EPSTEIN–BARR VIRUS AND A CELLULAR SIGNALING PATHWAY IN LYMPHOMAS FROM IMMUNOSUPPRESSED PATIENTS

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ABSTRACT

Background Epstein–Barr virus (EBV) is associated with various malignant and benign lymphoproliferative disorders. It also efficiently transforms human B lymphocytes in vitro. The latent membrane protein 1 (LMP1) of EBV-infected cells plays a central part in this process by mimicking members of the family of tumor necrosis factor (TNF) receptors, thereby transmitting growth signals from the cell membrane to the nucleus through cytoplasmic TNF-receptor–associated factors (TRAFs). I sought evidence of LMP1-mediated signal transduction through TRAFs in tumor tissue from patients with post-transplantation lymphoproliferative disease and non-Hodgkin's lymphomas related to the acquired immunodeficiency syndrome (AIDS).

Methods The association of LMP1 with TRAF-1 or TRAF-3 in tumor tissue was studied with double-immunofluorescence microscopy and immunoprecipitation assays. Evidence of LMP1–TRAF signaling was sought with an electrophoretic mobility shift assay for the nuclear factor-κB (NF-κB) transcription factor.

Results Tumors from eight patients with post-transplantation lymphoproliferative disease, two patients with AIDS-associated non-Hodgkin's lymphoma, and three patients with endemic Burkitt's lymphoma were analyzed. Tumors from six of the patients with post-transplantation lymphoproliferative disease were positive for EBV and expressed LMP1; two samples were EBV-negative. Tumors from both patients with AIDS-associated non-Hodgkin's lymphoma were EBV-positive and expressed LMP1, whereas tumors from all three patients with Burkitt's tumors were positive for EBV but negative for LMP1. Double-immunofluorescence microscopy showed that LMP1 localized with and immunoprecipitated with TRAF-1 and TRAF-3 in all eight of the EBV-positive, LMP1-positive samples. An electrophoretic mobility shift assay revealed activated NF-κB in all eight EBV-positive, LMP1-positive samples as well, but not in either of the EBV-negative, LMP1-negative samples or in the three EBV-positive, LMP1-negative samples.

Conclusions LMP1-mediated signaling through the TRAF system has a role in the pathogenesis of the EBV-positive lymphomas that arise in immunosuppressed patients. (N Engl J Med 1998;338:1413-21.)

EPSTEIN–BARR virus (EBV) is a human herpesvirus linked to endemic (African) Burkitt's lymphoma, post-transplantation lymphoproliferative disease, and non-Hodgkin's lymphoma associated with the acquired immunodeficiency syndrome (AIDS). In vitro, EBV efficiently transforms B lymphocytes, causing them to proliferate continually.[1] EBV-infected cells express only nine viral proteins, and they mediate the transforming role of EBV in B lymphocytes. One of these proteins, latent membrane protein 1 (LMP1), has potent transforming effects in cell culture and animal models[2-12] and is essential for the in vitro transformation of B cells by EBV.[13]

LMP1 is a viral analogue of the family of tumor necrosis factor (TNF) receptors in human cells. These receptors are embedded in the cell membrane, with one end facing the external milieu and the other the interior of the cell. Like the cellular TNF receptors, LMP1 has a cytoplasmic tail that binds to intracellular proteins called TNF-receptor–associated factors (TRAFs). These LMP1-bound proteins activate the nuclear factor-κB (NF-κB) transcription factor, thereby causing the cell to proliferate (Fig. 1).[14-17] Cellular members of the TNF-receptor family are important mediators of lymphocyte growth and activation. Two representatives of this family, CD30 and CD40, are expressed by Hodgkin's and non-Hodgkin's lymphomas and may be important in the pathogenesis of these diseases. It is plausible that LMP1 exerts its growth-promoting effects on human B lymphocytes by aggregating in the plasma membrane in association with members of the TRAF family. In this way, it would mimic an activated TNF-receptor–TRAF complex.

Despite knowledge of the in vitro transforming and biochemical properties of LMP1, there is no direct evidence that LMP1 contributes to the malignant phenotype of lymphoproliferative diseases. In fact, the role of EBV in the development and maintenance of these diseases is controversial, partly because LMP1 is not expressed in all types of EBV-

