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The Latent Membrane Protein 1 of Epstein-Barr Virus (EBV) Primes EBV Latency Cells for Type I Interferon Production

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Abstract

Epstein-Barr virus (EBV) latency has been associated with a variety of human cancers. Latent membrane protein 1 (LMP-1) is one of the key viral proteins required for transformation of primary B cells in vitro and establishment of EBV latency. We have previously shown that LMP-1 induces the expression of several interferon (IFN)-stimulated genes and has antiviral effect (Zhang, J., Das, S. C., Kottakul, C., Pattanaik, A. K., and Zhang, L. (2004) J. Biol. Chem. 279, 46335–46342). In this report, a novel mechanism related to the antiviral effect of LMP-1 is identified. We show that EBV type III latency cells, in which LMP-1 is expressed, are primed to produce robust levels of endogenous IFNs upon infection of Sendai virus. The priming action is due to the expression of LMP-1 but not EBV nuclear antigen 2 (EBNA-2). The signaling events from the C-terminal activator regions of LMP-1 are essential to prime primed cells for high IFN production. LMP-1-mediated activation of NF-κB is apparently necessary and sufficient for LMP-1-mediated priming effect in DG75 cells, a human B cell line. IFN regulatory factor 7 (IRF-7) that can be activated by LMP-1 is also implicated in the priming action. Taken together, these data strongly suggest that LMP-1 may prime EBV latency cells for IFN production and that the antiviral property of LMP-1 may be an intrinsic part of EBV latency program, which may assist the establishment and/or maintenance of viral latency.

Abbreviations: EBV, Epstein-Barr virus; LMP-1, latent membrane protein 1; HA, hemagglutinin; IFN, interferon; IRF-7, IFN regulatory factor 7; NF-κB, nuclear factor κB; IκB, inhibitor κB; ISG, IFN-stimulated gene; FBS, fetal bovine serum; RPA, RNase protection assay; BL, Burkitt’s lymphoma; EBNA, EBV nuclear antigen; CTAR, C-terminal activator region; GADD45, glyceraldehyde-3-phosphate dehydrogenase; ELISA, enzyme-linked immunosorbent assay.

Introduction

Epstein-Barr virus (EBV) is a human γ-herpesvirus and is an important cause of lymphomas in patients with advanced human immunodeficiency virus infection or AIDS and in severely immunocompromised people, especially organ transplant recipients. Also, EBV infection is associated with the development of nasopharyngeal carcinoma and Burkitt’s lymphoma (BL) (1, 2).

The biologic hallmark of EBV-cell interaction is latency. Three types of latency have been described, each having its own distinct pattern of gene expression. Type I latency is exemplified by BL tumors in vivo. EBV nuclear antigen 1 (EBNA-1) protein is expressed in this form of latency. Type II latency is exemplified by nasopharyngeal carcinoma and Hodgkin disease. EBNA-1, latent membrane protein 1 (LMP-1), and LMP2A and LMP2B proteins are expressed in type II latency. Type III latency is represented by lymphoblastoid cell lines. Nine viral proteins are expressed, including six nuclear proteins (EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, and EBNA-LP) and three integral membrane proteins (LMP-1, LMP-2A, and LMP-2B) (reviewed in references 1 and 2).

EBV LMP-1 is the principal oncoprotein required for EBV transformation of human B cells and establishment of latency in vitro. LMP-1 is an integral membrane protein with six transmembrane-spanning domains with a long C-terminal domain, which is located in the cytoplasm (2, 3). Two C-terminal activator regions (CTARs) have been identified to initiate signal transduction. LMP-1 acts as a constitutively active receptor-like molecule that does not need the binding of a ligand (4). LMP-1 appears to be a central effector of altered cell growth, survival, adhesive, invasive, and antiviral potential (5–9).

Type I interferons (IFNs) are cytokines with many functions including antiviral and anti-proliferation (10, 11). IFNs are produced upon the infection of cells by viruses. There are a large number of type 1 IFN genes in the human: 13 IFN-α genes, 1 IFN-β gene, and 1 IFN-ω gene (12). It is not known now why there are so many type 1 IFN genes. However, a unique role for IFN-β for a fully effective antiviral response, which cannot be compensated by IFN-α, has been documented (13).

