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Epstein-Barr Virus Gene Expression in Malignant Lymphomas Induced by Experimental Virus Infection of Cottontop Tamarins

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Inoculation of cottontop tamarins with a large dose of Epstein-Barr virus (EBV) leads to the induction of multiple EBV genome-positive lymphomas. These tumors have been characterized as oligoclonal or monoclonal large-cell malignant lymphomas that closely resemble the EBV genome-positive B-cell lymphomas that arise in human allograft recipients. The expression of latent and lytic EBV-encoded proteins was investigated in these virus-induced tamarin lymphomas and in derived cell lines. The tamarin tumors were found to express EBV nuclear antigen 1 (EBNA 1), EBNA 2, EBNA leader protein, and the latent membrane protein (LMP) as determined both by immunohistochemical staining and by immunoblotting. However, within the limits of the immunoblotting assays, no expression of the EBNA 3a protein family could be detected. Assays for lytic-cycle proteins by using both polyclonal human sera and monoclonal antibodies against viral capsid antigen, early antigen, and membrane antigen (gp340/220) showed minimal, if any, expression of these antigens in the lymphoma biopsies. In contrast, the cell lines derived from these lymphomas, even in early passage, expressed abundant levels of the lytic-cycle antigens and also expressed the EBNA 3a protein as well as EBNA 1, EBNA 2, EBNA leader protein, and LMP. This finding suggests that the virus-lymphoma cell interaction, in particular the switch to lytic cycle, is subject to some form of host control in vivo. The expression of EBNA 2 and LMP in these tamarin lymphomas strengthens their resemblance to posttransplant lymphomas in humans, since these human tumors are also EBNA 2 and LMP positive (L. S. Young, C. Alfieri, K. Hennessy, H. Evans, C. O'Hara, K. Anderson, A. Rickinson, E. Kieff, and J. I. Cohen, submitted for publication). Since both proteins are known to be important effector molecules of virus-induced B-cell growth transformation in vitro, their expression in these lymphomas constitutes the best evidence for a direct oncogenic role for EBV in vivo.

Epstein-Barr virus (EBV) readily infects human B cells in culture and induces their growth transformation into permanent lymphoblastoid cell lines (LCLs) in which every cell carries multiple episomal copies of the viral genome (12, 25). Only a restricted number of EBV-encoded proteins, the so-called latent gene products, are constitutively expressed in growth-transformed cells. These include six nuclear antigens, EBNA 1, encoded by the BKRF1 reading frame of the viral genome (33), EBNA 2, encoded by BYRF1 (14), EBNA leader protein (EBNA-LP), encoded by multiple exons 5′ of the EBNA 2 gene (32, 38), and EBNA 3a, 3b, and 3c, encoded by adjacent reading frames in the *Bam*HI-E region of the genome (15, 23, 24, 27, 31), plus a latent membrane protein (LMP), encoded by the BNLF1 reading frame (13). Virus-induced B-cell growth transformation in vitro appears to depend on the cooperative action of all of these latent gene products.

EBV is closely associated with two human malignancies of B-cell origin, the high-incidence or endemic form of Burkitt's lymphoma (BL), seen in subequatorial regions of Africa and New Guinea (8), and the oligoclonal B-cell lymphomas to which immunosuppressed allograft recipients are particularly prone (5). Strong but circumstantial evidence suggests an etiological role for EBV in the pathogenesis of BL, but only as one link in a complex chain of events culminating in the malignant transformation of a single virus genome-positive cell; another crucial factor is the deregulation of cellular *myc* gene expression after chromosomal translocation and mutation affecting the c-*myc* locus (3). The special nature of BL cells, as compared with in vitro-transformed LCLs, is emphasized by the recent finding that EBV latent gene expression is unusually restricted in tumor biopsies, with at least two of the important effector proteins of normal B-cell transformation, EBNA 2 and LMP, apparently having been down regulated (29).

Analogous studies of EBV latent gene expression in the oligoclonal B-cell lymphomas of immunosuppressed patients have not yet been systematically carried out, not least because of the difficulties of obtaining sufficiently large tumor biopsies for a detailed study of viral protein content. For this reason, we sought to examine EBV gene expression in virus-induced B-cell lymphomas in an animal model that is very similar to this human disease. Inoculation of cottontop tamarins with a large standard dose of virus regularly gives rise to multiple EBV genome-positive B-cell lymphomas appearing within 14 to 21 days. These lesions are very similar to human posttransplant lymphomas in their histological appearance as large-cell malignant lymphomas with well-circumscribed geographical necrosis, in their lack of any consistent chromosomal abnormality, and in their multifocal presentation, with each focus being composed of either one or a small number of individual clones. Individual tumors are mono- or oligoclonal (6). It is also interesting that a small proportion of experimentally infected tamarins subsequently show regression of these lesions (6), as has been observed with lymphomas in allograft recipients when immunosuppressive treatment is relaxed (5).

