The Journal of Microbiology, December 1999, p.256-262
Vol. 37, No. 4

Deletion Analysis of the Major NF-κB Activation Domain in Latent Membrane Protein 1 of Epstein-Barr Virus

Shin Cho and Won-Keun Lee*

Department of Biological Science and Institute of Bioscience and Biotechnology, Myongji University, Yongin, Kyunggi-do 449-728, Korea

(Received September 8, 1999 / Accepted November 25, 1999)

Latent membrane protein 1 (LMP1) of the Epstein-Barr virus (EBV) is an integral membrane protein with six transmembrane domains, which is essential for EBV-induced B cell transformation. LMP1 functions as a constitutively active tumor necrosis factor receptor (TNFR) like membrane receptor, whose signaling requires recruitment of TNFR-associated factors (TRAFs) and leads to NF-κB activation. NF-κB activation by LMP1 is critical for B cell transformation and has been linked to many phenotypic changes associated with EBV-induced B cell transformation. Deletion analysis has identified two NF-κB activation regions in the carboxy terminal cytoplasmic domains of LMP1, termed CTAR1 (residues 194-232) and CTAR2 (351-386). The membrane proximal C-terminal domain was precisely mapped to a PXQXT motif (residues 204-208) involved in TRAF binding as well as NF-κB activation. In this study, we dissected the CTAR2 region, which is the major NF-κB signaling effector of LMP1, to determine a minimal functional sequence. A series of LMP1 mutant constructs systematically deleted for the CTAR2 region were prepared, and NF-κB activation activity of these mutants were assessed by transiently expressing them in 293 cells and Jurkat T cells. The NF-κB activation domain of CTAR2 appears to reside in a stretch of 6 amino acids (residues 379-384) at the end of the carboxy terminus.

Key words: Epstein-Barr virus, Latent membrane protein 1, NF-κB activation

Epstein-Barr virus (EBV), a ubiquitous human herpes virus, efficiently transforms mature B-lymphocytes into continuously proliferating lymphoblastoid cell lines (LCLs). In such transformed cells, EBV establishes latent infection with little production of progeny virions but consistently expresses a number of so-called latent genes. They include six nuclear antigens (EBNA1, 2, 3A, 3B, 3C, and LP), three latent membrane proteins (LMP1, 2A, and 2B), and two closely related nonpolyadenylated small RNA transcripts (EBER1 and 2). Molecular genetic analyses have shown that most of these latent proteins including EBNA1, 2, 3A, 3C and LP as well as LMP1 are essential or critical for EBV-induced transformation of B cells in vitro (17, 21). EBV latent infection is also strongly associated with pathogenesis of a number of human malignancies including endemic Burkitts lymphoma (BL), undifferentiated nasopharyngeal carcinoma (NPC). However, in such EBV-associated tumors, EBV shows different types of latency, characterized by limited expression of viral latent genes, EBNA1 only or EBNA1 and LMPs.

Of the EBV latent genes essential for B cell transformation, LMP1 is unique in that it is oncogenic when transfected into rodent fibroblasts (1, 23). Furthermore, expression of LMP1 in EBV-negative B lymphoma cell lines and even in primary B cells can induce a plethora of cell surface phenotype changes that are associated with the activation process initiated by EBV latent infection; increases in the expression of intracellular adhesion molecules (ICAM-1, LFA-1, and LFA-3) and various B cell activation markers (CD40, CD23, and CD21) involved in cell growth regulation (20, 24, 25). In epithelial cells, LMP1 expression inhibits cellular differentiation, a property that might be important in the context of undifferentiated NPC tumors consistently infected with EBV (3, 5). LMP1 also induces the expression of antiapoptotic proteins such as Bcl-2, Mcl-1, and A20, indicating that it plays a role in preventing cells from apoptosis (11, 18, 22). Together, these observations indicate pivotal roles for LMP1 in EBV-induced B cell transformation and also in the pathogenesis of EBV-associated carcinoma where LMP1 is regularly detected.

* To whom correspondence should be addressed.
(Tel) 82-335-330-6194; (Fax) 82-335-335-8249
(E-mail) wklee@bio.myongji.ac.kr

LMP1 is an integral membrane protein of 386 amino acids with 6 hydrophobic transmembrane domains, and both its short amino- and long carboxy termini are on the cytoplasmic side of the membrane. The LMP1 protein aggregates in punctuate patches in the membrane. The aggregation requires all six transmembrane domains and is essential for the transforming function of LMP1.