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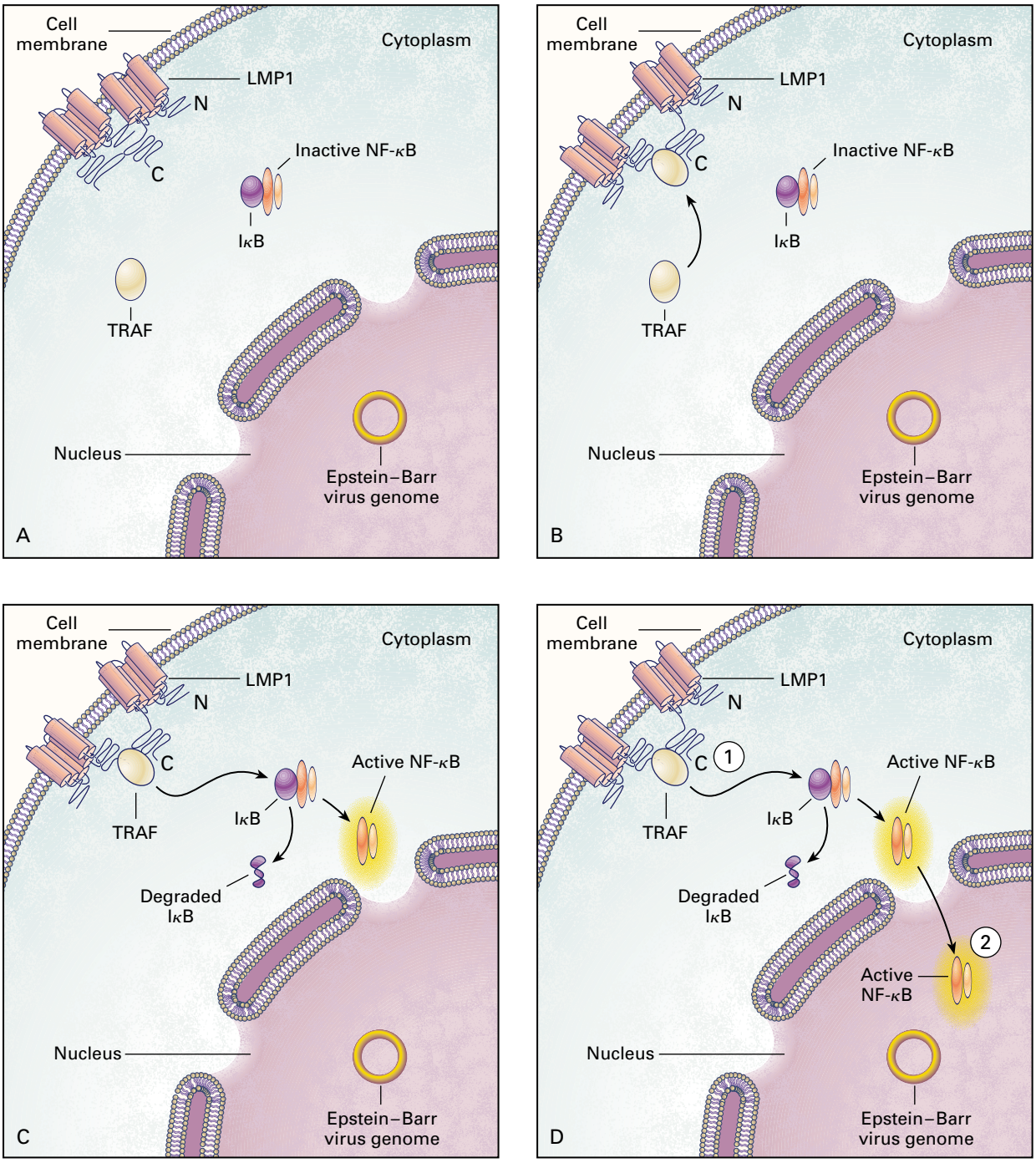

Figure 1. The Role of Epstein–Barr Virus Latent Membrane Protein 1 in Activating Cell Growth in Post-Transplantation Lymphoproliferative Disease and AIDS-Related Non-Hodgkin's Lymphoma.

The Epstein–Barr virus latent membrane protein 1 (LMP1), which is expressed in post-transplantation lymphoproliferative disease and AIDS-associated non-Hodgkin's lymphoma, aggregates in the plasma membrane of the cell (Panel A) and is a viral homologue of the family of cellular tumor necrosis factor (TNF) receptors. LMP1 binds members of the TNF-receptor–associated factor (TRAF) family (Panel B), which then activate the NF-κB transcription factor (Panel C). IκB is an inhibitory protein, which, when degraded, allows NF-κB nuclear translocation and activation. In vitro experimental evidence indicates that LMP1 probably stimulates cell growth through its association with TRAF molecules by mimicking an activated TNF-receptor complex in the plasma membrane of the cell. Double-immunofluorescence microscopy and immunoprecipitation were used in studies of post-transplantation lymphoproliferative disease and AIDS-associated non-Hodgkin's lymphoma to determine whether LMP1 forms aggregates in association with TRAF molecules (1 in Panel D). In addition, an electrophoretic mobility shift assay was used to determine whether the NF-κB transcription factor was activated in the tumors (2 in Panel D).

associated neoplasms.[1] LMP1 is typically found in post-transplantation lymphoproliferative disease, AIDS-related non-Hodgkin's lymphoma, and Hodgkin's disease, but not in endemic Burkitt's lymphoma, even though Burkitt's tumors nearly always carry the EBV genome.[18-21] Burkitt's tumors also contain chromosomal translocations that constitutively activate the *MYC* gene on chromosome 8. The consistent association of EBV with Burkitt's lymphoma and the lack of expression of LMP1 suggest that EBV stimulates B lymphocytes early in the disease, whereas continued effects of EBV are not necessary to maintain the malignant phenotype. Activation of *MYC* may obviate the need for EBV proteins to sustain the transformed state.

Post-transplantation lymphoproliferative disease comprises a heterogeneous group of EBV-associated lymphoproliferative disorders that occur in patients who receive immunosuppressive therapy after solid-organ or bone marrow transplantation. Pathologically, the tumors range from a polymorphic proliferation of B lymphocytes to monoclonal high-grade malignant lymphoma. From studies of Burkitt's lymphoma, one might expect that LMP1 may no longer be expressed in monoclonal cases of post-transplantation lymphoproliferative disease. Actually, these monoclonal cases can have Burkitt-like *MYC* translocations and also abundantly express LMP1,[22] suggesting that LMP1 is necessary for the malignant phenotype of post-transplantation lymphoproliferative disease.

In this study, I investigated the physical association of LMP1 with TRAF molecules in tumor specimens from representative cases of post-transplantation lymphoproliferative disease and EBV-positive AIDS-associated non-Hodgkin's lymphoma (Fig. 1). In each case, I also determined whether the NF-κB transcription factor was activated in the tumors. The finding of LMP1 in association with TRAF mole-

TABLE 1. CHARACTERISTICS OF PATIENTS WITH POST-TRANSPLANTATION LYMPHOPROLIFERATIVE DISEASE AND PATIENTS WITH AIDS-ASSOCIATED NON-HODGKIN'S LYMPHOMA.*

PATIENT NO.	MORPHOLOGIC FINDINGS	EBV STATUS	SIMPLIFIED CYTOGENETIC ANALYSIS	CELLULAR ONCOGENE INVOLVED
Post-transplantation lymphoproliferative disease				
1	Polymorphic	+	Normal findings	NA
2	Polymorphic	+	ND	NA
3	Monomorphic, diffuse large cell, centroblastic	+	ND	NA
4	Monomorphic, diffuse large cell, immunoblastic	+	Trisomy 9	NA
5	Monomorphic, diffuse large cell, immunoblastic	−	t(3;22)(q27q11)	*BCL6*
6	Monomorphic, diffuse large cell, immunoblastic	−	ND	NA
7	Monomorphic, Burkitt-like	+	t(8;22)(q24;q11)	*MYC*
8	Monomorphic, Burkitt-like	+	t(8;14)(q24;q32)	*MYC*
AIDS-associated non-Hodgkin's lymphoma				
9	Diffuse large-cell NHL, immunoblastic	+	t(8;14)	*MYC*
10	Diffuse large-cell NHL, immunoblastic	+	ND	ND

*EBV denotes Epstein–Barr virus, NA not applicable, ND not done, and NHL non-Hodgkin's lymphoma.

