The human herpes virus 8-encoded Viral FLICE Inhibitory Protein Physically Associates with and Persistently Activates the IκB Kinase Complex*

Received for publication, October 31, 2001, and in revised form, February 4, 2002
Published, JBC Papers in Press, February 5, 2002, DOI 10.1074/jbc.M110480200

Li Liu, Michael T. Eby, Nisha Rathore, Suwan K. Sinha, Arvind Kumar, and Preet M. Chaudhary‡

From the Hamon Center for Therapeutic Oncology Research and Division of Hematology-Oncology, University of Texas Southwestern Medical Center, Dallas, Texas 75390-8593

The Human Herpes Virus 8-encoded Viral FLICE Inhibitory Protein

PHYSICALLY ASSOCIATES WITH AND PERSISTENTLY ACTIVATES THE IKK COMPLEX

The human herpes virus 8 (HHV8, also called Kaposi’s sarcoma-associated herpesvirus) has been linked to Kaposi’s sarcoma and primary effusion lymphoma (PEL) in immunocompromised individuals. We demonstrate that PEL cell lines have a constitutively active NF-κB pathway, which is associated with persistent phosphorylation of IκBα. To elucidate the mechanism of NF-κB activation in PEL cell lines, we have investigated the role of viral FLICE inhibitory protein (vFLIP) in this process. We report that stable expression of HHV8 vFLIP in a variety of cell lines is associated with persistent NF-κB activation caused by constitutive phosphorylation of IκBα. HHV8 vFLIP gets recruited to a ~700-kDa IκB kinase (IKK) complex and physically associates with IKKα, IKKβ, NEMO/IKKγ, and RIP. HHV8 vFLIP is incapable of activating NF-κB in cells deficient in NEMO/IKKγ, thereby suggesting an essential role of an intact IKK complex in this process. Our results suggest that HHV8 vFLIP might contribute to the persistent NF-κB activation observed in PEL cells by associating with and stimulating the activity of the cellular IKK complex.

Nuclear factor κB (NF-κB)1 is a heterodimeric transcription factor that is primarily composed of 50- and 65-kDa subunits of the Rel family and that is required for regulated expression of several genes involved in inflammation and immune response (1–3). NF-κB is present in the cytoplasm of cells in association with a family of inhibitory proteins, called IκB (1, 4). IκB proteins retain NF-κB in the cytoplasm by masking its nuclear localization signal. Stimulation by a number of cytokines, such as TNFα and interleukin-1, results in the activation of a large molecular mass (600–900 kDa), IκB kinase complex that leads to inducible phosphorylation of the IκB proteins at two N-terminal serine residues (5, 6). This complex consists of two catalytic subunits, IKKα (IKK1) and IKKβ (IKK2), and a regulatory subunit, NEMO/IKKγ (7–13). The phosphorylation of IκB proteins results in their rapid ubiquitination and proteasome-mediated degradation, which releases NF-κB from their inhibitory influence. Once released, NF-κB is free to migrate to the nucleus and activate transcription of its target genes.

Some of the noteworthy genes activated by NF-κB include those for cytokines and growth factors, chemokines, cell adhesion molecules, acute phase proteins, anti-apoptotic proteins, and transcription factors p53 and c-Myc (2). The NF-κB pathway has been also shown to play a key role in the control of cell proliferation and oncogenesis. Several members of the NF-κB family have been associated with the development of tumors as a result of overexpression, gene amplification, or gene rearrangement (14). Activation of the NF-κB has been shown to be responsible for the transforming ability of human T-cell leukemia virus type I Tax and Epstein-Barr virus latent membrane protein 1 (15).

Caspase 8 (FLICE/MACH or Mch5) is one of the apical caspases of the caspase cascade, which is activated by signaling via the death receptors belonging to the TNF receptor family (16–18). Caspase 8 is recruited to the multimerized death-inducing signaling complex of these receptors via its N-terminal procaspase domain, which contains two homologous copies of a death effector domain. Death effector domain-containing procaspases are also found in two additional cellular proteins: caspase 10 (Mch4 and FLICE2) (18, 19), and MRIT (c-FLIP, Caspar, I-FLICE, FLAME, CASH, and CLARP), a caspase 8 homolog that is devoid of protease activity (20–26).