A breakthrough for how LMP1 regulates so many cellular genes came with the observations that LMP1 stimulates the A20 promoter and the HIV-1 promoter through activation of the cellular transcription factor, NF-κB (10, 18). Deletion mutation analyses of LMP1 have shown that the cytoplasmic C-terminus contains two regions important for activation of NF-κB, termed CTAR1 (residues 194-232) and CTAR2 (residues 351-386) (12, 19). CTAR1 contributes approximately 20 to 30% of total LMP1-induced NF-κB activation whereas CTAR2 is responsible for 70 to 80% of the total NF-κB activation by LMP1. The yeast two hybrid analysis using the LMP1 C-terminal tail as a bait and in vitro binding assays have shown that CTAR1 binds cellular proteins (TRAFs 1, 2, 3, and 5) that belong to the tumor necrosis factor receptor-associated factor (TRAF) family, through a PXQXT motif (residues 204-208). The interaction with CTAR1 and TRAF1 and TRAF2 has been shown to be critical for both NF-κB activation signaling and B cell transformation (4, 13, 15). Deletion of CTAR2 of LMP1 in the context of the whole virus results in a virus, which retains the B cell transforming activity, but the resulting transformed cell requires a fibroblast feeder for normal growth in culture (16). This observation indicates that LMP1 CTAR2 plays an important role in providing cell growth factor-related cell signaling. Therefore, LMP1 most likely contributes in part to the transforming activity by functioning as a constantly activated TNF receptor-like NF-κB signaling receptor, whose aggregation occurs without aid of a binding ligand (8).

However, the molecular nature of how CTAR2, the major effector domain of LMP1 for NF-κB activation, exerts its activity remains elusive. Although the CTAR2 region contains a TRAF binding PXQXT/S motif as in CTAR1, this motif fails to bind any TRAF proteins in binding assays (6). In this study, we thus carried out deletion mutation analysis of the CTAR2 region to reveal a core-sequence necessary for its NF-κB activation.

Methods and Materials

Cells and cell culture

BJAB is an EBV-negative Burkitts lymphoma cell line. Jurkat is a T-leukemia cell line. Both BJAB and Jurkat cells were maintained in RPMI 1640 media supplemented with 10% heat-inactivated FBS (Gibco BRL) and 3 μg/ml of gentamycin (Sigma). 293 cells, a human embryonic kidney (HEK) epithelial cell line (9) (kindly provided by C. Min, Ajou University) were cultured in Dulbecco's modified Eagle's medium (DMEM/high glucose, HyClone Laboratories, Inc) containing 10% FBS and 3 μg/ml gentamycin.

LMP1 mutant constructs

The pSG5-based LMP1 construct (pLMP1) has been described previously (2) and expresses the full-length LMP1 of B95-8 EBV. All LMP1 deletion constructs were derived from this construct. To facilitate construction of LMP1 deletion mutants, the 1.5-kb *Eco*R1-*Bam*HI fragment of the LMP1 gene in pLMP1 was first subcloned into pUC19 to yield pUC19-LMP1. The pUC19-based LMP1(Δ188/350) construct was then made by internally deleting a 0.5-kb *Nco*I fragment from pUC19-LMP1 and self-ligating the resulting plasmid. A series of pUC19-based LMP1 mutants (Δ188/354, Δ188/364, Δ188/371, Δ188/375, Δ188/378, and Δ188/380), which were consecutively deleted for the CTAR-2 region, were made by PCR amplifications of deleted CTAR-2 sequences and replacing resulting amplified DNAs for the wild type sequence of LMP1 (Δ188/350). For instance, to make pUC19-LMP1 (Δ188/354), the LMP1 3-terminal sequence was amplified using a 5 primer (5'-taccatggTGATCCACACCTTC-CTA-3'), the LMP1-R primer (5'-cgggatccAGTTAAG-GTGATTAGCTAA-3'), pLMP1 as the template, and Vent DNA polymerase (New England Biolabs). The resulting PCR product was purified, digested with *Nco*I and *Bam*HI, and used to replace the *Nco*I-*Bam*HI fragment of pUC19-LMP1 (Δ188/350), yielding pUC19-LMP1 (Δ188/354). Likewise, to make pUC19-based other LMP1 deletion mutants, the following 5 primers were made and used to amplify LMP1 3-sequences with the LMP1-R primer; 5'-taccatggTTCTTCTGGTTCCGGTG-3' (Δ188/364), 5'-taccatggAG-ATGATGACGACCCA-3' (Δ188/371), 5'-taccatgg- ACCACACGGGCCCGTT-3' (Δ188/375), 5-taccatggCCCA- GTTCAGCTAAGCTAC-3' (Δ188/378), and (Δ188/380).