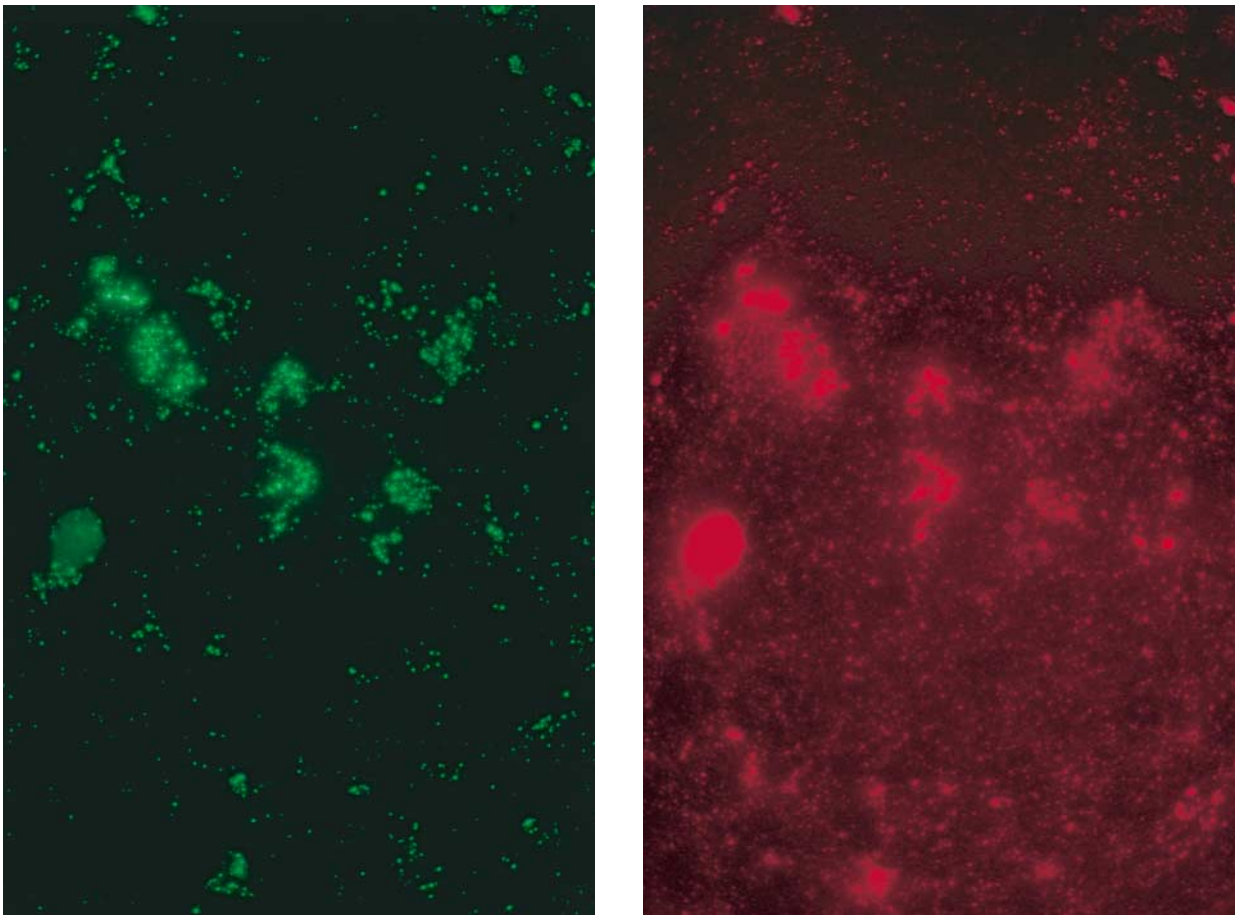

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Figure 2. Localization of Latent Membrane Protein 1 with TNF-Receptor–Associated Factor 1 in Tumor Samples from a Patient with Post-Transplantation Lymphoproliferative Disease (×1000).

Briefly, frozen tumor specimens were sectioned (thickness, 1 to 2 μm), fixed in methanol–acetone (3:1), and stained with a monoclonal antibody specific for latent membrane protein 1 (LMP1) or polyclonal rabbit antiserum to TNF-receptor–associated factor 1 (TRAF-1). Panel A shows the pattern of LMP1 staining (green fluorescence), Panel B the pattern of TRAF-1 staining (red fluorescence), and Panel C the dual staining of LMP1 and TRAF-1 (yellow fluorescence). LMP1 typically forms patches that aggregate into a large cap in the membrane; TRAF-1 has adopted the same distribution pattern as LMP1.

cules and activated NF-κB in post-transplantation lymphoproliferative disease and AIDS-associated non-Hodgkin's lymphoma tumors is evidence that LMP1 signaling contributes to the transformed phenotype of EBV-associated tumors in vivo.