Here we demonstrate by in situ hybridization the presence of EBV DNA in every cell of the experimentally induced B-cell lymphomas of tamarins and report on the expression of EBV-encoded proteins both in the lymphoma biopsies

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themselves and in subsequently derived lymphoma cell lines in early-passage culture.

MATERIALS AND METHODS

Cell culture. All cells were cultured in RPMI 1640 medium (GIBCO, Paisley, United Kingdom) supplemented with 10% fetal calf serum, 2 mM glutamine, penicillin (100 IU/ml), streptomycin (100 μg/ml), and gentamicin (50 μg/ml). The cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air and fed twice weekly.

Tamarin tumor biopsies and derived cell lines. Adult cottontop tamarins (*Saquinus oedipus oedipus*) from a successful breeding colony (17) were inoculated with a standard 100% tumorigenic dose of EBV (strain B95.8; $10^{5.3}$ lymphocyte-transforming doses per animal) as described in earlier work (6). Tumors were removed at autopsy; part of each tumor was snap frozen in liquid nitrogen, and the remainder was teased out to give a single-cell suspension. The cells were cultured at a concentration of 10^6 cells per ml in 2-ml Linbro wells (Flow Laboratories, Rickmansworth, United Kingdom), and cell lines were established by direct outgrowth. In addition, an EBV-transformed LCL was established from the normal peripheral blood B cells of tamarin B77 as previously described (10). The following control cell lines were also included: BL2, BL30, and EB4 as EBV genome-negative controls, X50/7 as an EBV-positive nonproducer line, and B95.8 as an EBV-positive virus-producing line.

In situ hybridization. Plasmid pSL76 (1), containing a double EBV *Bam*HI-W insert, was biotinylated by nick translation with biotin-11-dUTP (Bethesda Research Laboratories, Inc., Gaithersburg, Md.), followed by purification on spun chromatography columns. Control biotinylated DNA probes (pBR322, bacteriophage λ) were prepared in the same manner. Snap-frozen biopsies of tamarin lymphoma were cryosectioned (5-μm section), mounted on acid-washed sterile glass slides, fixed at −20°C in 3:1 (vol/vol) methanol-acetic acid for 10 min, air dried, and stored at −20°C. Slides with control cell lines (B95.8, Raji, and HSB2) were prepared by cytocentrifugation, fixed, and stored as described above for tissue sections. In situ hybridization to tissue sections and cells with the biotinylated probes was performed as previously described (4). The hybridized biotinylated probe was detected indirectly by using a mouse antibiotin monoclonal antibody (MAb) (Dako Ltd.), followed by the streptavidin AB complex-horseradish peroxidase system (Dako Ltd.). After development with 3,3′-diaminobenzidine tetrahydrochloride and hydrogen peroxide, the signal was amplified by using a 3,3′-diaminobenzidine tetrahydrochloride enhancement kit (Amersham Ltd.). Finally, tissues and cells were counterstained with hematoxylin, washed, and mounted in DPX.

Antisera and MAbs. Expression of nuclear antigens EBNA 1, EBNA 2, EBNA 3, and EBNA-LP in the various tumor specimens was determined by immunoblotting with selected human sera (Mo, PG, RM, and RS22) having strong reactivity against one or more EBNAs, often with relatively weak reactivity against lytic-cycle antigens. Expression of these antigens was independently checked by immunoblotting with a human serum EE reactive predominantly against the early-antigen (EA) and virus capsid antigen complexes of the virus productive cycle. Each of these sera has already been well characterized (29, 30, 41).

MAbs were also used to probe for EBNA 2, for LMP, and for certain lytic-cycle antigens. Expression of EBNA 2 was detected by using MAb PE2 (41; L. S. Young, C. Alfieri, K. Hennessy, H. Evans, C. O'Hara, K. Anderson, A. Rickinson, E. Kieff, and J. I. Cohen, submitted for publication). Expression of LMP was detected by using a pool of four MAbs, CS1 to -4 (28). MAbs against EA-D (R3; 22), EA-R (R63; 22), and virus capsid antigen (V3; 34) were used to detect these lytic-cycle antigens. Expression of gp340 was detected by using MAb 72A1 (16) and a rabbit antiserum prepared against purified virus from the B95.8 cell line (21) that has a high antibody titer to gp340.