The pUC19-based LMP1 Δ350/354, Δ350/364, Δ350/371, Δ350/375, Δ350/378, and Δ350/380 deletion mutants were made by inserting the 490-bp *Nco*I-*Nco*I fragment of the B95-8 LMP1 gene into the *Nco*I site of pUC19-LMP1 Δ188/354, Δ188/364, Δ188/371, Δ188/375, Δ188/378, and Δ188/380 deletion mutants, respectively. Subsequent subcloning of the *Eco*R1-*Bam*HI fragments of each of these pUC-based LMP1 mutant constructs into the *Eco*R1-*Bam*HI site of the pSG5 vector yielded pSG5-based LMP1 deletion mutant constructs, pLMP1(Δ350/354), pLMP1(Δ350/364), pLMP1

(Δ350/371), pLMP1(Δ350/375), pLMP1(Δ350/378), and pLMP1(Δ350/380).

pLMP1(1-187), pLMP1(1-350), and pLMP1(1-379), all pSG5-based LMP1 C-terminal truncated mutant constructs, were derived from pUC19-LMP1(1-187), pUC19-LMP1(1-350), and pUC19-LMP1(1-379), respectively, that were constructed as follows. The pUC19-LMP1(1-187) was made by blunting the *Nco*I site of pUC19-LMP1 and inserting a duplex DNA of a stop codon-carrying linker (5'-GATAGTCTAGAC-TATC-3'). To prepare the pUC19-LMP1(1-350) construct, the 0.6 kb *Nco*I-*Bam*HI fragment of pUC19-LMP1 was first replaced with the *Nco*I-*Bam*HI digest of a PCR product obtained by use of a stop-codon carrying primer (5'-taccatggCTAGGGTGATCCACACCT-TCCTACGCTG-3') and the LMP1-R primer. The pUC19-LMP1(1-350) was then made by inserting the 490-bp *Nco*I-*Nco*I fragment of the wild type LMP1 gene into the *Nco*I site of the resulting plasmid. To prepare the pUC19-LMP1(1-379) construct, a LMP1 mutant was made in which an *Apa*I site was created in the C-terminal cytoplasmic region by mutating a C to a G at the Gly-378 codon and an A to a C at the Pro-379 codon. The pUC19-LMP1 (1-379) was then made by inserting in this *Apa*I site a stop-codon containing duplex DNA generated by annealing two oligonucleotides, 5'-TTAGTCTAGAGGCC-3' and 5'-TCT-AGACTAAGGCC-3'.

pLMP1(1-383) and pLMP1(1-384) constructs were made by replacing the 0.1-kb *Esp*I-*Bam*HI fragment of pLMP1 with that amplified from pLMP1 using a forward primer containing an *Esp*I site and a stop codon, 5'-cagctaagcTAATATGACTAACCTTTCTTT-3' or 5'-cagctaagcTACTAAGACTAACCTTTCTTTACT-3' and the LMP1-R primer, respectively. Mutated sequences in all LMP1 mutant constructs were confirmed by sequence analysis.

Transfections, reporter assays, and immunoblotting

For transient transfections, 293 cells (9×10^5) were plated out on a 60-mm plate and 24 h later were transfected using a calcium phosphate technique. To measure NF-κB activation activity of the wild type LMP1 and its deletion mutants, 293 cells were given with 3 μg of pLMP1 or each of the pSG5-based LMP1 deletion constructs or the pSG5 vector, 3 μg of p3X-κB-Luc or p3X-mutκB-Luc, and 3 μg of pCMV-βgal. At 30-48 h post-transfection, cell extracts were made. The p3X-κB-Luc and p3X-mutκB-Luc contain a firefly luciferase gene under control of a minimal fos promoter with either three copies of the major histocompatability complex (MHC) class I κB element or its mutated form, respectively (kindly provided by B. Sugden, University of Wisconsin at Madison, USA) (19). Luciferase reporter and β-galactosidase assays were performed as described previously (2). Luciferase activity was normalized with β-galactosidase activity.

To detect expression of LMP1 deletion mutants, BJAB cells (8×10^6) were electroporated with 3 μg each of the pSG5-based LMP1 deletion constructs or the pSG5 vector at 260 V and 960 μF using Gene Pulsar (Bio-Rad). At 40 h post-transfection, cells were counted, harvested, and suspended in 400 μl of SDS lysis buffer (40 mM Tris-HCl, pH 7.0, 1.4% SDS, 7% glycerol, and 0.01% bromophenol blue). Cell proteins equivalent to 4×10^5 cells were resolved on a SDS-10% polyacrylamide gel and blotted onto a nitrocellulose membrane (Amersham). LMP1 was then detected by incubating the blot with CS1-4 (1:100, Novocastra), a LMP1 monoclonal antibody, and a color reaction using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Sigma).

