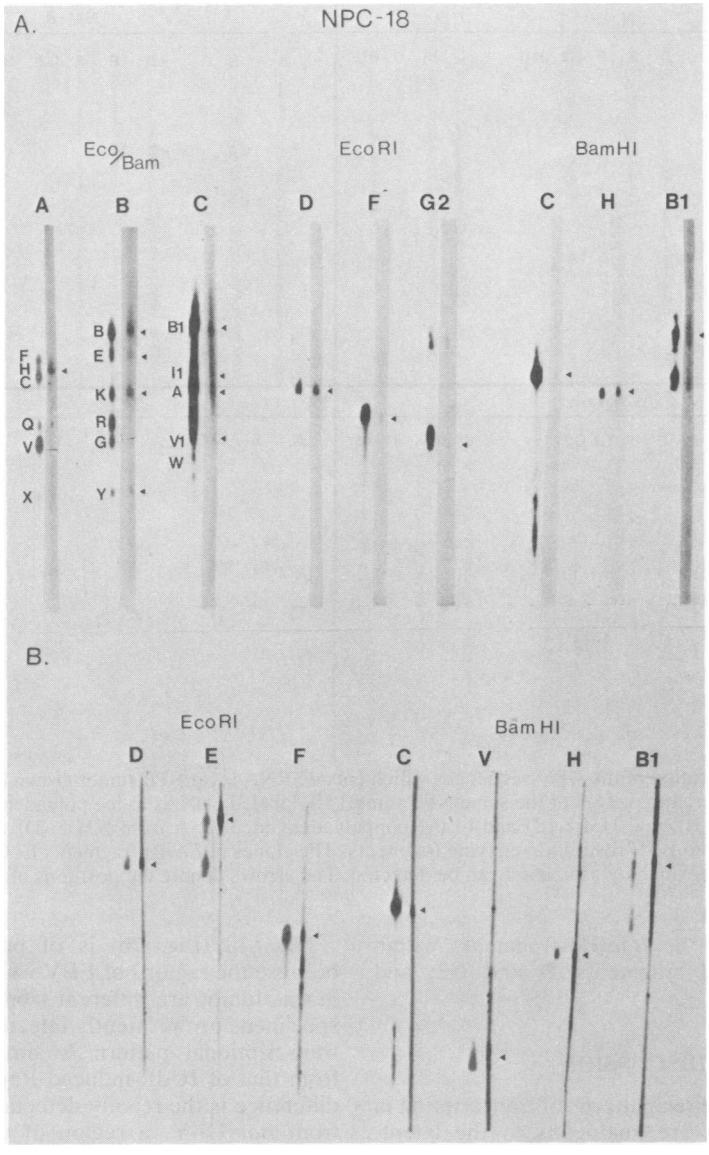

FIG. 3. EBV sequences which encode RNA in NPC 18. Hybridization with cDNAs to the polyadenylated RNA (A) and nonpolyadenylated RNA (B) to restriction enzyme fragments of EBV are shown in the right strip of each blot. The arrows indicate hybridization with the cDNA probe. Hybridization with ^{32}P-labeled HR1 viral DNA is presented on the left. The *Bam*HI-B1 clone contains a subpopulation of deleted fragments to which hybridization is also detected. The *Eco*RI-G2 clone also contains *Eco*RI-C, to which hybridization can be detected. The arrow denotes the position of G2.

ed. The pattern of relative abundance indicated by these hybridization results is similar to that found in a productively infected cell line. This is the only sample from which we have detected strong hybridization to *Bam*HI-F, which encodes 11 RNAs in a productive infection (21).

(iii) NM 3. NM 3 is the 31st passage in nude mice of a lymphoblastoid tumor which resulted from the inoculation of a lymphoblastoid cell line established from NPC tissue. The cDNA to the polyadenylated RNA from this tumor hybridized very strongly to *Bam*HI-H in addition to *Bam*HI fragments C, V, E, K, and B1 and *Eco*RI-Dhet (Fig. 6). Hybridization with the cDNA to the nonpolyadenylated RNA detected *Bam*HI fragments C, V, H, X, and F within