Affinity purification of human sera. The β-galactosidase fusion protein containing sequences of EBNA 3a (15) was partially purified from bacterial lysates by salt precipitation with 40% ammonium sulfate. The precipitate was washed once with 30% ammonium sulfate, reconstituted in a minimal volume of water, dialyzed extensively against isotonic phosphate-buffered saline, pH 7.2 (PBS), and clarified by centrifugation. An affinity column was prepared by coupling 10 mg of partially purified EBNA 3a fusion protein to a 1.0-ml prepacked activated column (HEMA-cart; Anachem) according to the instructions of the manufacturer. A 1-ml amount of RS22 human serum was diluted with 3 ml of 20 mM Tris-buffered saline, pH 7.5 (TBS), and recirculated through the column for 4 h. The column was washed extensively with TBS–0.3% Tween 20–0.1% bovine serum albumin before specifically bound antibodies were eluted with 50 mM diethylamine–150 mM NaCl, pH 11.5, which was recirculated through the column for 30 min. The eluted antibodies were neutralized with 0.2 M phosphate buffer, pH 2.0, and diluted 1:50 in TBS containing 5% dried milk (TBS-milk). The affinity-purified anti-EBNA 3a antibodies were then used to probe immunoblots.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. Protein extracts were prepared as previously described both from tumor tissue (41) and from established cell lines (26). Samples corresponding to 10^6 cells per track were separated by discontinuous gel electrophoresis (18) and blotted onto nitrocellulose filters essentially as described elsewhere (26). A 5.5% acrylamide gel was used for detection of gp340, and transfer onto nitrocellulose was carried out at 80 V for 4 h. Excess protein-binding sites on the blotted filters were blocked by incubation for 2 h in TBS-milk. The filters were then incubated overnight at 4°C with selected antibodies diluted appropriately in TBS-milk and subsequently washed in PBS–0.1% Tween 20 (PBS-Tween). In experiments where the first incubation used one of the MAbs, the filters were further incubated with rabbit anti-mouse immunoglobulin (Dakopatts) diluted 1:5,000 in TBS-milk for 1 h at room temperature and again washed in PBS-Tween. Specifically bound antibody was detected exactly as described previously (42), using ^{125}I-labeled protein A (Amersham) and autoradiography.

RESULTS

Detection of EBV DNA in lymphoma biopsy cells. Several of the lymphoma biopsies from EBV-infected tamarins were examined as frozen sections by in situ hybridization, using a biotinylated probe from the *Bam*HI-W (large internal repeat) region of the EBV genome. Preliminary studies had shown that this probe was capable of specifically detecting EBV DNA in the nonproductively infected cells of virus genome-positive BL cell lines and of EBV-transformed LCLs (data not shown). The tamarin lymphomas gave positive labeling with the EBV DNA probe in essentially every cell (Fig. 1B). Control plasmid probes used in parallel, including pBR322 (Fig. 1A) and phage λ, gave no significant labeling.

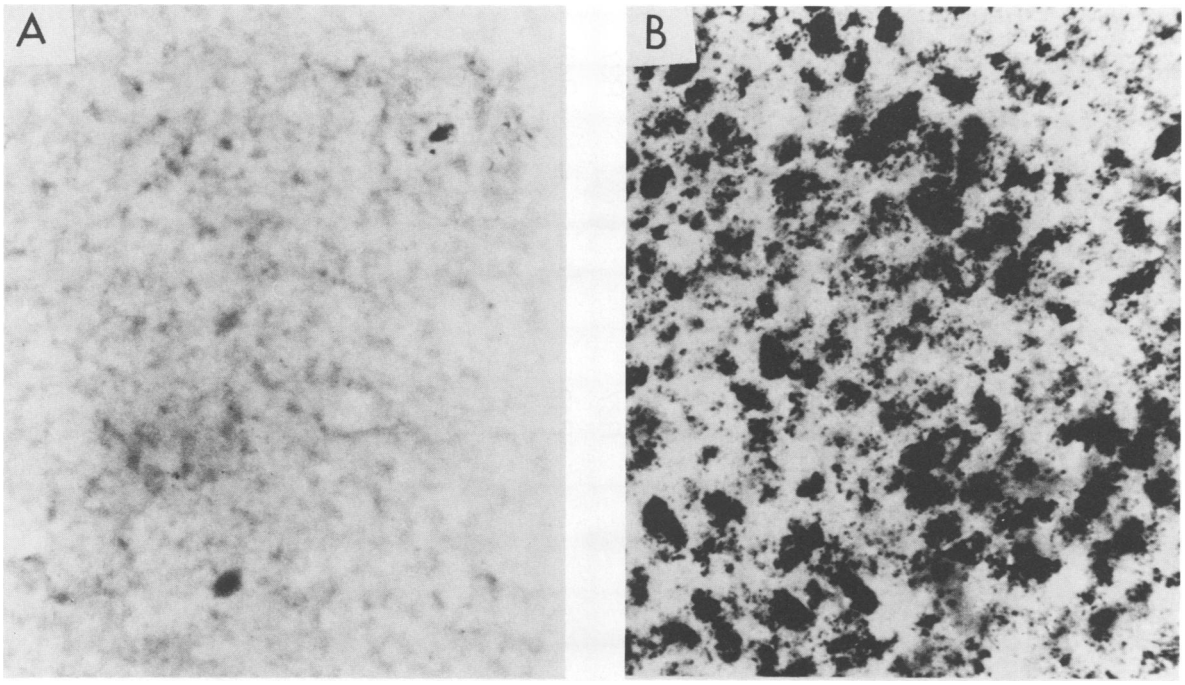

FIG. 1. In situ hybridization for EBV DNA on a section from a tamarin lymphoma. (A) Control hybridization with a biotinylated pBR322 probe; (B) positive hybridization with a biotinylated EBV *Bam*HI-W probe.