Results and Discussion

LMP1 expression activates the NF-κB transcription factor

To determine the effect of LMP1 on NF-κB activation, the B95-8 LMP1 construct in the pSG5 expression vector or the pSG5 vector was co-transfected in 293 cells with a NF-κB-dependent luciferase reporter (3X-κB-Luc or 3X-mutκB-Luc) and a β-galactosidase expression plasmid as the internal control. At 40 h post-transfection, the cells were harvested and cell extracts were prepared. Luciferase and β-galactosidase activities in the extracts were measured to indirectly monitor levels of NF-κB transcription factors activated by LMP1. The luciferase activities were then normalized by the β-galactosidase activities to minimize experimental errors due to different transfection efficiencies.

Expression of the full-length LMP1 expression construct in 293 cells resulted in a 50-fold increase in expression of luciferase from the co-transfected reporter carrying 3 copies of the wild-type (3x-κB-Luc) of the κB element relative to that in pSG5 vector-transfected cells (Fig. 1A). Little or negligible luciferase activity was observed in cells transfected with the same LMP1 construct and luciferase reporter carrying 3 copies of the mutated version of the κB element. The increases in amounts of the LMP1 expression vector transfected lead to more luciferase activities in a dosage-dependent manner (Fig. 1B). These results thus confirm that ectopic expression of LMP1 in 293 cells results in activation of the cellular NF-κB factor.

It has been shown that the LMP1 C-terminal cytoplasmic domain contains two different NF-κB acti-

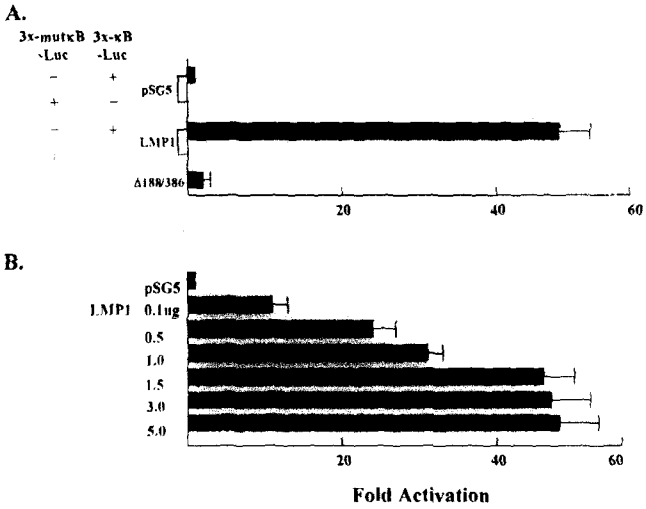

Fig. 1. Expression of LMP1 in 293 cells results in activation of NF-κB transcription factor. A. The B95-8 LMP1 expression construct (pLMP1) or the pSG5 expression vector was cotransfected into 293 cells along with a NF-κB luciferase reporter (p3X-κB-Luc or p3X-mutκB-Luc), and an internal control plasmid expressing the *E. coli* β-galactosidase (pCMV-βgal) using the $CaPO_4$-DNA precipitate method. A cell extract was prepared 30 h post-transfection. Activation of NF-κB was determined by measurement of luciferase activity in the extract, which was then normalized to β-galactosidase activity therein for transfection efficiency. B. NF-κB activation assay was carried as in A except that increasing amounts of pLMP1 as indicated were used. Fold activation was determined relative to the pSG5 vector. The data are the mean (+/- S.D.) of three separate experiments.