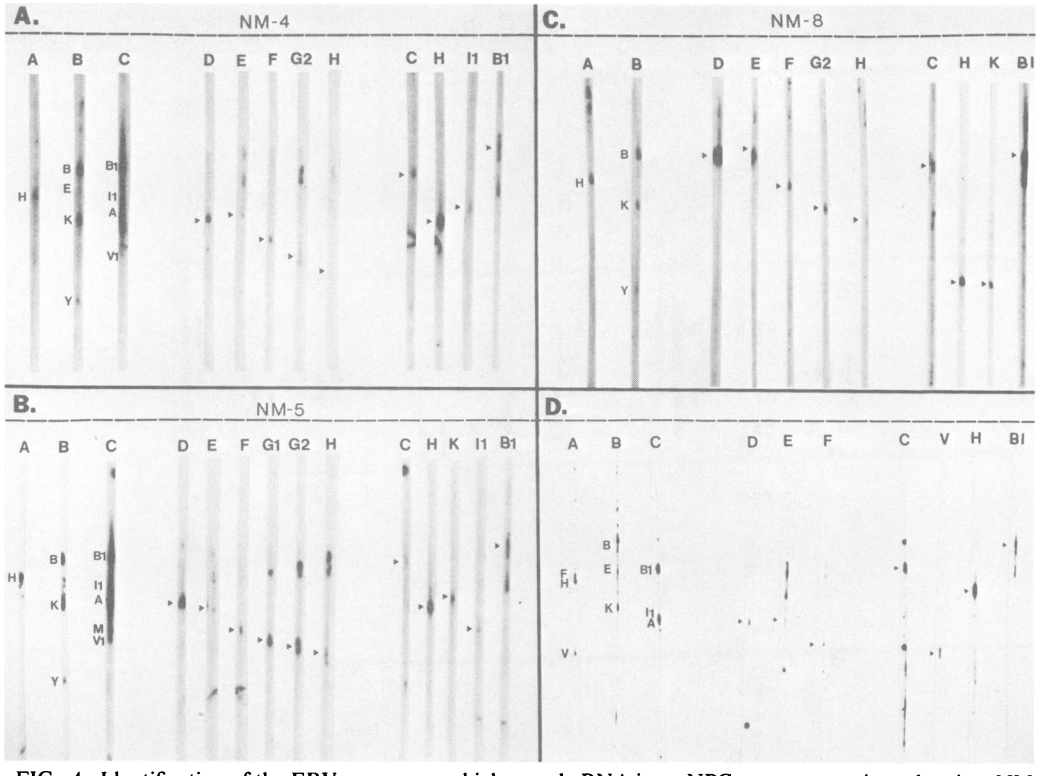

FIG. 4. Identification of the EBV sequences which encode RNA in an NPC tumor grown in nude mice. NMs 4, 5, and 8 are different passages of the same NPC tumor. ^{32}P-labeled cDNAs to the polyadenylated RNAs from NM 4 (A), NM 5 (B), and NM 8 (C) and to the nonpolyadenylated RNA from NM 8 (D) were hybridized to duplicate blots of the EBV restriction enzyme fragments. The clones of *Eco*RI fragments E, G1, G2, and H also contain *Eco*RI-C to which hybridization can be detected. The arrows denote the positions of E, G1, G2, and H.

*Eco*RI-A; all of the *Bam*HI fragments within *Eco*RI-B; *Eco*RI fragments D and G2; and *Bam*HI-B1.

DISCUSSION

We identified three patterns of transcription in NPC tissue that are analogous to the latent, abortive, and productive infections identified in latently infected and induced lymphoblastoid cell cultures. To present and contrast the NPC transcriptional patterns best, we grouped them according to pattern and compared them with the corresponding pattern in the EBV lymphoblastoid cell lines. In Fig. 7a, the transcriptional patterns in NPC tissues F, C, 49, and 55 and in the lymphoblastoid tumor grown in nude mice, NM 3, are compared with that of a latently infected cell line (23). The patterns of expression in these tissues are quite similar. Whether the apparent differences in the relative abundance of transcription from particular fragments in the NPC material represent fluctuations in transcription or reflect differences in preservation of template RNA is at this point unknown.