Expression of EBV latent proteins in lymphoma biopsy cells. Tumor biopsy samples from 10 tamarins were examined by immunoblotting for expression of EBV latent proteins (Fig. 2). With use of a selected human serum, Mo, whose reactivity in immunoblotting was almost entirely restricted to EBNA 1 (Fig. 2A), each tumor gave two bands, one at the expected 80-kilodalton (kDa) molecular size for the B95.8 virus-encoded EBNA 1 protein (compare with the major band in the positive control track, X50/7) and another, equally intense band at approximately 65 kDa. Several other human sera with selective EBNA 1 reactivity gave identical bands.

This finding was reminiscent of results obtained in our recent analysis of EBNA 1 expression in virus genome-positive nasopharyngeal carcinoma biopsies, in which multiple EBNA 1-related bands were observed and in which antibody elution experiments had confirmed their antigenic relatedness (41). Using exactly the same approach, we were able to confirm that antibodies eluted from either the 80-kDa or the 65-kDa band in Fig. 2A were capable of recognizing both species when reapplied to lymphoma biopsy protein extracts (data not shown). This finding strongly suggested that both proteins were EBNA 1 species. Note that the less intense band at 50 kDa in Fig. 2A was due to the heavy chains of immunoglobulin molecules which are regularly found within B-cell lymphoma biopsies; these have protein A-binding capacity and so were picked up by the particular detection system used here.

Recently developed MAbs specific for EBNA 2 (PE2; 41; Young et al., submitted) and LMP (CS1 to -4; 28) were used to examine expression of these proteins in the same 10 biopsies. EBNA 2 was detectable as a characteristic protein doublet in the 85-kDa region of the gel (Fig. 2B). Generally, the relative levels of EBNA 2 present in different tumors correlated with differences already noted in the intensity of EBNA 1 expression in the tumors; thus, the biopsy giving the weakest EBNA 1 signal (R49) was the only one in which detection of EBNA 2 required exposure of the immunoblot longer than that illustrated in Fig. 2b. Likewise, LMP was also clearly detectable in every biopsy as a characteristic 60-kDa band whose relative intensity between tumors again tended to mirror that already observed for the EBNA 1 and EBNA 2 proteins (Fig. 2C). We infer that these differences relate primarily to the relative proportions of tumor cells within the individual biopsy specimens. The EBNA 2-positive, LMP-positive status of the tamarin lymphomas was further confirmed by immunofluorescence staining (data not shown).

Immunoblotting studies were also carried out with an EBNA-LP-specific MAb (JF 186; 11) that was originally raised against a synthetic peptide epitope from the B95.8 EBNA-LP sequence. This antibody recognizes the characteristic ladder of EBNA-LP species in B95.8-transformed human B cells but gives, at best, only borderline reactivity with the B95.8 tamarin cell line itself (11). When protein extracts of five available tamarin lymphoma biopsies were probed with JF 186, specific labeling of the characteristic EBNA-LP ladder was observed, but only after a very long period of autoradiography (data not shown). The same very weak labeling of EBNA-LP was also observed when the lymphoma extracts were probed with selected human sera with proven EBNA-LP reactivity.

Several attempts using selected human sera were made to examine the lymphoma biopsies for expression of the EBNA 3a, 3b, and 3c family of proteins. It was never possible to detect these proteins unequivocally in the biopsies with this approach. This question was pursued further as part of the next series of experiments, in which EBV latent gene expression in selected biopsies was compared directly with that seen in the same cells immediately after their establishment as cell lines in vitro.

EBV latent proteins in biopsy cells versus derived cell lines.