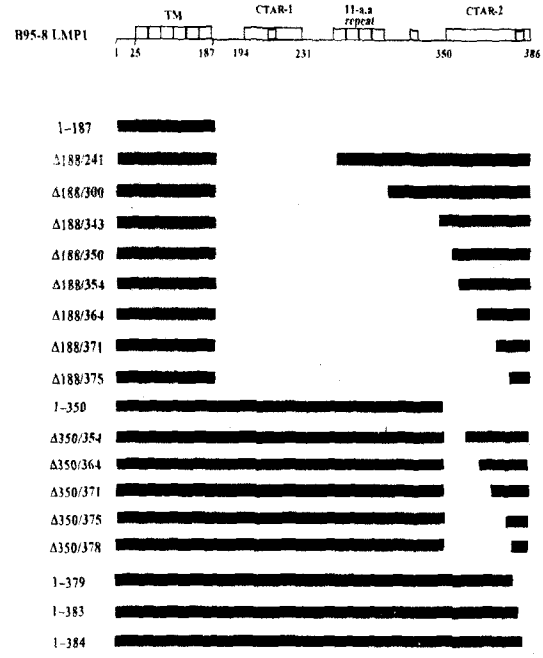

Fig. 2. The map of LMP1 deletion mutant constructs. All LMP1 deletion constructs were derived from the B95-8 LMP1 gene as described in Methods and Materials. Indicated on top of the diagram of B98-8 LMP1 are structural features of LMP1 such as six hydrophobic transmembrane domains (TM) and four copies of the 11-amino acid repeat. Two C-terminal NF-κB activating regions (CTAR1 and CTAR2) are also shown. Gray boxes within CTAR1 and CTAR2 as well as one between the 11-aa repeats and CTAR2 indicate a PXQXT sequence motif.

vating regions, CTAR1 and CTAR2 (12). Transfection of the pLMP1(1-187) construct expressing a LMP1 mutant truncated for the entire C-terminal cytoplasmic domain failed to induce luciferase activity from 3X-κB-Luc reporter, confirming that the NF-κB activation requires the C-terminal cytoplasmic domain of LMP1 (Fig. 1A). LMP1(1-187) could not be detected in immunoblots using anti-LMP1 CS1-4 mAbs which recognized epitopes in the C-terminal region. However, LMP1(1-187) expressed in BJAB cells was detected by immunofluorescence microscopy using the OT-22 mAb recognizing the LMP1 N-terminal cytoplasmic region (data not shown).

Construction and expression analysis of LMP1 deletion constructs

Of the two C-terminal NF-κB activating regions of LMP1, CTAR-1 is located at the proximal end of the LMP1 C-terminal tail, whereas CTAR2 has been mapped to the distal end of the LMP1 C-terminal tail. In order to map the core NF-κB activation region of CTAR2, a series of LMP1 CTAR2-deleted mutants were made (Fig. 2). They included a mutant deleted for the entire CTAR2 region [LMP1(1-350)] and six mutants, which were internally deleted for CTAR2 starting at residue 350 toward the end of the C-terminal tail [LMP1(Δ350/354), LMP1(Δ350/364), LMP1 (Δ350/371), LMP1(Δ350/375), and LMP1(Δ350/378)].

These CTAR2 deleted LMP1 mutant constructs were then electroporated in Jurkat cells, and cellular proteins obtained at 40 h post-transfection were sub-

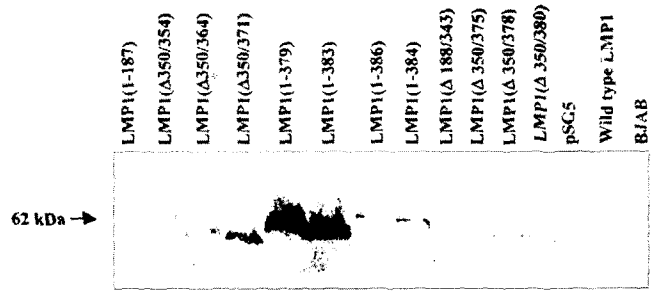

Fig. 3. Immunoblot assay of the LMP1 mutants expressed following transfection into Jurkat cells. At 30 h post electroporation, cells were harvested, lysed, and separated by 8% SDS-PAGE. Following transfer to nitrocellulose filters, the blot was probed with the CS.1-4 pool of monoclonal antibodies as described in Materials and Methods.

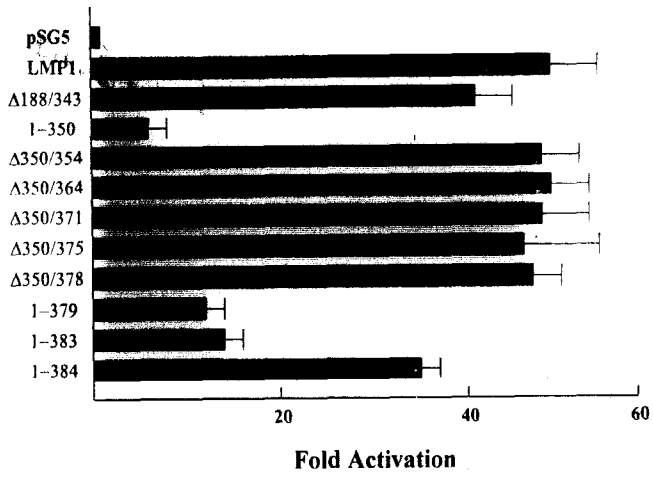

Fig. 4. NF-κB activation assay for LMP1 mutants in 293 cells. Each of the LMP1 mutant expression plasmids was transfected into 293 cells along with p3X-κB-Luc and pCMV-βgal using $CaPO_4$-DNA precipitates. NF-κB activation activity was determined 48 h post transfection as described in Fig. 1A. Fold activation was determined relative to the pSG5 vector. The data are the mean of three separate experiments. Bars indicate standard deviations.