The amount of IFNs produced by viruses or double-stranded RNA can be increased robustly by treating cells with IFN before infection, a phenomenon known as IFN priming (14–16). The stimulation of IFN production observed upon priming results from an increase in the rate of IFN gene transcription and requires an IFN-inducible factor(s) (17, 18).

The mechanism of the transcriptional activation of IFNs has been under intensive investigation. One of the major players in the IFN production is IFN regulatory factor 7 (IRF-7). IRF-7 was cloned in the context of EBV latency and has an intimate relation with EBV (19–25). IRF-7 is inducible by type I IFNs and can be further activated by phosphorylation and nuclear translocation upon viral infection, and activated IRF-7 is partially responsible for the robust transcriptional activation of IFNs (23, 26–29).

Previously, we have shown that LMP-1 induces several antiviral IFN-stimulated genes (ISGs) without triggering IFN production and that LMP-1 possesses antiviral activity (30). In this report, we extend our earlier discovery and show that EBV latency cells have enhanced ability to produce IFN upon viral superinfection. We further show that LMP-1 primes EBV latency cells for IFN production, similar to IFN primed cells. To our knowledge, this is the first report that a viral latent gene primes cells for IFN production.

Experimental Procedures

Plasmids, Antibodies, and Viruses—Expression plasmids of LMP-1 and its signaling-defective mutant, LMP-DM, as well as IRF-7 and its DNA-binding mutant, IRF7-K92E, were described previously (24, 31). Expression plasmid of EBNA-2 (pAG155) was a gift from Dr. Paul Ling. NF-κB-related plasmids p65 and p50, IκB ex-
expression plasmids, plus NF-κB reporter construct were gifts from Dr. Albert Baldwin. EBNA-2 antibody (PE2) and LMP-1 antibody (CS1–4) were purchased from Dako. STAT-1 antibody was purchased from Santa Cruz Biotechnology. Tubulin antibody was purchased from Sigma. Anti-Sendai virus antibody was purchased from U. S. Biological. Recombinant human IFN-α was purchased from Schering-Plough. Sendai virus stock was purchased from Spafas, Inc. For virus infection, 200 HA units/ml Sendai virus were added to the target cells for 6 h, and cells were then collected for RNA isolation. However, 7-h infection was used for examining the endogenous IFN production in the media.

Cell Culture, Transient Transfection, and Isolation of Transfected Cells—DG75 is an EBV-negative Burkitt’s lymphoma cell line (32). BL41 is an EBV-negative BL line, and BL41-EBV was generated by in vitro infection of BL41 with EBV B95-8 strain (33). Sav I and Sav III are genetically identical cell lines that differ only in their latency types (34). Akata (type I) and IB4 (Type III) are EBV-positive cell lines. These cells were maintained in RPMI 1640 plus 10% fetal bovine serum (FBS). 293 cells are human fibroblasts and are maintained in Dulbecco’s modified Eagle’s medium plus 10% FBS. Electroporation (320 V; 925 microfarads) was used for transfection of the B cells as described previously (20, 21, 24). A total 10 µg of DNA was used for transfection of DG75 cells. 1 µg of LMP-1-expression plasmids were always used in transfection because similar LMP-1 expression levels in transfected and EBV type III latency cells could be achieved under such conditions. Enrichment for CD+4-positive cells was performed with the use of anti-CD-4-antibody conjugated to magnetic beads according to the manufacturer’s recommendation (Dynal, Inc.). DG75 cells were transfected with CD-4 expression and other plasmids. One day after the transfection, the cells were used for isolation of CD-4-positive cells with the use of Dynabeads CD4 (Dynal Inc.)