Several viruses also encode proteins containing two death effector domains (27–29). These virally encoded death effector domain-containing proteins (also called viral FLICE inhibitory proteins or vFLIPs) include the orf-K13 from the human herpes virus 8 (HHV8/KS-associated herpesvirus, MC159L and MC160L from the Molluscum contagiosum virus, and E8 from the equine herpesvirus 2. Recently, similar vFLIPs have been found in other Gammaherpesviridae of the genus Rhadinovirus, including rhesus rhadinovirus, herpesvirus saimiri, and bovine herpesvirus 4 (30–32).

We and others have previously demonstrated that overexpression of HHV8 vFLIP can protect against death receptor-induced apoptosis in vitro and to promote tumor growth in vivo (33–35). Furthermore, unlike MC159L and E8, HHV8 vFLIP was found to activate the NF-κB pathway when overexpressed in 293T and NIH3T3 cells by transient transfection (33). The present study was undertaken to better understand the mechanism of NF-κB activation by HHV8 vFLIP.
HHV8 vFLIP Associates with the IKK Complex to Activate NF-κB

EXPERIMENTAL PROCEDURES

Plasmids—pGEX-KG IκBα (1–54) and pGEX-KG IκBα (S32A/S36A) were generous gifts from Dr. Richard Gaynor. Retrovirus constructs containing C-terminal FLAG epitope tag vFLIP or MRIT/cFLIP were constructed in MSCV-based retroviral vectors, and the amphotropic retroviruses were generated as described previously (36).

Expression of Bacterially Produced GST-IκBα Proteins—The wild-type and mutant GST-IκBα pGEX-KG constructs were transfected into Escherichia coli BL21 DE3. Cultures (400 ml) of E. coli were grown to an A600nm of 0.6–0.8 and induced with 0.5 mM isopropyl-b-D-thiogalactopyranoside for 3 h. The cells were pelleted, resuspended in buffer A (20 mM HEPES, pH 7.9, 400 mM NaCl, 5 mM dithiothreitol (DTT), 50 mM manitol, 10 mM sodium acetate, 10% glycerol, 0.1 mM EDTA, 0.1% Nonidet P-40, 1 mM phenylmethylsulfon fluoride (PMSF), 50 mM NaF, 5 mM β-glycosphosphate, and protease inhibitor mixture (Roche Molecular Biochemicals) at 37 °C for 30 min. 30 μl of reaction solution was added to each sample. 10 μl of 4× SDS-PAGE sample buffer was added at the end of reaction, and the mixture was heated at 95 °C for 5 min and pelleted by centrifugation. The supernatants were then resolved on SDS-12% polyacrylamide gels followed by autoradiography. For in-solution kinase assay, protein samples (10 μl each of fractions of Superdex 200 column) were incubated with soluble GST-IκBα protein in the same reaction conditions mentioned above.

Western Blot Analysis—100–200 μg of whole cell extracts, cytoplasmic extracts, or proteins immunoprecipitated on agarose beads were heated in the presence of SDS-PAGE sample buffer and loaded on 12% SDS-PAGE gel followed by transferring to nitrocellulose membranes. These membranes were incubated with antibodies against specified proteins in Tris-buffered-saline with Tween 20 followed by incubation with secondary antibody and development with enhanced chemiluminescence (Pierce). The primary antibodies used in these experiments were α−IκBα (Santa Cruz Biotechnology, SC-371, 1:5000), p-IκBα (New England Biolabs, 29415, 1:1000), α−FLAG (Santa Cruz Biotechnology, SC-807, 1:5000), α−NEMO (Santa Cruz Biotechnology, SC-8330, 1:4000), α−RIP (Transduction Laboratories, R41220, 1:1000), α−IKKα (Santa Cruz Biotechnology, SC-7182, 1:4000), and α−IKKβ (Santa Cruz Biotechnology, SC-7807, 1:5000).