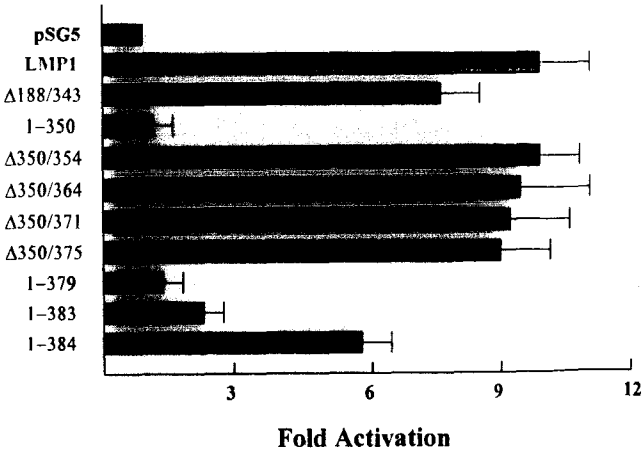

Fig. 5. NF-κB activation assay for LMP1 mutants in Jurkat T cells.

jected to immunoblotting using the CS1-4 LMP1 mAbs. As shown in Fig. 3, all constructs expressed LMP1 proteins with expected molecular sizes. Interestingly, LMP1(1-379) and LMP1(1-383) were expressed at higher levels than wild type LMP1.

NF-κB activation assays for CTAR2-deleted LMP1 mutants

To determine the ability of these CTAR-2 deleted mutants to activate NF-κB, transient NF-κB activation assays in 293 cells were then carried out as described above. The results of these assays are shown in Fig. 4.

The entire CTAR2 deletion mutant, LMP1(1-350), stimulated NF-κB activity by 7-fold, compared with the vector control, which is only 15% of the activity of full-length LMP1. This result thus confirms that the CTAR2 region is the major effector for the NF-κB activation signaling by LMP1. Three consecutive CTAR2 internal deletion mutants, LMP1(Δ350/354), LMP1 (Δ350/364), and LMP1(Δ350/371), showed NF-κB activation activities comparable to that of the wild type LMP1, indicating that residues 350 to 371 in CTAR2 are dispensable for NF-κB activation. Further deletions to residues 375 [LMP1(Δ350/375)] or 378 [LMP1(Δ350/378)] resulted in a consistent marginal decrease in the activity by 3%. In sum, the results of the CTAR2 internal deletion analyses indicated that the NF-κB activation domain of CTAR2 must reside in the last 8 residues of the C-terminal.

To test this possibility, LMP1 C-terminal truncated were prepared and tested for their ability to activate NF-κB (Fig. 4). Removal of the carboxy terminal last 2 residues, LMP1(1-384), reduced activity to 68% of the wild type activity, which is still significantly higher than that of the CTAR1-deleted mutant, LMP1(Δ188/343). This suggests that the last 2 residues may not be important but necessary for full activity. However, LMP1(1-383) lacking the last 3 consecutive residues showed only 30% activity of wild type LMP1, indicating that residue 384 is critical for NF-κB activation by CTAR2. LMP1(1-379) with deletion of the last 7 residues showed similar activation activity to that of LMP1(1-383), 28% of full-length LMP1.

Similar NF-κB activation assays were also performed Jurkat T cells to determine if there are any differences in activity depending upon the cell type. As shown in Fig. 5, wild type LMP1 and all CTAR2 mutants analyzed showed consistently 5-fold less activity in Jurkat cells compared with those in 293 cells.

The results of analyses of the C-terminal truncated and the CTAR2-internal deletion mutants indicated that the NF-κB activation domain of CTAR2 resides in the stretch of six residues 379 to 384 (Pro-Val-Gln-Leu-Ser-Tyr). Recently, the same six residues have been identified as the NF-κB activation domain by Floetmann and Rowe, who analyzed a series of glycine substitutions of CTAR2 (7). These six residues thus should provide a structural interface interacting with a cell signaling protein(s) mediating NF-κB activation.