NPC 18 (Fig. 7b) is of particular interest because the regions of EBV which encode RNA in this tumor are different from the other NPC specimens or the latently infected cell lines. The transcriptional pattern is similar yet distinct from that of IUdR-induced Raji cells (24). One difference is the readily detectable transcription from *Bam*HI-Y, a region of the genome that does not encode RNA in latent or IUdR-induced Raji cells but does generate five RNAs, two of which are early, in a productive infection (21). The other obvious difference is the lack of polyadenylated RNA from *Bam*HI-V. Transcription from this fragment is present in Raji cells and is characteristic of latent infection. However, this transcription persists after induction of Raji cells with IUdR. This may be the result of incomplete induction, and the resultant transcriptional pattern may be a composite of the RNAs expressed in latently infected and induced cells. One perspective of the transcriptional pattern in NPC 18 is that it represents a state of abortive infection not simulated by any of the in vitro systems. This finding also suggests that in vivo there may be a transition from

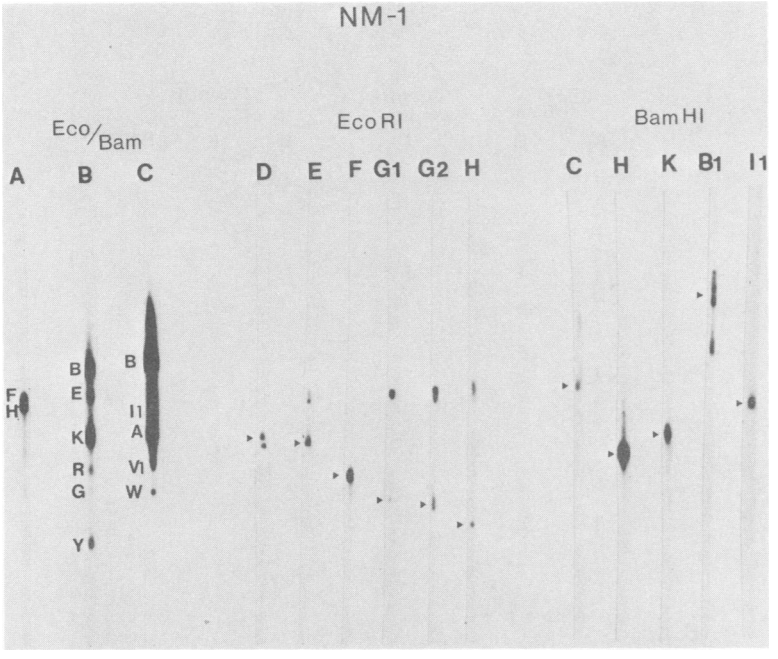

FIG. 5. Identification of the EBV sequences which encode RNA in an NPC grown in nude mice which produces infectious virus.

latent infection to an intermediate state of activated transcription. Such transitions, in some way tied to the course of the disease, may explain the different levels of EBV antibody response detected in patients with NPC.

As it is known that NPC tumor tissue grown in nude mice can produce virus (11, 34), the EBV transcription in these tumors may represent an artificial derepression of the genome. The effect of this derepression is quite different in the epithelial tumors, NMs 4, 5, 8, and 1, compared with the lymphoblastoid tumor NM 3. The lymphoblastoid tumor retains a restricted pattern of expression, with the most abundant transcription encoded by *Bam*HI fragments V and H and *Eco*RI-D. The strong hybridization to *Bam*HI-H probably reflects some activation and may be due to the abundant 2.5-kilobase early replicative RNA encoded by this fragment in a productive infection (21). In contrast, the epithelial tumors grown in nude mice do not contain polyadenylated RNA from *Bam*HI-V and contain transcripts from other additional regions of the EBV genome. The similarity of the transcriptional patterns for NMs 4, 5, and 8 to that of NPC 18 is striking, with the most abundant transcription encoded by *Bam*HI fragments H, B, K, Y, B1, I1, and A. Moreover, comparison of the transcriptional patterns for NMs 4, 5, and 8, or NPC 18 with that in NM 1, which is apparently producing virus, reveals several differences between a productive infection and the state of activation in NMs 4, 5, and 8. The sequence complexity of RNA is greatest in NM 1 and includes, in addition to all the sequences transcribed in NMs 4, 5, and 8, abundant transcription from *Bam*HI-F and all of the sequences within *Eco*RI-B.