The transfected cells were incubated with Dynabeads-CD4 at 72 µl of beads/107 cells for 20–30 min at 4 °C with gentle rotation. CD4-positive cells were isolated by placing the test tubes in a magnetic separation device (Dynal magnet). The supernatant was discarded while the CD4-positive cells were attached to the wall of the test tube. The CD4-positive cells were washed 4–5 times in phosphate-buffered saline plus 2% FBS and resuspended in 100 µl of RPMI 1640 plus 1% FBS. Cells were detached from the Dynabeads CD4 by incubating for 45–60 min at room temperature with 10 µl of DETACHaBEAD (Dynal). The detached beads were removed by using the magnet separation device. The released cells were washed 2–3 times with 500 µl of RPMI 1640 plus 10% FBS and resuspended in RPMI 1640 plus 10% FBS at 5 x 105 cells/ml. The isolated cells were used to extract total RNAs or prepare cell lysates immediately or recovered overnight before infection by viruses.

Western Blot Analysis with Enhanced Chemiluminescence (ECL)—Separation of proteins on SDS-PAGE was carried out following standard protocol. After the proteins were transferred to a nitrocellulose or Immobilon membrane, the membrane was blocked with 5% nonfat dry milk in TBST (50 mM Tris-HCl, pH 7.5, 200 mM NaCl, 0.05% Tween 20) at room temperature for 10 min. It was then washed briefly with TBST and incubated with the primary antibody in 5% milk in TBST for 1 h at room temperature or overnight at 4 °C. After washing with TBST three times (10 min each), the membrane was incubated with the secondary antibody at room temperature for 1 h. It was then washed three times with TBST, treated with ECL detection reagents (Amersham Biosciences), and exposed to Kodak XAR-5 film.

RNA Extraction, RNase Protection Assays (RPA), and Reporter Assays—Total RNA was isolated from cells using the RNAsesy total RNA isolation kit (Qiagen, Valencia, CA) or TRIzol extraction. RPA was performed with 10 µg of total RNA using the RNase protection assay kit II (Ambion, Houston, TX) at 55 °C. Sometimes, gradient temperatures were performed for RPA when difficulties in RPA were encountered (35). The GAPDH probe was from U. S. Biochemicals. The probe for IFN-β was a gift from Dr. Ganes Sen. The luciferase reporter assays were performed using the assay kit from Promega according to manufacturer’s recommendation.

Removal of Sendai Virus from Culture Medium—The removal of Sendai virus was achieved by the use of 15-nm Planova virus removal filters (gifts from Asahi Kasei, Pharma Corp.). Seven hours after Sendai virus infection, cell culture media were collected and passed through the Planova filters following the manufacturer’s protocol. To test whether the filtered media contain Sendai viruses, the filtered conditional media were used to treat fresh BL41 cells, and the cell lysates were made 24 h later. Western blot with anti-Sendai antibody was used to detect any Sendai proteins in cell lysates. Sendai virus-treated (20 HA units/ml) cell lysates were used as positive controls.

IFN-α Measurement and Functional Assay—The concentration of IFN-α was determined by a commercially available human interferon-α (Hu-IFN-α) ELISA kit (PBL Biomedical Laboratories; catalog number 41100) according to the manufacturer’s recommendations. The kit is able to detect human IFN-α, IFN-α2, IFN-αA/D, IFN-αd, IFN-αk, and IFN-α4b. However, it cannot detect IFN-β, IFN-ω, and other IFN-α subtypes. Samples were examined in triplicates. To test the functions of IFNs in cell culture media, the conditional media were first passed through a 15-nm filter to remove Sendai viruses. These media were then used to treat fresh BL41 cells at desired dilutions for 24 h, and cell lysates were used for the detection of STAT-1 expression. The human IFN-α neutralization antibody (RDI-PB31130) was purchased from Fitzgerald Industries Intl. The antibody is able to react with several kinds of IFN-α subtypes but not IFN-β and IFN-ω. 100,000 neutralization units/ml were added to BL41 cells along with suitable concentration of conditional media and incubated for 24 h. The expression of STAT-1 in BL41 cells was then measured by Western blot analyses.