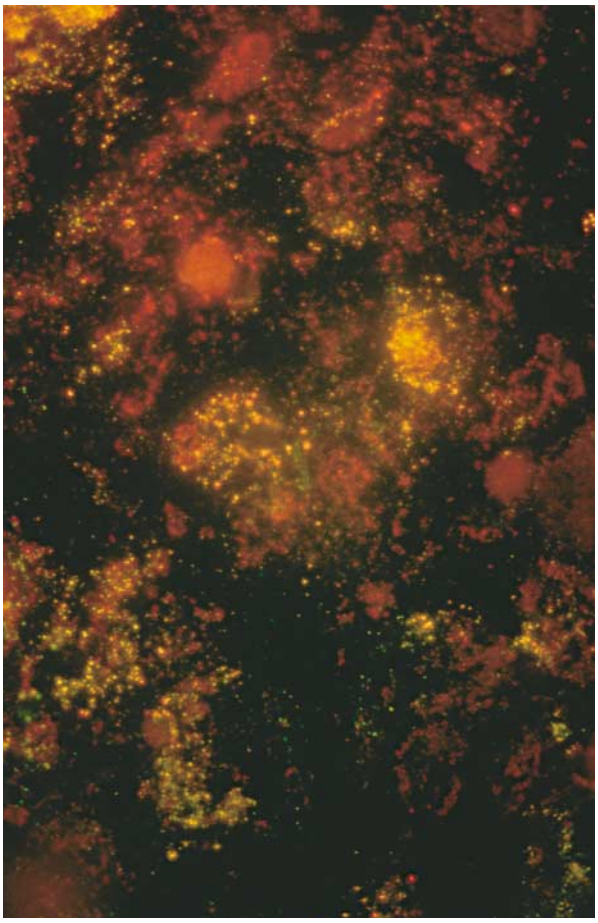
METHODS

Tumor Tissue, Cells, and Cell Culture

Specimens from eight patients with post-transplantation lymphoproliferative disease, which had been analyzed previously,[22] and from two patients with AIDS-associated non-Hodgkin's lymphoma were studied. Tumor tissue was obtained from fresh biopsy specimens that were flash-frozen in liquid nitrogen at the time of collection. Three frozen biopsy specimens of Burkitt's tumor were generously provided by African physicians over a span of several years. Control cell lines were grown in continuous cultures at 37°C in 5 percent carbon dioxide in RPMI-1640 medium supplemented with 10 percent fetal-calf serum (BioWhittaker, Walkersville, Md.). IB4 is an EBV-infected cord-blood B lymphoblastoid cell line that does not allow viral replication. BJAB is an EBV-negative human B lymphoma cell line.

Antibodies and Antiserum

The S12 monoclonal antibody recognizes the C-terminal portion of LMP1 and was kindly provided by Elliott Kieff (Harvard Medical School, Boston). The CS1, CS2, CS3, and CS4 anti-LMP1 monoclonal antibodies were from Dako (Carpinteria, Calif.). Antiserum to the TRAF molecules was obtained from Santa Cruz Biotech (Santa Cruz, Calif.). Rabbit anti-TRAF1 antiserum (N19) was used for immunofluorescence staining and immunoprecipitation, and rabbit anti-TRAF1 antiserum (S19) was used for Western immunoblotting. Rabbit anti-TRAF2 antiserum (C20 and N19) was used for immunofluorescence staining and Western immunoblotting. Antiserum N19 anti-TRAF1 and N19 anti-TRAF2 have distinct specificities and do not cross-react. The rabbit anti-TRAF3 antibody H20 was used for immunofluorescence staining, immunoprecipitation, and Western immunoblotting. Normal goat and rabbit serum was purchased from Sigma



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Chemical (St. Louis). Goat antimouse antibodies (H and L) or goat antirabbit secondary antibodies conjugated with Oregon green 488 and Texas red X were obtained from Molecular Probes (Eugene, Oreg.).

Western Immunoblotting

Proteins were solubilized from fresh or frozen tissue in sample buffer (62.5 mM TRIS–hydrochloride, pH 6.8; 2 percent sodium dodecyl sulfate, 10 percent glycerol, and 5 percent 2-mercaptoethanol) at 95°C for 10 minutes. The lysates were cleared by microcentrifugation for five minutes, and the supernatants were collected for separation by discontinuous sodium dodecyl sulfate–polyacrylamide-gel electrophoresis according to standard procedures.[8] After separation, proteins were transferred to nitrocellulose and the membranes were probed with a biotin-conjugated anti-LMP1 (S12) monoclonal antibody, washed, and then probed with ^{125}I-labeled streptavidin (Amersham, Arlington Heights, Ill.). After a final washing, the reactive protein bands were detected by autoradiography with Kodak XAR-5 film (Eastman Kodak, Rochester, N.Y.).

Immunofluorescence Microscopy

The sections were fixed in methanol–acetone (3:1 vol/vol) at −20°C for 5 to 10 minutes. After fixation, the slides were briefly washed in phosphate-buffered saline and then blocked with 10 percent (vol/vol) normal goat serum for 20 minutes and stained with monoclonal antibodies for LMP1 and rabbit antiserum for TRAF1, TRAF2, or TRAF3 as follows. All antibody dilutions were in 10 percent normal goat serum in phosphate-buffered saline. The tissue sections were incubated for one hour with S12 (anti-LMP) hybridoma supernatant (dilution, 1:100) and one of the following: 400 ng of N19 (anti-TRAF1) per milliliter, 400 ng of H20 (anti-TRAF3) per milliliter, 400 ng of C20 (anti-TRAF2) per milliliter, or 400 ng of N19 (anti-TRAF2) per milliliter. The samples were then washed three times in phosphate-buffered saline for 10 minutes. The secondary antibodies Oregon green 488 goat antimouse IgG (H and L) and Texas red X goat antirabbit IgG (H and L) were incubated at a concentration of 2 μg per milliliter with the sections for 30 minutes followed by three 10-minute washes in phosphate-buffered saline. The stained sections were air-dried, mounted in ProLong Antifade (Molecular Probes) according to the manufacturer's directions, and photographed on a microscope (model ES800, Nikon, New York) equipped with an epifluorescent system for detecting fluorescein isothiocyanate, Texas red, or a combination of the two.