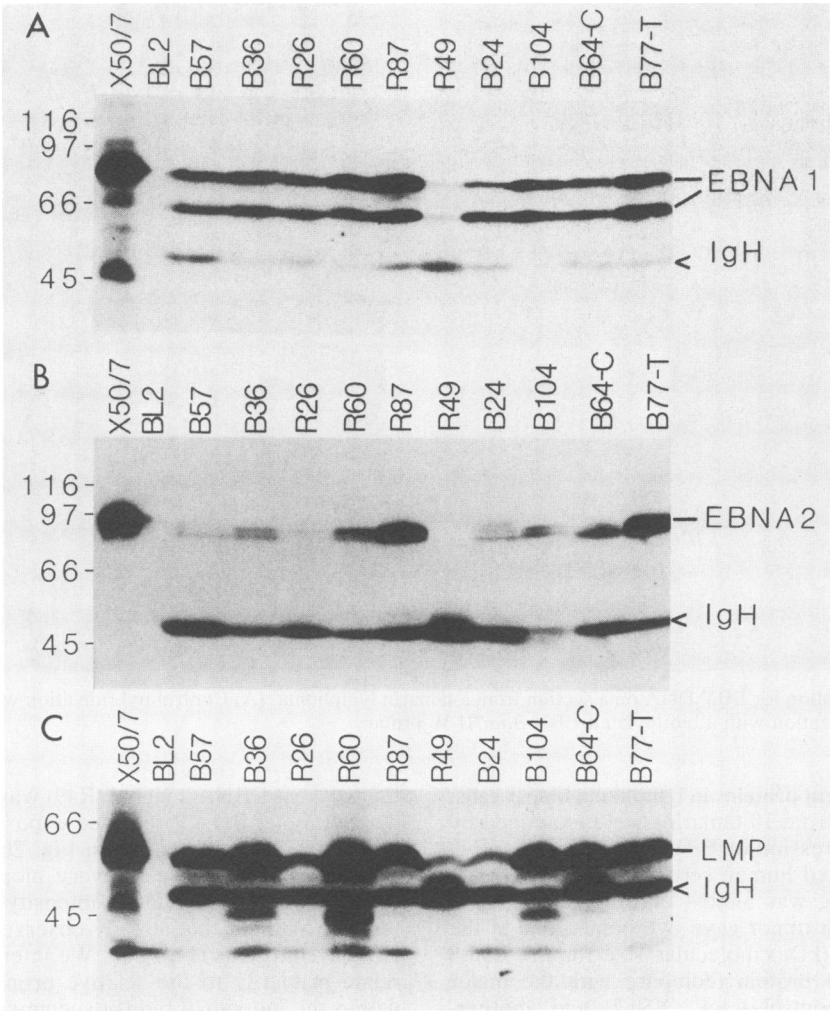

FIG. 2. Immunoblots showing levels of EBNA 1 (A), EBNA 2 (B), and LMP (C) expression in tamarin lymphomas. EBNA 1 expression was detected with a human serum (Mo) having high anti-EBNA 1 reactivity. EBNA 2 and LMP expression was detected with MAbs PE2 and CS1 to -4, respectively. The 50-kDa reactivity was nonspecific, being derived from the heavy chains of immunoglobulin molecules (IgH) that contaminate fresh biopsy material and cross-react in the protein A step of the immunoblotting procedure. The anti-LMP MAbs, CS1 to -4, also cross-react with a 41-kDa cellular protein, as previously described (28). X50/7 and BL2 are EBV-positive and -negative reference control lines, respectively.

Figure 3A illustrates the pattern of results obtained when protein extracts of tumor biopsies and of their derived cell lines were probed with human sera, in this case serum PG, which is reactive against EBNA 1, EBNA 2, and EBNA-LP. EBNA 1, detected as 80- and 65-kDa species in the biopsies, appeared as a single 80-kDa band in the derived cell lines, whereas EBNA 2, an 85-kDa doublet in the biopsies, appeared as a single band running slightly above the position of the original doublet. This latter finding was subsequently confirmed by using the EBNA 2-specific MAb PE2 (data not shown). Figure 3A also illustrates the relatively weak expression of the EBNA-LP ladder of lower-molecular-weight proteins which was noted both in the biopsies and in their derived cell lines.

Further immunoblotting studies with selected human sera on this same panel of cell extracts suggested that although the EBNA 3 family of proteins could not be detected in biopsies, they were present in the derived cell lines (data not shown). The point was pursued by using an affinity-purified anti-EBNA 3a antibody preparation obtained by absorbing the relevant reactivities in a human serum onto a β-galactosidase–EBNA 3a bacterial fusion protein (15). This procedure confirmed that EBNA 3a was detectable in the derived cell lines, even in very early passage, but not in the fresh biopsies (Fig. 3B).