The activated state of EBV in the NMs and in NPC 18 is not entirely surprising in that whenever EBV has been introduced into nonlymphoid host cells, either by microinjection (12), transfection (13), or receptor implantation (36), the early antigen has been expressed but not EBV nuclear antigen. It is clear from the data presented here that latent, abortive, and productive infections can occur in epithelial cells. However, essential for the maintenance of latency in epithelial cells may be the presence of EBV-reactive T lymphocytes. Therefore, the loss of the infiltrating lymphocytes during the growth of the tumor tissue in nude mice might promote an activated state of viral expression.

NPCs are classified into three histopathological subtypes by the WHO, which differ in the extent of differentiation of the malignant epithelial cell and the degree of lymphocyte infiltration. The undifferentiated carcinomas, class WHO 3, which are heavily infiltrated with lymphocytes, are clearly associated with EBV, since most patients have elevated EBV antibody titers. The association of EBV with the most differentiated NPC, class WHO 1, is not as consistent, since patients usually do not have

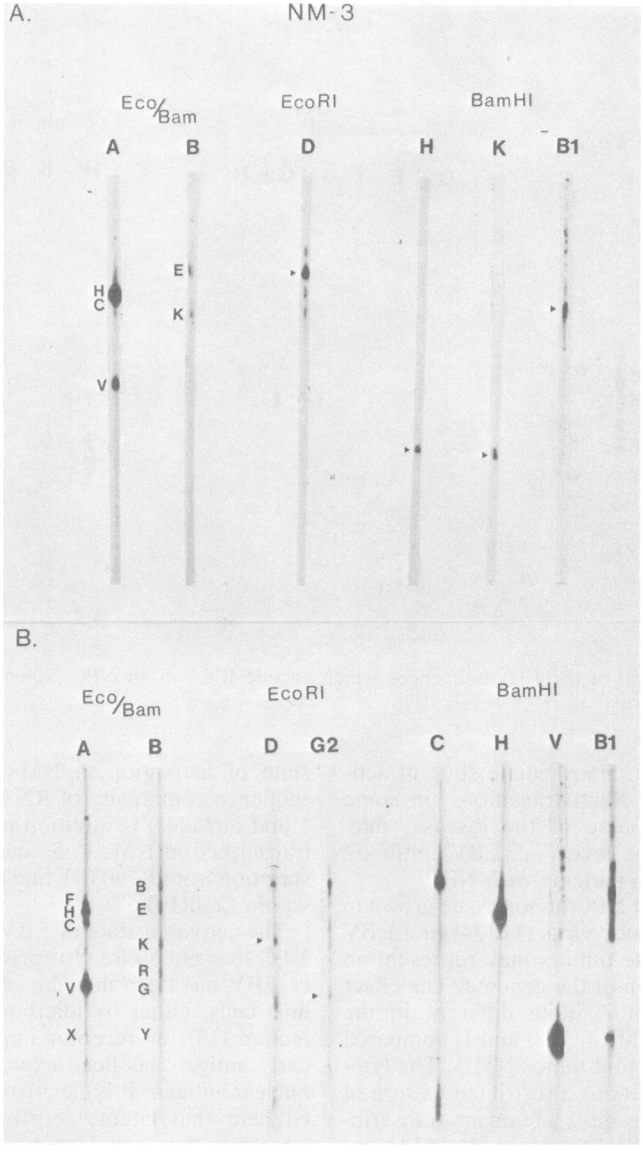

FIG. 6. Transcription in a lymphoblastoid tumor grown in nude mice. The sequences which encode polyadenylated RNA (A) or nonpolyadenylated RNA (B) are identified.

elevated EBV titers. NPC 18 is a keratinizing squamous cell carcinoma, class WHO 1. Perhaps the lack of lymphocyte infiltration or the differentiated state of the cell influences EBV expression in such tumors. One paradox of this situation is that although many of the sequences transcribed in NPC 18 are thought to encode components of EA (22), this patient did not have an elevated titer to EA or viral capsid antigen. It is possible that the EBV expression within the tumor had recently changed and that the patient did not yet have a detectable immune response or that EBV expression is blocked at some step past adenylation. Alternatively, the class of RNAs expressed in this tumor may represent functions expressed before the appearance of the early antigen. A class of proteins has been identified in a permissive EBV system that is transiently expressed early after infection (9). These proteins are not precipitated with antisera to the early antigen. If the RNAs transcribed in NPC 18 encode these proteins, the state of viral expression would be intermediate between latent and replicative and would not necessarily imply cell death.