Electrophoretic Mobility Shift Assay

Nuclear extracts were prepared from cultured cells or tissues as described previously.[16] The protein content was determined by the Bradford assay (Bio-Rad Laboratories, Hercules, Calif.). A probe was generated from a pair of complementary oligonucleotides containing a specific binding site for the NF-κB transcription factor: 5′AGCTTGGGGACTTTCCACTAGTACG3′ and 5′AATTCGTACTAGTGGAAAGTCCCCA3′. The oligonucleotides were designed so that, when annealed, the double-stranded probe DNA contained single-stranded 5′ ends. The probe was labeled by filling in the single-stranded 5′ ends with the Klenow fragment in the presence of [^{32}P]deoxy-ATP. Complementary oligonucleotides were annealed by heating to 95°C for five minutes, then allowed to cool on the benchtop. The labeled probe was isolated, and binding reactions and electrophoresis were performed as described previously.[16] Autoradiography was performed on the dried gel. The specificity of the shifted band was determined by comparison with appropriate positive and negative controls and by using excess cold oligonucleotide to compete with the radiolabeled probe, thereby leaving no visible shifted band on the autoradiograph.

DNA Amplification

A pair of oligonucleotide primers 5′GTTCGCGTTGCTAGGCCACC3′ and 5′AGGACCACTTTATACCAGGG3′ were used for DNA amplification that flank part of the *Bam*W repeat sequence of the EBV nuclear antigen 2 gene, resulting in a product of 100 bp. DNA was amplified for 30 to 40 rounds in a DNA thermal cycler (model 2400, Perkin–Elmer Cetus, Norwalk, Conn.; or Rapidcycler, Idaho Technology, Boise, Idaho) according to the manufacturer's recommendations. For the Perkin–Elmer thermal cycler, the amplification consisted of denaturation for 60 seconds at 94°C, primer annealing for 90 seconds at 56°C, and extension for 90 seconds at 72°C; for the Rapidcycler, amplification consisted of denaturation for 5 seconds at 94°C, annealing for 10 seconds at 56°C, and extension for 15 seconds at 72°C. The amplification products were separated by agarose-gel electrophoresis (NuSieve, FMC Bioproducts, Rockland, Me.) and analyzed by ethidium bromide staining for the appropriate size DNA band. EBV-positive and EBV-negative control cell lines were also amplified.

RESULTS

The morphologic findings, clonality, cytogenetic characteristics, and pattern of *EBV* gene expression in samples of tumor from eight patients with post-transplantation lymphoproliferative disease[22] and two patients with AIDS-associated non-Hodgkin's lym-