EBV productive-cycle antigens in biopsy cells versus derived cell lines. In the final set of experiments, extracts of biopsies and of derived cell lines were probed for antigens of the virus productive cycle. Immunoblotting with the selected human serum EE preferentially detected proteins of the early-antigen (EA-D) complex in the 45- to 60-kDa region of the gel, plus a number of other productive-cycle antigens both above and below this size range. Figure 4 shows a deliberately overexposed autoradiograph of an immunoblot probed with serum EE; only very weak expression of EA-D was detectable in the biopsy tissue itself, but this was greatly enhanced upon in vitro passage such that the lymphoma cell lines soon became almost as productive as the high-producer B95.8 cell line itself. In confirmatory experiments, frozen sections of the lymphoma biopsies were stained by indirect

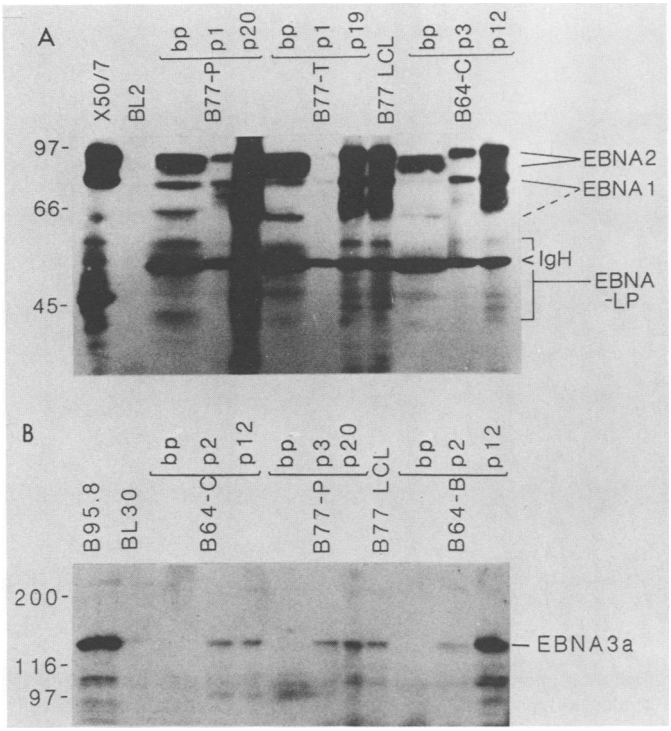

FIG. 3. Immunoblots showing levels of EBNA 1, EBNA 2, and EBNA-LP (A) and of EBNA 3a (B) expression in tamarin lymphoma biopsies and their derived cell lines. (A) Immunoblot probed with a human serum, PG, which recognizes EBNA 1, EBNA 2, and EBNA-LP. (B) Immunoblots probed with an affinity-purified human antibody preparation specific for EBNA 3a. bp, Lymphoma biopsy; p1, etc., passage numbers of lymphoma-derived cell lines; LCL, lymphoblastoid cell line obtained from EBV infection of normal tamarin peripheral blood B cells in vitro.

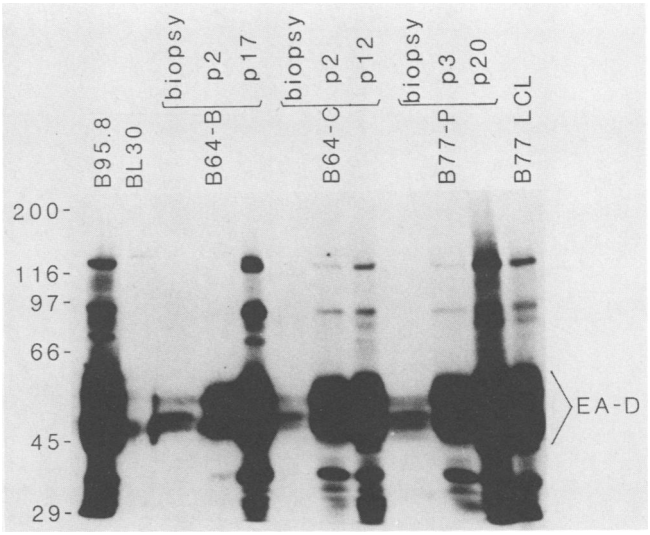

FIG. 4. Immunoblot showing levels of EBV lytic-cycle antigen expression in tamarin lymphoma biopsies and their derived cell lines. The immunoblot was probed with human serum EE, which reacts predominantly with lytic-cycle-associated antigens such as the EA-D complex. The B95.8-producing line and EBV-negative BL30 line are included as positive and negative controls, respectively. Designations are as for Fig. 3.

immunofluorescence with MAbs to EA-D (R3; 22), EA-R (R63; 23), and VCA (V3; 34). Specific labeling was detectable with each of these reagents but at very low levels, usually less than 0.1% of the biopsy cells being scored as positive; in contrast, 5 to 10% of cells in the derived cell lines were positive for the productive-cycle antigens (data not shown).

An essentially similar pattern of results was obtained when these same biopsy and cultured cell populations were analyzed for expression of the virus envelope glycoprotein gp340. Immunoblotting either with a rabbit antiserum prepared against purified B95.8 virus which has a high titer of antibodies to gp340 (Fig. 5) or with the specific MAb 72A1 (16; data not shown) indicated that biopsies contained little if any detectable gp340 but that expression soon rose to high levels with cell line outgrowth in culture.