It is apparent from these data that the same

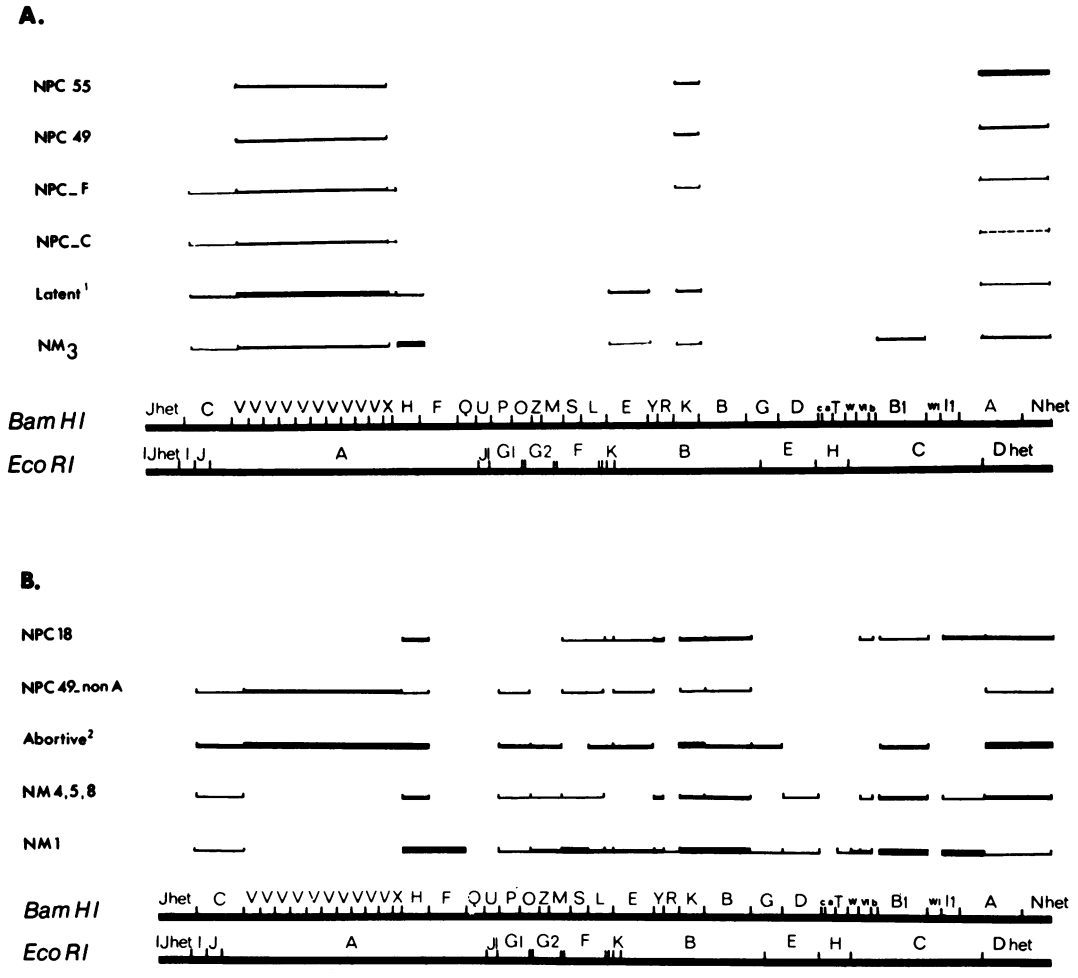

FIG. 7. Summary of EBV transcription in NPC. (A) Latent patterns of expression. Comparison of NPCs F, C, 49, and 55 and NM 3 to a latently EBV-infected lymphocyte line (23). (B) Activated patterns of expression. Comparison of the NPC 18 and NMs 4, 5, 8, and 1, to an induced EBV-infected lymphocyte line (24).

sequences which are transcribed in growth-transformed, latently infected lymphoblastoid cell lines are also transcribed in NPC tissue. However, it is also clear that other sequences can be transcribed and that at times the state of infection may be more analogous to an abortive infection. Based on the similar transcriptional patterns of the four tumors in this activated state, NPC 18 and NMs 4, 5, and 8, it is possible that particular functions are consistently expressed in such abortive infections in NPC tissue.

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