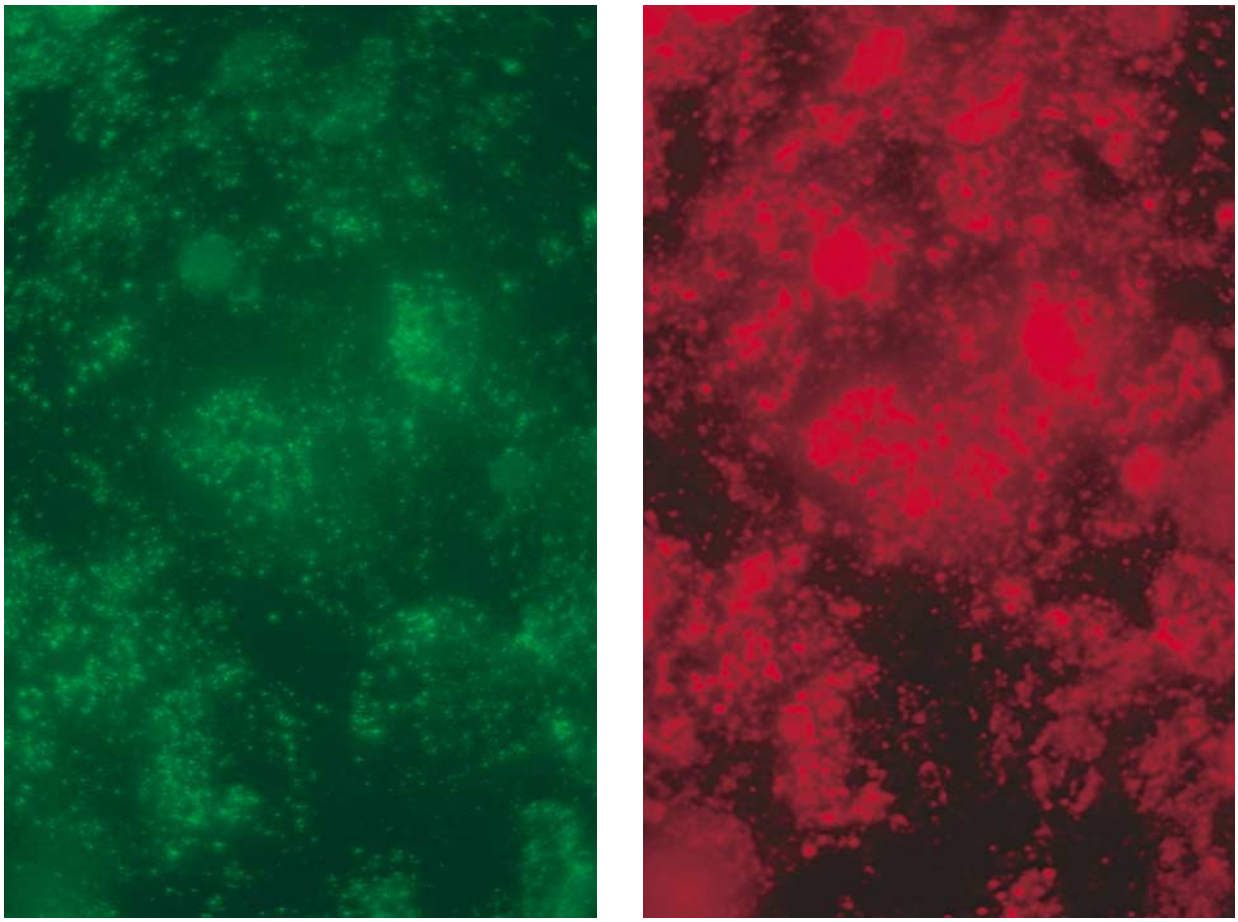

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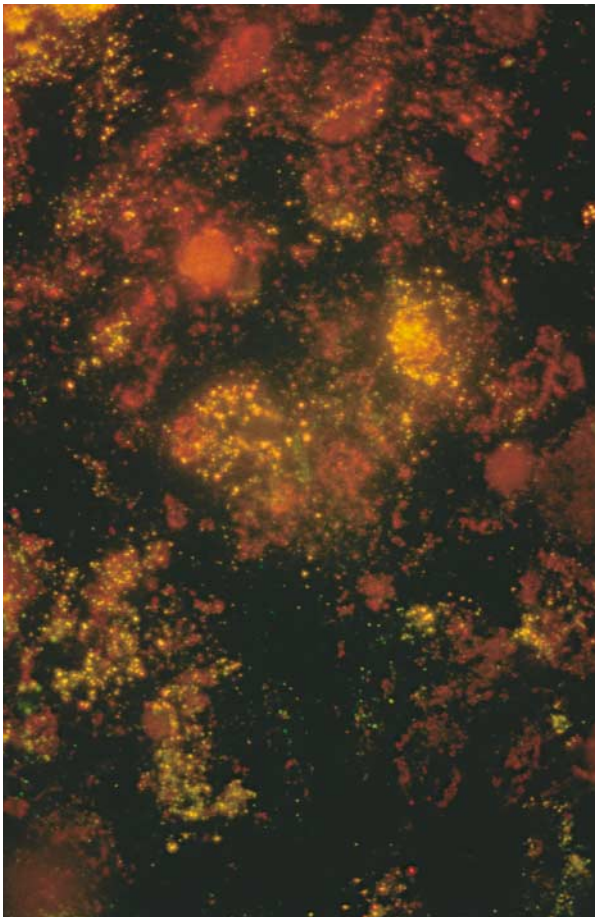
Figure 3. Localization of Latent Membrane Protein 1 with TNF-Receptor–Associated Factor 3 in Tumor Samples from a Patient with Post-Transplantation Lymphoproliferative Disease (×1000).

Briefly, frozen tumor specimens were sectioned (thickness, 1 to 2 μm), fixed in methanol–acetone (3:1), and stained with a monoclonal antibody specific for latent membrane protein 1 (LMP1) or polyclonal rabbit antiserum to TNF-receptor–associated factor 3 (TRAF-3). Panel A shows the pattern of LMP1 staining (green fluorescence), Panel B the pattern of TRAF-3 staining (red fluorescence), and Panel C the dual staining of LMP1 and TRAF-3 (yellow fluorescence). LMP1 typically forms patches that aggregate into a large cap in the membrane; TRAF-3, like TRAF-1, has adopted the same distribution pattern as LMP1.

phoma are shown in Table 1. Two of the 10 specimens — both from patients with post-transplantation lymphoproliferative diseases — were EBV-negative.[22] The other specimens were EBV-positive and expressed the LMP1 protein on immunofluorescence microscopy and Western immunoblotting (data not shown). All three specimens of endemic Burkitt's lymphoma were EBV-positive by the polymerase chain reaction, but in none was LMP1 protein detectable by immunofluorescence microscopy or Western immunoblotting. In these three lymphomas, *MYC* gene rearrangements were found by Southern blotting (unpublished observations).

Two methods were used to examine tumor specimens for interactions between LMP1 and TRAF molecules: double-immunofluorescence microscopy and immunoprecipitation. Tissue from all eight of the LMP1-positive specimens was examined to establish whether LMP1 was found with antibodies against either TRAF-1 or TRAF-3 on double-immunofluorescence microscopy (LMP1 associates with both TRAF-1 and TRAF-3 in vitro[16]). In every case, LMP1 was found with TRAF-1 (Fig. 2) and TRAF-3 (Fig. 3) in plasma-membrane aggregates. This result indicates that the two TRAF signaling molecules associate with membrane aggregates of LMP1 in post-transplantation lymphoproliferative disease and in AIDS-associated non-Hodgkin's lymphoma.

To confirm these findings, an immunoprecipitation assay was performed. If LMP1 is physically associated with TRAF-1 and TRAF-3 molecules in these tumors, then immunoprecipitation with specific



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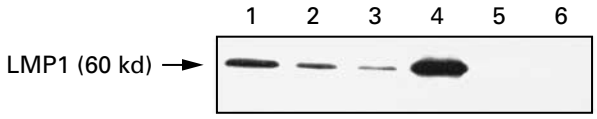

Figure 4. Immunoprecipitation of Latent Membrane Protein 1 with TNF-Receptor–Associated Factors 1 and 3 from Tumor Tissue from a Patient with Post-Transplantation Lymphoproliferative Disease.

Protein lysates from tumor tissue were prepared and immunoprecipitated with antibodies against TNF-receptor–associated factor (TRAF) or latent membrane protein 1 (LMP1) and analyzed for LMP1 by Western immunoblotting. Lane 1 shows an LMP1-positive control containing 20 percent of the amount of lysate used for the immunoprecipitation assays shown in the other lanes. Lane 2 shows the LMP1 that was immunoprecipitated with the anti–TRAF-1 antibody. Lane 3 shows the LMP1 that was immunoprecipitated with the anti–TRAF-3 antibody. Lane 4 shows the LMP1 that was immunoprecipitated with the anti-LMP1 antibody. No LMP1 was detected when nonimmune normal mouse serum (lane 5) or rabbit serum (lane 6) was used in the immunoprecipitation assay.

antiserum to TRAF-1 or TRAF-3 should also precipitate LMP1. Antibodies against TRAF-1, TRAF-3, or LMP1 were used to promote immunoprecipitation of their specific target molecules. The presence of LMP1 in the immunoprecipitates was analyzed by Western immunoblotting. Figure 4 shows a representative experiment with a specimen from a patient with post-transplantation lymphoproliferative disease in which 10 percent of the total LMP1 in the tumor lysate was immunoprecipitated with either anti-TRAF-1 or anti-TRAF-3 antibodies. The findings were similar for all four of the samples from patients with post-transplantation lymphoproliferative disease that were tested in this way and for both samples from the patients with AIDS-associated non-Hodgkin's lymphoma.