Results

EBV Type III Latency Cells Have Higher Capacity to Produce IFN-β—It is well known that EBV type III latency expresses high levels of IRF-7 and other ISGs, similar to IFN-treated cells. Because IFN can prime the cell for robust IFN production, whether these EBV latency cells are primed for higher IFN production upon viral superinfection was examined. BL41 is an EBV-negative Burkitt’s lymphoma line, and BL41-EBV is its EBV-infected derivative with type III latency. Sav I (type I latency) and Sav III (type III latency) are genetically identical sister cell lines that differ only in their types of latency. These two pairs of cell lines were used to address the presence of EBV type III latency on the production of IFN. Sendai virus was used to infect these cells, and total RNA was isolated 6 h after infection. RPA experiments were used for the detection of IFN-β RNA production. As shown in Figure 1A, whereas BL41 and Sav I cells had little or no detectable IFN-β production, high IFN-β RNAs were observed in BL41-EBV and Sav III cells, both of which are type III latency cells with LMP-1 expression (Figure 1B). We also tested IFN-β production in Akata (type I latency) and IB4 (type III latency) cells upon Sendai infection. Although IB4 could produce high levels of IFN-β, Akata cells failed to produce a significant amount (data not shown). These data suggest that the induction of IFN-β upon viral infection is enhanced in EBV type III latency cells.

EBV Type III Latency Cells Produce Functional Type I IFNs upon Superinfection—Type I IFNs are a family of IFN-α, one IFN-β, and one IFN-ω. However, using IFN-β as an indicator for type I IFN
production is well established and appreciated in the field (26, 28, 29, 31). Thus, the results in Figure 1 suggest that type I IFNs were robustly induced in EBV latency cells. Because IFN-β RNA is synthesized in EBV-infected cells and because EBV and other herpesviruses encode genes to shut off host gene expression (36–40), it is thus necessary to examine whether IFN proteins are synthesized and secreted properly.

ELISA was used for the detection of IFN-α proteins in the culture media. As shown in Figure 2A, the Sendai virus-infected BL41-EBV cells produced huge amount of IFN-α. However, uninfected cells and infected BL41 cells produced undetectable levels of IFN-α, which was in agreement with the data in Figure 1. These results indicate that IFNs are properly synthesized and secreted from EBV latency cells. Whether these secreted IFNs were functional was tested. The media from Sendai virus-infected or -uninfected cells were collected and passed through 15-nm filters. It is well established that a 15-nm filter is able to eliminate Sendai viruses (41). We confirmed that the filtered media did not contain detectable Sendai viruses as determined by viral protein expression (data not shown). These conditional media were then used to treat fresh BL41 cells with various dilutions and to determine the expression of STAT-1 24 h later. The reasons to use STAT-1 as an indicator of IFN functions are because STAT-1 is highly inducible by IFN and Sendai virus blocks IFN signaling (42–45). Therefore, a successful induction of STAT-1 may suggest the presence of functional IFNs and absence of Sendai viruses. As shown in Figure 2B, the conditional medium from BL41-EBV-Sendai infected cells was ~10-fold more potent than that from BL41-infected cells for induction of STAT-1. The apparent discrepancy between Figure 2A (column 2) and 2B (lanes 3–6) might be due to the fact that the ELISA kit only detects a subset of IFN-α (see “Experimental Procedures” for details) and/or that viral superinfection has produced other secretable factors that are capable of inducing STAT-1. Of note is the fact that LMP-1 has been shown to induce STAT-1. Therefore, type III latency cells had produced functional IFNs, and IFN-β RNA was a good indicator for the robust induction of STAT-1. Therefore, type III latency cells had produced functional IFNs, and IFN-β RNA was a good indicator for the robust induction of STAT-1. Figure 2C shows that the human IFN-α neutralization antibody partially inhibited the induction of STAT-1. Therefore, type III latency cells had produced IFNs without detectable Sendai viruses, as determined by viral protein expression (data not shown). These conditional media were then used to treat fresh BL41 cells with various dilutions and to determine the expression of STAT-1 24 h later. The reasons to use STAT-1 as an indicator of IFN functions are because STAT-1 is highly inducible by IFN and Sendai virus blocks IFN signaling (42–45). Therefore, a successful induction of STAT-1 may suggest the presence of functional IFNs and absence of Sendai viruses. As shown in Figure 2B, the conditional medium from BL41-EBV-Sendai infected cells was ~10-fold more potent than that from BL41-infected cells for induction of STAT-1. The apparent discrepancy between Figure 2A (column 2) and 2B (lanes 3–6) might be due to the fact that the ELISA kit only detects a subset of IFN-α (see “Experimental Procedures” for details) and/or that viral superinfection has produced other secretable factors that are capable of inducing STAT-1. Of note is the fact that LMP-1 has been shown to induce STAT-1. Therefore, type III latency cells had produced functional IFNs, and IFN-β RNA was a good indicator for the robust type I IFN production in this system. The data in Figures 1 and 2 collectively suggest that EBV type III latency cells are primed for type I IFN production.