RESULTS

Constitutive Activation of NF-κB in HHV8-infected PEL Cell Lines Is Due to Persistent Activation of the IKK Complex—NF-κB is normally sequestered in the cytoplasm of cells because of its association with a family of inhibitory proteins, called IκB (1, 4). However, NF-κB is persistently present in the nuclei of human T-cell leukemia virus type I- and Epstein-Barr virus-transformed cells and has been shown to contribute to the transforming ability of these viruses (15). We were interested in checking whether infection by HHV8 virus might also lead to persistent NF-κB activation. To test this hypothesis we used electrophoretic mobility shift assay (EMSA) to examine the DNA binding activity of nuclear NF-κB in three HHV8-infected PEL cell lines, BC-1, BC-3, and BCBL1, respectively. We also used two non-HHV8-infected lymphoid cell lines, CEM and Jurkat, respectively, as controls for the above experiment. Consistent with a recent report (38), persistent NF-κB activation was seen in all three HHV8-infected PEL cell lines, with the BC-1 cell line showing the highest and BCBL1 cell line showing the least NF-κB activation. In contrast, NF-κB binding activity was absent in the nuclear extracts of CEM and Jurkat cell lines (Fig. 1A). We next sought to analyze the nature and composition of the observed complex in the BC-1 cell line. As shown in Fig. 1B, the observed complex in the BC-1 cell line could be effectively competed with an excess cold probe containing κB binding sites both unafected by competition with a nonspecific DNA duplex. Finally, a supershift assay utilizing subunit-specific antibodies demonstrated that the observed complex contained the p65 and p50 subunits of NF-κB (Fig. 1B).

To determine the mechanism of persistent NF-κB activation in PEL cell lines, we examined the phosphorylation status of IκBα protein by Western blot analysis. Consistent with the EMSA results, phosphorylation of IκBα was totally absent in...
HHV8 vFLIP Associates with the IKK Complex to Activate NF-κB

Fig. 1. PEL cell lines have constitutive NF-κB activation. A, EMSA demonstrating constitutive NF-κB DNA binding activity in the PEL cell lines. The position of the NF-κB complex is marked with an arrow. Nuclear extracts from the following cell lines were used: Lane 1, Jurkat; lane 2, CEM; lane 3, BC-1; lane 4, BC-3; lane 5, BCBL. B, EMSAs demonstrating the nature and specificity of NF-κB complex in BC-1 cell line. The following nuclear extracts were used: Lane 1, BC-1 + cold nonspecific competitor oligonucleotide; lane 2, BC-1 + cold κB competitor oligonucleotide; lane 3, BC-1 + p65 antiserum; lane 4, BC-1 + p50 antiserum; lane 5, BC-1 + c-Rel antiserum. The position of the NF-κB complex is marked with arrow, whereas an asterisk marks the position of a nonspecific band. The arrowheads mark the position of the supershifted complexes. C, status of phosphorylated and total IκBα in PEL cell lines. Cellular extracts containing equal amount of protein were resolved by SDS-PAGE, and phosphorylation of IκBα was analyzed by Western blot using phospho-IκBα antibody (top panel). The blot was stripped and reprobed with antibodies directed against IκBα to demonstrate degradation of IκBα. Western blotting with antibodies against IKKα, IKKβ, and actin is used to demonstrate equal loading of proteins in different lanes. Treatment of Jurkat cells with TNFa (10 ng/ml) was carried out for 15 min and served as a positive control.

CEM and Jurkat cells (Fig. 1C). In contrast, phosphorylation of IκBα was readily detected in all three PEL cell lines and was present in the following order of magnitude: BC-1 > BC-3 > BCBL (Fig. 1C). Of interest, persistent phosphorylation of IκBα observed in BC-1 and BC-3 cell lines was significantly stronger than the TNF-induced IκBα-phosphorylation observed in Jurkat cells. Reprobing of the above blot with an IκBα antibody revealed a decrease in the level of total IκBα protein in the PEL cell lines (Fig. 1C). The above results suggest that the constitutive NF-κB activation in the PEL cell lines is probably due to persistent phosphorylation and subsequent degradation of the IκBα protein.