The immunoprecipitation of LMP1 with anti-TRAF-1 or anti-TRAF-3 antiserum confirms that the LMP1 transforming protein is physically associated with these TRAF signaling molecules in the lymphoproliferative disorders under investigation. Since in vitro studies have shown that LMP1 mimics an activated TNF-receptor complex by aggregating with TRAF molecules,[14] my findings suggest that the same mechanism contributes to abnormal cell growth in post-transplantation lymphoproliferative disease and AIDS-associated non-Hodgkin's lymphoma.

In vitro, a functional consequence of the binding of LMP1 to TRAF-1 is activation of the NF-κB transcription factor. To test the hypothesis that aggregates of LMP1 and TRAF have a similar effect in vivo, an electrophoretic mobility shift assay was used to detect activated NF-κB in the nucleus of tumor cells. There was enough material to perform these assays on LMP1-positive samples from four patients with post-transplantation lymphoproliferative disease and both patients with AIDS-associated non-Hodgkin's lymphoma, and on LMP1-negative samples from three patients with endemic Burkitt's lymphoma and two patients with post-transplantation lymphoproliferative disease (Fig. 5). In all the LMP1-positive samples, the electrophoretic mobility shift assay showed activated NF-κB, whereas samples of EBV-negative, LMP1-negative tissue from patients with post-transplantation lymphoproliferative disease and samples of EBV-positive, LMP1-negative tissue from patients with Burkitt's lymphoma did not have detectable activated NF-κB. In all samples showing activated NF-κB, there was complete correlation between aggregates of LMP1 and TRAF signaling molecules in the plasma membrane and signs of activation of NF-κB in the cell nucleus.

DISCUSSION

This study demonstrates an interaction between the EBV transforming protein LMP1 and the TRAF-1 and TRAF-3 signal-transduction molecules in post-

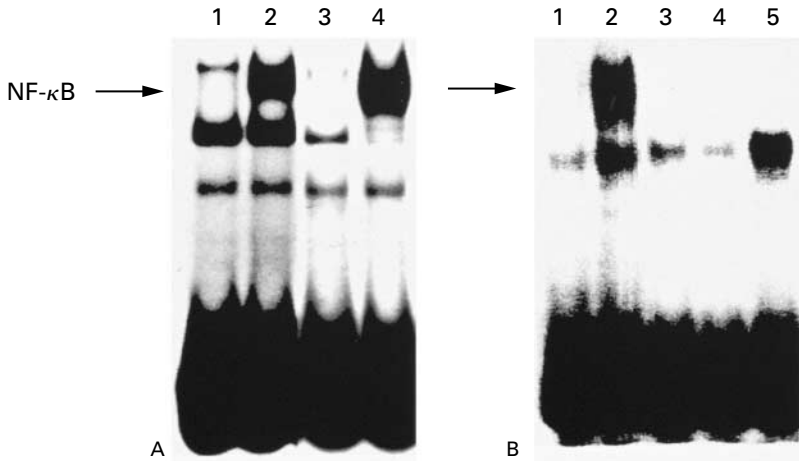

Figure 5. Activation of NF-κB in Tumors Expressing Latent Membrane Protein 1 (Panel A), but Not in Tumors That Do Not Express the Protein (Panel B).

Nuclear lysates from tumors were prepared, and electrophoretic mobility shift assays were performed. In Panel A, lane 1 shows the negative control, a human B-cell line (BJAB) that is negative for Epstein–Barr virus (EBV). Lane 2 shows the positive control, the BJAB cell line transfected with latent membrane protein 1 (LMP1). Lane 3 shows an EBV-negative tumor sample that was also negative for LMP1 from a patient with post-transplantation lymphoproliferative disease (Patient 6 in Table 1). Lane 4 shows an EBV-positive, LMP1-positive tumor sample from a patient with post-transplantation lymphoproliferative disease (Patient 8 in Table 1). In Panel B, lane 1 shows the BJAB negative control. Lane 2 shows an EBV-positive (LMP1-positive) specimen from a patient with AIDS-associated non-Hodgkin's lymphoma (Patient 9 in Table 1). Lanes 3, 4, and 5 show EBV-positive, LMP1-negative tumor samples from three patients with endemic Burkitt's lymphoma.

transplantation lymphoproliferative disease and AIDS-associated non-Hodgkin's lymphoma. This finding provides evidence that LMP1 functions as a signaling protein in the cells of these tumors. Additional support for this conclusion is provided by the finding of activated NF-κB in tumors in which LMP1 was bound to TRAF-1 and TRAF-3 and, conversely, the absence of activated NF-κB in tumors lacking LMP1. Evidence from genetic and biochemical studies of cell cultures indicates that LMP1 is a constitutively activated viral analogue of the cellular TNF-receptor family. These investigations also show that LMP1 transduces an NF-κB activation signal that is partially mediated through TRAF molecules. Through its potent growth-promoting effects, LMP1 has been postulated to mediate cell transformation in EBV-associated cancers in humans, although direct evidence of this effect has been lacking.[13-17] The frequent expression of LMP1 in tumor tissue from patients with post-transplantation lymphoproliferative disease and AIDS-associated non-Hodgkin's lymphoma supports the notion that LMP1 may contribute to the development of these diseases.

In contrast to the findings in specimens from patients with endemic Burkitt's lymphoma, all EBV-positive specimens from patients with post-transplantation lymphoproliferative disease expressed LMP1, including those with Burkitt-like lymphoma who had *MYC* gene translocations.[22] This suggests that in post-transplantation lymphoproliferative disease, LMP1 may be necessary for the proliferation of malignant cells, even after activation of cellular oncogenes. In AIDS-associated non-Hodgkin's lymphoma, less is known about the pattern of LMP1 expression as it relates to cellular oncogene activation. Notably, Patient 9, who had AIDS-associated non-Hodgkin's lymphoma (Table 1), had both LMP1 and a *MYC* gene translocation, suggesting similarities in the pathogenesis of this kind of lymphoma and post-transplantation lymphoproliferative disease.