DISCUSSION

The constitutive expression of EBV latent gene products that is found in all in vitro-transformed LCLs strongly implies that one or more of these proteins are the effector molecules of the transformation process. Particular attention has been focused recently on EBNA 2 and LMP, which on transfection and expression in human B cells can induce transformation-related phenotypic changes through specific activation of cellular genes (36, 37). Moreover, LMP also has growth-transforming ability in rodent fibroblast culture systems (2, 35). In contrast, EBNA 1 appears to be primarily involved in episomal maintenance of the virus genome in EBV-transformed cells (39, 40) and has not as yet been shown to have direct effects on cellular phenotype. The functions of the other latent proteins, EBNA 3a, 3b, and 3c and EBNA-LP, with respect to cell transformation are not known.

The clearest evidence that EBV has direct oncogenic capacity in vivo, rather than just cell-transforming ability in vitro, comes from experimental infections in subhuman primates, leading to monoclonal or oligoclonal B-cell lymphomas (6). However, such experimentally induced tumors have never been analyzed for expression of the full range of EBV latent proteins, most particularly for the expression of the known effector molecules EBNA 2 and LMP. The importance of this point has recently been highlighted by the finding that in endemic BL, in which EBV infection is just one event in a complex multistep pathogenesis, the malignant clone that finally emerges appears to have become independent of both EBNA 2 and LMP for its continued growth (29). If EBV is the major proliferative influence driving malignant B-cell outgrowth in experimentally infected tamarins, then one would expect the pattern of virus latent gene expression in those lymphomas to resemble that seen in in vitro-transformed LCLs more closely than that seen in BL cell lines.

The results presented here show that this is indeed the case. First, in situ hybridization confirmed that essentially every lymphoma cell carried the EBV genome (Fig. 1). Second, every biopsy analyzed by immunoblotting was positive for EBNA 1, EBNA 2, EBNA-LP, and LMP (Fig. 3A and 5). Third, immunofluorescence staining of cytological preparations showed patterns of EBNA 2 and LMP expression in tumor populations which mimicked those seen in in vitro-transformed LCLs (data not shown).

There were, nevertheless, some unusual aspects of the results with lymphoma biopsies. We routinely detected two distinct EBNA 1 bands of equal intensity, one at the ex-

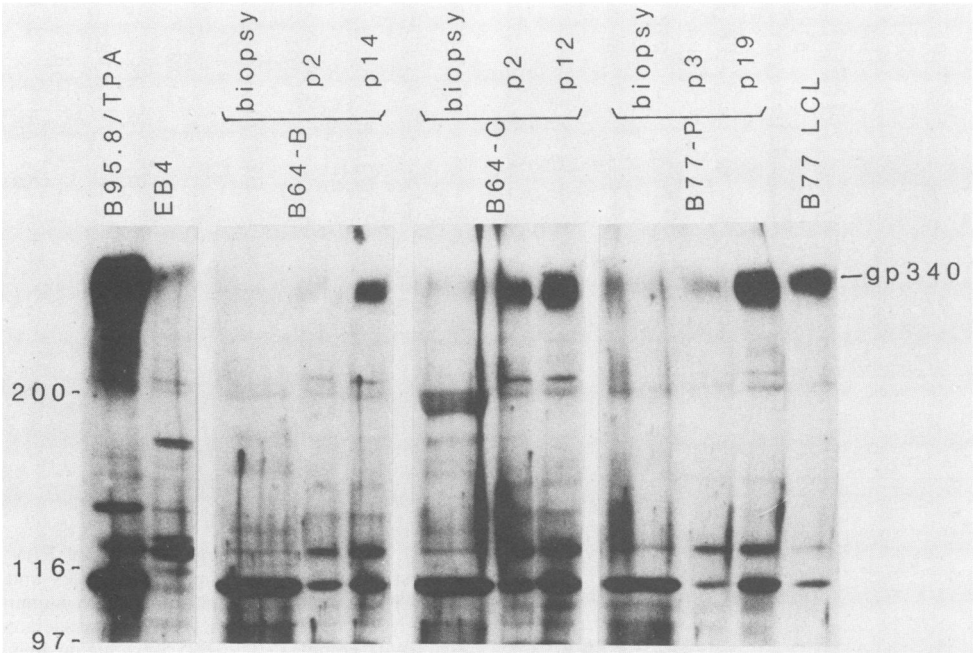

FIG. 5. Immunoblot showing levels of gp340 expression in tamarin lymphomas and their derived cell lines. The immunoblot was probed with a polyclonal rabbit antiserum containing antibodies to gp340. 12-*O*-Tetradecanoylphorbol 13-acetate-induced B95.8 cells and the EB4 cell line were used as positive and negative controls, respectively. Designations are as for Fig. 3.