Retroviral-mediated Expression of HHV8 vFLIP Leads to Persistent NF-κB Activation—We have previously demonstrated that transient transfection-based overexpression of HHV8 vFLIP can lead to NF-κB activation (33). We were interested in determining whether the constitutive NF-κB activation observed in PEL cell lines might be mediated by HHV8 vFLIP. To test this hypothesis, we began by checking whether stable expression of HHV8 vFLIP can lead to constitutive NF-κB activation. For this purpose, we used retroviral-mediated gene transfer to generate mass culture of H460 cells with stable expression of FLAG epitope-tagged HHV8 vFLIP or MRIT/cFLIP. As shown in Fig. 2A, stable expression of HHV8 vFLIP in H460 cells lead to strong NF-κB binding activity as measured by gel shift assay. In contrast, cells expressing an empty vector or MRIT/cFLIP demonstrated low level basal NF-κB binding activity. The specificity of the NF-κB complex seen in the vFLIP-expressing cells was confirmed by competition with a cold NF-κB probe or a nonspecific probe (Fig. 2A). A supershift assay utilizing subunit specific antibodies demonstrated that the complex was a heterodimer of p65 and p50 subunits of NF-κB (Fig. 2A). Essentially similar results were obtained upon stable expression of HHV8 vFLIP in 293T and TF-1 cells (Fig. 2B and data not shown). Finally, expression of vFLIP in 293T and H460 cells was associated with an increase in NF-κB transcriptional activity as measured by a luciferase-based reporter assay (Fig. 2C and data not shown).

Next, we examined the status of total and phosphorylated IκBα in H460 cells expressing empty vector, HHV8 vFLIP, or MRIT/cFLIP. Consistent with previous results with the PEL cell lines, expression of HHV8 vFLIP was associated with a decrease in the steady-state level of total IκBα and an increase in its phosphorylated form (Fig. 2D). Taken together, the above results suggest that HHV8 vFLIP leads to persistent NF-κB activation by constitutive phosphorylation of IκBα.

HHV8 vFLIP Complex Possesses IKK Activity—To test the possibility that HHV8 vFLIP leads to persistent IκBα phosphorylation by interacting with and activating the IKK complex, FLAG-tagged HHV8 vFLIP was immunoprecipitated from the cytosolic extracts of H460-vFLIP cells using FLAG antibody and assayed for the IκBα antibody (Fig. 3A). The vFLIP-associated IKK activity was demonstrated with a cytosolic complex that possesses IKK activity. For this purpose, we used coimmunoprecipitation experiments were carried out.
HHV8 vFLIP Associates with the IKK Complex to Activate NF-κB

HHV8 vFLIP Associates with the IKK Complex to Activate NF-κB

Fig. 2. Expression of HHV8 vFLIP leads to persistent NF-κB activation. A, electrophoretic mobility shift assays. Upper panel, nuclear extracts were prepared from parental H460 cells (lane 1) or those expressing empty vector (lane 2), vFLIP (lane 3), or MRIT/cFLIP (lane 4), and the assay was carried out as described under “Experimental Procedures.” The position of the NF-κB complex is marked by an arrow. The specificity of the complex is demonstrated by competition with excess cold nonspecific probe (lane 5), wild-type NF-κB probe (lane 6), or mutant NF-κB probe (lane 7). Supershift assay was performed using antisera against p65 (lane 8), p50 (lane 9), or c-Rel (lane 10) subunits of NF-κB or a control antiserum (lane 11). Lower panel, expression of vFLIP does not affect the SP1 binding activity. B, retroviral-mediated expression of vFLIP leads to persistent NF-κB activation in 293T cells as measured by EMSA. C, increased NF-κB transcriptional activity in 293T cells with stable expression of vFLIP as measured by a luciferase-based reporter assay. 293T cells were transfected with an NF-κB/H9260 construct (75 ng/well) and a Rous sarcoma virus promoter-driven LacZ (β-galactosidase) reporter construct (75 ng/well), and the experiment was performed as described previously (65). The values shown are the averages of one representative experiment of two in which each transfection was performed in duplicate. D, Western blot analysis demonstrating increased phosphorylation (top panel) and decrease in the total IκBα protein (bottom panel) in H460 cells expressing vFLIP.