EBV was first identified in endemic Burkitt's lymphoma cells more than 30 years ago and has since been linked to several malignant diseases. Nevertheless, direct evidence of an EBV-mediated signaling process in these neoplasms has been elusive. Given the evidence that LMP1 signaling through aggregation with TRAF molecules is essential for transformation in vitro, my findings suggest that this pathway is also critical to the malignant phenotype in vivo. The presence of cellular (nonviral) members of the TNF-receptor family, including CD30 and CD40, is characteristic of other types of lymphoma. TRAF-mediated signaling processes may occur in a similar manner in these diseases and may be important in the transformation process.

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REFERENCES

1. Kieff E. Epstein-Barr virus and its replication. In: Fields BN, Knipe DM, Howley PM, eds. Fields virology. 3rd ed. Vol. 2. Philadelphia: Lippincott-Raven, 1996:2343-96.
2. Wang D, Liebowitz D, Kieff E. An EBV membrane protein expressed in immortalized lymphocytes transforms established rodent cells. Cell 1985;43:831-40.
3. *Idem.* The truncated form of the Epstein-Barr virus latent-infection membrane protein expressed in virus replication does not transform rodent fibroblasts. J Virol 1988;62:2337-46.
4. Baichwal VR, Sugden B. Transformation of Balb 3T3 cells by the BNLF-1 gene of Epstein-Barr virus. Oncogene 1988;2:461-7.
5. Dawson CW, Rickinson AB, Young LS. Epstein-Barr virus latent membrane protein inhibits human epithelial cell differentiation. Nature 1990; 344:777-80.
6. Wilson JB, Weinberg W, Johnson R, Yuspa S, Levine AJ. Expression of the BNLF-1 oncogene of Epstein-Barr virus in the skin of transgenic mice induces hyperplasia and aberrant expression of keratin 6. Cell 1990;61: 1315-27.
7. Fahraeus R, Rymo L, Rhim JS, Klein G. Morphological transformation of human keratinocytes expressing the LMP gene of Epstein-Barr virus. Nature 1990;345:447-9.
8. Wang D, Liebowitz D, Wang F, et al. Epstein-Barr virus latent infection membrane protein alters the human B-lymphocyte phenotype: deletion of the amino terminus abolishes activity. J Virol 1988;62:4173-84.
9. Henderson S, Rowe M, Gregory C, et al. Induction of bcl-2 expression by Epstein-Barr virus latent membrane protein 1 protects infected B cells from programmed cell death. Cell 1991;65:1107-15.
10. Hammarskjold ML, Simurda MC. Epstein-Barr virus latent membrane protein transactivates the human immunodeficiency virus type 1 long terminal repeat through induction of NF-kappa B activity. J Virol 1992;66: 6496-501.
11. Laherty CD, Hu HM, Opipari AW, Wang F, Dixit VM. The Epstein-Barr virus LMP1 gene product induces A20 zinc finger protein expression by activating nuclear factor kappa B. J Biol Chem 1992;267:24157-60.
12. Rowe M, Peng-Pilon M, Huen DS, et al. Upregulation of bcl-2 by the Epstein-Barr virus latent membrane protein LMP1: a B-cell-specific response that is delayed relative to NF-kappa B activation and to induction of cell surface markers. J Virol 1994;68:5602-12.
13. Kaye KM, Izumi KM, Kieff E. Epstein-Barr virus latent membrane protein 1 is essential for B-lymphocyte growth transformation. Proc Natl Acad Sci U S A 1993;90:9150-4.
14. Mosialos G, Birkenbach M, Yalamanchili R, VanArsdale T, Ware C, Kieff E. The Epstein-Barr virus transforming protein LMP1 engages signaling proteins for the tumor necrosis factor receptor family. Cell 1995;80: 389-99.
15. Kaye KM, Devergne O, Harada JN, et al. Tumor necrosis factor receptor associated factor 2 is a mediator of NF-κB activation by latent infection membrane protein 1, the Epstein-Barr virus transforming protein. Proc Natl Acad Sci U S A 1996;93:11085-90.
16. Devergne O, Hatzivassiliou E, Izumi KM, et al. Association of TRAF1, TRAF2, and TRAF3 with an Epstein-Barr virus LMP1 domain important for B-lymphocyte transformation: role in NF-κB activation. Mol Cell Biol 1996;16:7098-108.
17. Liebowitz D, Mannick J, Takada K, Kieff E. Phenotypes of Epstein-Barr virus LMP1 deletion mutants indicate transmembrane and amino-terminal cytoplasmic domains necessary for effects in B-lymphoma cells. J Virol 1992;66:4612-6.
18. Liebowitz D, Kieff E. Epstein-Barr virus. In: Roizman B, Whitley RJ, Lopez C, eds. The human herpesviruses. New York: Raven Press, 1993: 107-72.
19. Rowe DT, Rowe M, Evan GI, Wallace LE, Farrell PJ, Rickinson AB. Restricted expression of EBV latent genes and T-lymphocyte-detected membrane antigen in Burkitt's lymphoma cells. EMBO J 1986;5:2599-607.
20. Hatzubai A, Anafi M, Masucci MG, et al. Down-regulation of the EBV-encoded membrane protein (LMP) in Burkitt lymphomas. Int J Cancer 1987;40:358-64.
21. Gregory CD, Rowe M, Rickinson AB. Different Epstein-Barr virus-B cell interactions in phenotypically distinct clones of a Burkitt's lymphoma cell line. J Gen Virol 1990;71:1481-95.
22. Liebowitz D, LeBeau MM, Olopade OI. Evidence for signal transduction through the TNF receptor pathway in post-transplant lymphoproliferative disorders. J Acquir Immune Defic Syndr Hum Retrovirol 1997;14: A51. abstract.