pected 80-kDa position in the gel and another at 65 kDa; dual bands were not an artifact of snap freezing per se since they were also observed in lymphoma cells that had been liberated from the biopsy by gentle teasing and then immediately cryopreserved in the presence of 10% dimethyl sulfoxide (S. Finerty, unpublished results). Although some form of breakdown of the EBNA 1 protein remains a feasible explanation, the possibility that both EBNA 1 species are genuine products of the EBV genome in the tumor cells in vivo cannot yet be discounted. Only the 80-kDa EBNA 1 protein was detectable, however, in derived lymphoma cell lines (Fig. 3A). It was also interesting to note that the EBNA 2 protein expressed in cultured cells (itself often resolved into a doublet) migrated slightly more slowly in sodium dodecyl sulfate-polyacrylamide gel electrophoresis than did the more obvious doublet detectable in the biopsy cells (Fig. 3); the precise reason for this result is not known. There was no difference between biopsies and cell lines in expression of either LMP or EBNA-LP, and we believe that the very low levels of EBNA-LP seen here in the lymphoma cells simply reflect what is seen in all EBV-infected B cells of tamarin rather than human origin. Thus, even in vitro-transformed tamarin LCLs, including the B95.8 cell line itself, express very little detectable EBNA-LP (e.g., Fig. 3A, B77 LCL).

The most obvious distinguishing feature of the lymphoma biopsies was the apparent absence of the EBNA 3 protein family. It must be stressed, however, that these proteins are not as efficiently detected by conventional immunoblotting as are other latent gene products such as EBNA 1, EBNA 2, or LMP. From the work with an affinity-purified antibody preparation, it did seem that EBNA 3a was down regulated in biopsy cells in comparison with the corresponding derived cell lines (Fig. 3B). This potentially interesting observation requires further study. However, without access to better serological reagents and in particular to specific MAbs, it would be premature to conclude that the EBNA 3 proteins are not expressed at all in the biopsy cells.

The overall results strongly suggest that cell proliferation in these malignant B-cell lymphomas of tamarins is being driven directly and solely by EBV. This is significant both because it establishes the direct oncogenic potential of this virus and because the animal model under investigation closely resembles the human B-cell lymphoma to which immunosuppressed patients are particularly prone (5, 6). The inference is that these human B-cell lymphomas, already known to be EBV genome positive (5), will show a similar pattern of virus latent gene expression which includes the important effector molecules EBNA 2 and LMP. Since completing this study, we have had access to several such tumors, arising in patients undergoing bone marrow transplants, and have shown by immunofluorescent staining that the malignant cells are indeed EBNA 2 and LMP positive (Young et al., submitted).

This most recent finding strengthens the relationship between the tamarin and human lymphomas and furthermore suggests why both types of tumors sometimes regress (5, 6). There is strong circumstantial evidence that this regression is mediated by immune T cells, and we now know that LMP, and perhaps also EBNA 2, can provide the target epitopes against which EBV-specific cytotoxic T-cell responses are directed (20). Lymphomas whose proliferation depends on the continued expression of these effector molecules will, of necessity, remain subject to immune T-cell control. Their in vivo outgrowth will therefore be possible only if the number of B cells infected initially is so large as to constitute an irresistible challenge to the immune response, as may be the case in the tamarin model, or if the T-cell response itself is impaired, as happens in allograft recipients (5).

Finally, it is important to note that the EBV-induced lymphomas of tamarins are almost entirely nonproductive in vivo (Fig. 4 and 5). This is particularly interesting since tamarin B cells are known to be generally more permissive of EBV replication than are human B cells (7), as evidenced by the fact that the lymphoma-derived cell lines soon became

very productive with in vitro passage. There appears, therefore, to be some in vivo control over the switch from latency to a productive cycle; the mechanism of control is not understood. Although the infected tamarins had developed detectable serum antibodies to virus structural antigens by the time lymphoma biopsies were taken (A. J. Morgan and S. Finerty, unpublished results), in vivo control over entry into the virus productive cycle is not necessarily antibody mediated or even immunologically mediated.

The findings discussed above, in particular the virtual absence of gp340 expression in the tumor biopsy cells (Fig. 5), are relevant to the question of how a gp340-based subunit vaccine is able to protect tamarins from subsequent challenge with a lymphomagenic dose of EBV (9, 19). The results of vaccine work to date in this animal model suggest that gp340-induced protection is not necessarily achieved via anti-gp340 neutralizing antibodies and that it may involve a cell-mediated mechanism (10). If this is the case, and we have recently shown in humans that gp340-specific immune T cells do exist (33a), then the target cell population for such T-cell responses in the tamarin model is very unlikely to be the lymphoma itself, since this is gp340 negative. Any such cell-mediated control of lymphomagenesis in vaccinated animals must therefore be exercised early after virus challenge, perhaps on target B cells soon after their infection with the virus when gp340, along with other virion components, can be re-presented on the B-cell surface.

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