Fig. 3. HHV8 vFLIP has associated IKK activity. A, cellular extracts from H460 cells expressing empty vector, FLAG-vFLIP, or FLAG-MRIT/cFLIP were immunoprecipitated (IP) using a control antiserum (Con), FLAG (M2) antibody, or NEMO antibody and subjected to immune complex kinase assay using wild-type (wt) or mutant (mt) GST-IκBα (1-54) fusion proteins as substrates. The presence of vFLIP- associated IKK activity is demonstrated by the in vitro phosphorylation of wild-type but not mutant GST-IκBα in the lanes containing FLAG immunoprecipitate. In addition, immune complex kinase assay with the NEMO antibody demonstrates an increase in the total IKK activity in the vFLIP-expressing cells. B, Coomassie blue-stained gel demonstrating that equal amounts of wild-type and mutant GST-IκBα (1-54) substrate were used in each of the in vitro kinase reactions. FLAG-tagged vFLIP and MRIT/cFLIP were immunoprecipitated using a FLAG monoclonal or a control mouse antibody, and the nature of the coimmunoprecipitated proteins was determined by Western analysis. As shown in Fig. 4A, IKKα, IKKβ, and NEMO/IKKγ readily coimmunoprecipitated with vFLIP but were not detected in the immunoprecipitate of MRIT/cFLIP. In contrast, RIP was detected in the immunoprecipitates of both vFLIP and MRIT/cFLIP.

HHV8 vFLIP Physically Interacts with an ~700-kDa IKK Signalsome Complex—Previous studies have demonstrated that cytokine-induced IKK activity is present in a multiprotein signalsome complex of ~700 kDa (5, 6). To determine whether HHV8 vFLIP stimulates IKK activation by interacting with this large molecular mass complex, we compared the chromatographic distribution of vFLIP in extracts prepared from H460-vFLIP cells. A parallel experiment with cellular extracts prepared from H460-MRIT/cFLIP cells served as a negative control.
control. Following Superdex-200 fractionation of the above extracts, the column fractions were immunoprecipitated with FLAG (M2) monoclonal antibody. The immunoprecipitate was subsequently used for Western analysis with a rabbit polyclonal antibody against the FLAG tag to detect the presence of FLAG-tagged vFLIP or MRIT/cFLIP as well as in an in vitro kinase assay using GST-IκBα as a substrate. As shown in Fig. 5, A and B, the majority of vFLIP was found migrating between 600 and 700 kDa, which also correlated with the fraction containing the IKK activity. In contrast, MRIT/cFLIP was found migrating between 443 and 200 kDa (Fig. 5A).

We also examined the distribution of NEMO/IKK in the cell extracts prepared from vFLIP- and MRIT/cFLIP-expressing cells. Although the majority of NEMO/IKK was found migrating between 600 and 700 kDa, a smaller peak migrating near 450 kDa was detected in both cell lines (Fig. 6, A and B). As compared with MRIT-expressing cells, a relatively larger amount of NEMO/IKK in vFLIP-expressing cells was found in the ~700-kDa fraction. Because this fraction has been previously shown to contain the IKK activity (5, 6), these results suggest that expression of vFLIP leads to incorporation of NEMO/IKK into a constitutively active high molecular mass IKK complex. Finally, we examined the elution profile of IKKα and IKKβ in the cell extracts prepared from vFLIP-expressing cells using Western blot analysis as shown in Fig. 6C, both of the above kinases were found to coelute with vFLIP in the column fractions 8–10, thereby demonstrating that vFLIP coelutes with both the catalytic and regulatory subunits of the IKK complex.

Because the majority of vFLIP and the IKKs were detected in the same elution fractions, we next examined whether they are physically associated with each other. For this purpose, FLAG-tagged vFLIP or MRIT/cFLIP present in the various column fractions obtained following Superdex-200 fractionation were immunoprecipitated using the M2 FLAG antibody, and the presence of any associated NEMO/IKK was detected using Western blot analysis. As shown in Fig. 7A, NEMO/IKKα was found to coimmunoprecipitate with vFLIP in the column fractions 8–10, suggesting that the two proteins not only comigrate but are the components of the same IKK signalsome complex. Similarly, both IKKα and IKKβ were found to coimmunoprecipitate with vFLIP (see below). Consistent with previous results, no NEMO/IKKα was found to coimmunoprecipitate with MRIT/cFLIP (Fig. 7B).

We next examined the proportion of the IKKs that associate with vFLIP in various column fractions. For this purpose, various column fractions were immunoprecipitated with M2 FLAG antibody beads, and the amount of IKKs found to coimmunoprecipitate with the beads and the unbound fraction remaining in the supernatant was examined by Western blot analysis. As shown in Fig. 7C, significant proportions of IKKα, IKKβ, and NEMO/IKKγ were found to coimmunoprecipitate with vFLIP in the column fractions 8–10. A densitometry analysis revealed that 27 and 38% of IKKα, 36 and 42% of IKKβ, and 54 and 51% of NEMO/IKKγ were associated with vFLIP in the column fractions 8 and 9, respectively.