Interestingly, the minimal NF-κB activation sequence of CTAR2 includes a PXQXT motif, which is known to mediate NF-κB signaling via interaction with TRAF2 by LMP1 CTAR1 and some TNF receptor members such as CD30 and CD40. Recently, based on crystal structures of the TRAF domain of human TRAF2 complexed with peptides from TNRF family

members including CD40, CD30, and LMP1, Ye *et al.* (26) have shown that the major and minor TRAF2-binding consensus sequence consists of (P/S/A/T)X(Q/E)E and PXQXXD, respectively. The actual TRAF2-binding sequence in CTAR1 would likely be PXQXXD (204-209), rather than PXQXT (204-209) as previously determined. The absence of such TRAF2-binding consensus sequences within the CTAR2 is consistent with the previous reports that CTAR2 fails to bind TRAF2 (6). However, Kaye *et al.* (15) have shown that the LMP1 CTAR2-induced NF-κB activation is partially inhibited by a dominant negative TRAF2 mutant, indicating that TRAF2 acts as a downstream effector for CTAR2. Indeed, recently, it has been shown that CTAR2 binds the TNFR-associated death domain (TRADD), which is known to interact with TRAF2 to mediate NF-κB signaling of TNFR1 (14). The LMP1 CTAR2 is thus likely to induce NF-κB activation by indirect binding to TRAF2 via its association with TRADD as does TNRF1.

Acknowledgments

We are grateful to Dr. Bill Sugden (University of Wisconsin at Madison, USA) for the p3X-κB-Luc and p3X-mutκB-Luc plasmids and Dr. C. Min (Ajou University) for 293 cells. This work was supported by a grant from the Ministry of Education in Korea (1997) to W.-K. Lee.

References

1. **Baichwal, V.R. and B. Sugden**. 1988. Transformation of Balb 3T3 cells by the BNLF-1 gene of Epstein-Barr virus. *Oncogene* **2,** 461-467.
2. **Cho, S., S.-G. Cho, Y.-S. Shim, and W.-K. Lee**. 1998. Sequence analysis of the latent membrane protein 1 genes of Epstein-Barr virus isolates in Korea. *J. Microbiol.* **36,** 130-138.
3. **Dawson, C.W., A.B. Rickinson, and L.S. Young**. 1990. Epstein-Barr virus latent membrane protein inhibits human epithelial cell differentiation. *Nature* **344,**777-780.
4. **Devergne, O., E. Hatzivassiliou, K.M. Izumi, K.M. Kaye, M.F. Kleijnen, E. Kieff, and G. Mosialos**. 1996. Association of TRAF1, TRAF2, and TRAF3 with an Epstein-Barr virus LMP1 domain important for B-lymphocyte transformation: role in NF-kappaB activation. *Mol. Cell. Biol.* **16,** 7098-7108.
5. **Fahraeus, R., L. Rymo, J.S. Rhim, and G. Klein**. 1990. Morphological transformation of human keratinocytes expressing the LMP gene of Epstein-Barr virus. *Nature* **345,** 447-449.
6. **Franken, M., O. Devergne, M. Rosenzweig, B. Annis, E. Kieff, and F. Wang**. 1996. Comparative analysis identifies conserved tumor necrosis factor receptor-associated factor 3 binding sites in the human and simian Epstein-Barr virus oncogene LMP1. *J. Virol.* **70,** 7819-7826.
7. **Floettmann, J.E. and M. Rowe**. 1997. Epstein-Barr virus latent membrane protein-1 (LMP1) C-terminus activation region 2 (CTAR2) maps to the far C-terminus and requires oligomerization for NF-κB activation. *Oncogene* **17,** 1851-1858.
8. **Gires, O., U. Zimber-Strobl, R. Gonnella, M. Ueffing, G. Marschall, R. Zeidler, D. Pich, and W. Hammerschmidt**. 1997. Latent membrane protein 1 of Epstein-Barr virus mimics a constitutively active receptor molecules. *EMBO J.* **16,** 6131-6140.
9. **Graham, F.L., J. Smiley, W.C. Russel, and R. Nairn.** 1977. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J. Gen. Virol.* **36,** 59-74.
10. **Hammarskjold, M.L., and M.C. Simurda**. 1992. 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.* **66,** 6496-6501.
11. **Henderson, S., M. Rowe, C. Gregory, D. Croom-Carter, F. Wang, R. Longnecker, E. Kieff, and A.B. Rickinson.** 1991. Induction of bcl-2 expression by Epstein-Barr virus latent membrane protein 1 protects infected B cells from programmed cell death. *Cell* **65,** 1107-1115.
12. **Huen, D.S., S.A. Henderson, D. Croom-Carter, and M. Rowe.** 1995. The Epstein-Barr virus latent membrane protein-1 (LMP1) mediates activation of NF-kappa B and cell surface phenotype via two effector regions in its carboxy-terminal cytoplasmic domain. *Oncogene* 10, 549-560.
13. **Izumi, K.M., K.M. Kaye, and E.D. Kieff.** 1997. The Epstein-Barr virus LMP1 amino acid sequence that engages tumor necrosis factor receptor associated factors is critical for primary B lymphocyte growth transformation. *Proc. Natl. Acad. Sci. USA* **94,** 1447-1452.
14. **Izumi, K.M. and E.D. Kieff.** 1997. The Epstein-Barr virus oncogene product latent membrane protein 1 engages the tumor necrosis factor associated death domain protein to mediate B lymphocyte growth transformation and activates NF-κB. *Proc. Natl. Acad. Sci. USA* **94,** 12592-12597.
15. **Kaye, K.M., O. Devergne, J.N. Harada, K.M. Izumi, R. Yalamanchili, E. Kieff, and G. Mosialos.** 1996. Tumor necrosis factor receptor associated factor 2 is a mediator of NF-kappa B activation by latent infection membrane protein 1, the Epstein-Barr virus transforming protein. *Proc. Natl. Acad. Sci. USA* **93,** 11085-11090.
16. **Kaye, K.M., K.M. Izumi, G. Mosialos, and E. Kieff.** 1995. The Epstein-Barr virus LMP1 cytoplasmic carboxy terminus is essential for B-lymphocyte transformation; fibroblast cocultivation complements a critical function within the terminal 155 residues. *J. Virol.* **69,** 675-683.
17. **Kaye, K.M., K.M. Izumi, and E. Kieff.** 1993. Epstein-Barr virus latent membrane protein 1 is essential for B-lymphocyte growth transformation. *Proc. Natl. Acad. Sci. USA* **90,** 9150-9154.
18. **Laherty, C.D., H.M. Hu, A.W. Opipari, F. Wang, and V.M. Dixit**. 1992. The Epstein-Barr virus LMP1 gene product induces A20 zinc finger protein expression by activating nuclear factor kappa B. *J. Biol. Chem.* **267,** 24157-24160.
19. **Mitchell, T. and B. Sugden**. 1995. Stimulation of NF-kappa B-mediated transcription by mutant derivatives of the latent membrane protein of Epstein-Barr virus. *J. Virol.* **69,** 2968-2976.