**NEMO/IKKγ Is Essential for HHV8 vFLIP-induced NF-κB**—We were next interested in demonstrating that the IKK complex not only associates with HHV8 vFLIP but is also essential for its ability to activate NF-κB. For this purpose we took advantage of the murine pre-B-cell lines 70Z/3 and 1.3E2, respectively. The 1.3E2 cell line is a NEMO-deficient mutant of 70Z/3 cells and has been previously shown to be incapable of activating NF-κB in response to multiple stimuli (39). We used retroviral-mediated gene transfer to generate stable clones of the above cell lines expressing empty vector
HHV8 vFLIP Associates with the IKK Complex to Activate NF-κB

**DISCUSSION**

KS is the most common malignancy found in the patients with HIV infection. The isolation of a novel gamma herpesvirus, designated HHV8, as a potential etiological agent for KS was a major step in understanding the pathogenesis of KS (40). HHV8 genomes have also been consistently found in patients with PEL, also known as body cavity-associated lymphoma, a rare form of B-cell lymphoma characterized by malignant pleural, pericardial, or peritoneal effusion in the absence of a tumor mass (41). In addition to KS and PEL, HHV8 genome has been detected in multicentric Castleman’s disease, angioimmunoblastic lymphadenopathy, and some cases of reactive lymphadenopathies (42–44).

Despite the increasing evidence linking the presence of KS-associated herpesvirus/HHV8 with KS and lymphoproliferative disorders, the mechanism by which this virus leads to a transformed phenotype is still unknown. In the present study, we have demonstrated that IκBa is persistently phosphorylated in the PEL cell lines and is associated with constitutive NF-κB activation in these cells. Because constitutive NF-κB activation has been previously implicated in cellular transformation seen in association with infection by Epstein-Barr virus and human T-cell leukemia virus type I (15), it may play a causative role in the pathogenesis of KS and HHV8-associated lymphoproliferative disorders as well.

We have discovered that stable expression HHV8 vFLIP in both hematopoietic and nonhematopoietic cell lines can lead to constitutive NF-κB activation. We would like to point out that although we have used a retroviral vector to express vFLIP, it did not result in the expression of abnormally high levels of this protein. On the contrary, vFLIP protein was undetectable in the cellular lysates using a highly sensitive Western blot analysis and could be detected only after severalfold concentration of the protein by immunoprecipitation. Furthermore, PEL cell lines are known to harbor multiple copies (50–100) of HHV8 genome (41, 45). Therefore, taken together, it is highly unlikely that the NF-κB activation observed in the present study is due to expression of abnormally high and supraphysiological levels of vFLIP protein. In addition to vFLIP, two other HHV8-encoded proteins have been shown to lead to NF-κB activation, i.e., K1 and viral G-protein-coupled receptor (vGPCR), respectively (46–50). However, among these proteins, only vFLIP is expressed in latently infected KS spindle and PEL cells (51–54), making it a prime candidate for the constitutive NF-κB activation observed in the PEL cell lines.

Our study suggests that persistent NF-κB activation seen in the PEL cell lines is due to constitutive phosphorylation of IκBa, a feature also seen in vFLIP-expressing cells. Inducible phosphorylation of IκBa at Ser-32 and Ser-37 followed by its destruction by the ubiquitin-proteasome-dependent pathway is a known mechanism for NF-κB activation by cytokines, such as TNF and interleukin-1. This signal-dependent phosphorylation of IκBa has been shown to be mediated by the activation of a ~700-kDa signalsome complex comprising IKKα, IKKβ, and IKKγ/NEMO (7–13). Our results suggest that HHV8 vFLIP may resemble human T-cell leukemia virus type I Tax protein, which has been also shown to lead to NF-κB activation by associating with and persistently activating the IKK complex (55–60).