20. **Peng, M. and E. Lundgren**. 1992. Transient expression of the Epstein-Barr virus LMP1 gene in human primary B cells induces cellular activation and DNA synthesis. *Oncogene* **7**, 1775-1782.
21. **Rickinson, A.B. and E. Kieff**. 1996. Epstein-Barr virus, in *Fields Virology*, 3rd ed. edited by B. N. Fields, D. M. Knipe, and P. W. Howley, *et al.*, Lippincott-Raven Publishers, Philadelphia, USA, 2397-2446.
22. **Rowe, M., M. Peng-Pilon, D.S. Huen, R. Hardy, D. Croom-Carter, E. Lundgren, and A.B. Rickinson**. 1994. 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.* **68**, 5602-5612.
23. **Wang, D, D. Liebowitz, and E. Kieff**. 1985. An EBV membrane protein expressed in immortalized lymphocytes transforms established rodent cells. *Cell* **43**, 831-840.
24. **Wang, D., D. Liebowitz, F. Wang, C. Gregory, A. Rickinson, R. Larson, T. Springer, and E. Kieff**. 1988. Epstein-Barr virus latent infection membrane protein alters the human B-lymphocyte phenotype: deletion of the amino terminus abolishes activity. *J. Virol.* **62**, 4173-4184.
25. **Wang, F., C. Gregory, C. Sample, M. Rowe, D. Liebowitz, R. Murray, A. Rickinson, and E. Kieff**. 1990. Epstein-Barr virus latent membrane protein (LMP1) and nuclear proteins 2 and 3C are effectors of phenotypic changes in B lymphocytes: EBNA-2 and LMP1 cooperatively induce CD23. *J. Virol.***64**, 2309-2318.
26. **Ye, H., Y.C. Park, M. Kreishman, E. Kieff, and H. Wu**. 1999. The structural basis for the recognition of diverse receptor sequences by TRAF2. *Mol. Cell* **4**, 321-330.