The mechanism by which interaction of HHV8 vFLIP with the IKK complex results in persistent increase in IKK activity remains to be determined. It is conceivable that HHV8 vFLIP recruits an upstream kinase to the IKK complex. For example,
we have demonstrated that in addition to the various IKKs, HHV8 vFLIP also interacts with RIP, a protein kinase known to be crucial for TNFα-mediated NF-κB activation. RIP in turn may recruit and activate NF-κB-inducing kinase or mitogen-activated protein kinase kinase kinase, which are known to activate the IKK complex (61–64). Studies to address the role of RIP, NF-κB-inducing kinase, and mitogen-activated protein kinase kinase in vFLIP-induced NF-κB activation are currently in progress.

Acknowledgments—We thank Dr. Richard Gaynor’s laboratory for assistance with the gel filtration experiment, Dr. John Minna and Carol Sibley for cell lines, and the National Cell Culture Center (Minneapolis, MN) for large scale culture of H460 cells expressing vFLIP and MRIF/vFLIP.

REFERENCES
1. Baeuerle, P. A., and Baltimore, D. (1996) Cell 87, 13–20
2. Baeuerle, P. A., and Baichwal, V. R. (1997) Adv. Immunol. 65, 111–137
3. Baichwal, V. R., and Baeuerle, P. A. (1997) Curr. Biol. 7, 894–896
4. Baldwin, A. S., Jr. (1996) Annu. Rev. Immunol. 14, 649–683
5. Mercurio, F., Young, D. B., and Manning, A. M. (2000) Methods Mol. Biol. 99, 105–129
6. Karin, M., and Delhase, M. (2000) Semin. Immunol. 12, 85–98
7. Regnier, C. H., Song, H. Y., Gao, X., Goeddel, D. V., Cao, Z., and Rothe, M. (1999) Cell 96, 373–383
8. Wroniecki, J. D., Gao, C., Cao, Z., Rothe, M., and Goeddel, D. V. (1997) Science 278, 866–869
9. DiDonato, J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E., and Karin, M. (1997) Nature 388, 548–554
10. Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J., Young, D. B., Barbas, M., Mann, M., Manning, A., and Rao, A. (1997) Science 278, 860–866
11. Zandi, E., Rothwarf, D. M., Delhase, M., Hayakawa, M., and Karin, M. (1997) Cell 91, 243–252
12. Yamashita, S., Courtois, G., Bessia, C., Whiteside, S. T., Weil, R., Agou, F., Kirk, H. E., Kay, R. J., and Israel, A. (1998) Cell 93, 1231–1240
13. Rothwarf, D. M., Zandi, E., Natoli, G., and Karin, M. (1998) Nature 395, 297–300
14. Laque, I., and Gelinna, C. (1997) Semin. Cancer Biol. 8, 103–111
15. Mestallos, G. (1997) Semin. Cancer Biol. 8, 121–129
16. Boldin, M. P., Goncharov, T. M., Goltsev, Y. V., and Wallach, D. (1996) Curr. Opin. Immunol. 8, 253–260
17. Baeuerle, P. A., and Baichwal, V. R. (1997) Mol. Cell. Biol. 17, 1414–1419
18. Chang, Y. C., Epp, E., Zhou, X., Zhu, X., Zhang, X., and Wallach, D. (1996) Nat. Immunol. 4, 62–73
19. Boldin, M. P., Goncharov, T. M., Goltsev, Y. V., and Wallach, D. (1996) J. Biol. Chem. 271, 1475–1482
20. Boldin, M. P., Goncharov, T. M., Goltsev, Y. V., and Wallach, D. (1996) Science 275, 860–869
21. Han, D. K., Chaudhary, P. M., Wright, M. E., Friedman, C., Trask, B. J., Schwartz, S. M., and Hood, L. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5915–5921
22. Sudeith, K. A., Akira, S., Gresser, I., Lam, D. Y., and Goeddel, D. V. (2001) Curr. Opin. Immunol. 13, 602–608
The Human Herpes Virus 8-encoded Viral FLICE Inhibitory Protein Physically Associates with and Persistently Activates the IκB Kinase Complex
Li Liu, Michael T. Eby, Nisha Rathore, Suwan K. Sinha, Arvind Kumar and Preet M. Chaudhary

J. Biol. Chem. 2002, 277:13745-13751.
doi: 10.1074/jbc.M110480200 originally published online February 5, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M110480200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 65 references, 36 of which can be accessed free at http://www.jbc.org/content/277/16/13745.full.html#ref-list-1