**Figure 1.** EBV latency cells are primed to produce robust IFN-β upon superinfection. A, EBV type III latency cells produce higher levels of IFN-β upon superinfection of Sendai virus. BL41, BL41-EBV, Sav I, and Sav III cells were infected by Sendai virus (200 HA units/ml) for 6 h. Total RNAs were isolated and used for RPA with IFN-β and GAPDH probes. Yeast RNA was used as negative control. Specific protections of IFN-β and GAPDH RNAs are indicated. B, expression of LMP-1 in EBV latency cells. Lysates from BL41, BL41-EBV, Sav I, and Sav III were used for Western blot analysis with LMP-1 and tubulin antibodies. The identity of proteins is as shown. +/-, with or without virus infection.

**Figure 2.** IFNs produced by superinfection of EBV latency cells are functional. A, IFNs are secreted into the culture media. BL41 and BL41-EBV cells were infected by Sendai virus (200 HA units/ml) for 7 h. The cell culture media were collected, and the concentrations of IFN-α were measured with the use of ELISA. Standard deviations are shown. +/-, with or without Sendai virus infection. B, the conditional media from EBV type III latency cells infected by Sendai virus induces higher levels of STAT-1. BL41 and BL41-EBV were infected with (+) or without (−) Sendai (200 HA units/ml) for 7 h. Cell culture media were collected, and Sendai viruses were removed by passing through 15-nm Planova virus removal filters. The filtered conditional media were used to treat fresh BL41 cells at indicated dilution factors. After 24 h, cell lysates were prepared and used for Western blot analyses with STAT-1 and tubulin antibodies. The identity of proteins is as shown. C, IFN-α is involved in the induction of STAT-1. BL41 cells were treated with the conditional media (1:100, from BL41-EBV cells superinfected with Sendai), the neutralization antibody (Ab) of IFN-α, and the same amounts of normal rabbit serum (NRS) in various combinations as shown on the top. The expression of STAT-1 was examined 24 h later. The identity of proteins is as shown.
NF-κB Is Necessary and Sufficient for LMP-1-mediated Priming in DG75 Cells—Next, the roles of intracellular molecules involved in LMP-1-mediated priming action were examined. Because both LMP-1 CTARs can activate NF-κB and because NF-κB plays a pivotal role in the viral latency, whether NF-κB is involved in LMP-1-mediated priming was examined. As shown in Figure 5A, LMP-1 alone primed cells for high IFN production. However, in the presence of IκB, the priming effect of LMP-1 was completely abolished. IκB was able to block LMP-1-mediated activation of NF-κB (data not shown).

Because overexpression of NF-κB is capable of induction of IRF-7 in DG75 cells, we asked whether NF-κB activation alone might be contributing to the priming process of LMP-1. As shown in Figure 5A, overexpression of NF-κB (p65 + p50) was sufficient to prime cells for IFN production in DG75 cells. The activation of NF-κB by p65 and p50 was confirmed by NF-κB reporter assays (data not shown). Thus, the data in Figure 5 suggest that NF-κB is involved in LMP-1-mediated priming action.

IRF-7 May Be a Factor Involved in the Induction—Because LMP-DM failed to induce IRF-7 and NF-κB is required for induction of IRF-7 (24), the data in Figures 4 and 5 also suggest the potential involvement of IRF-7 in the priming process of LMP-1. In addition, the essential role of IRF-7 in type I IFN production has been well established (26–28, 50). IRF-7 and its DNA-binding mutant (IRF-7K92E) were used for transfection, and their roles in LMP-1-mediated priming action were examined. As shown in Figure 6, whereas wild-type IRF-7 was capable of priming the transfected cells, IRF-7 DNA-binding mutant (IRF-7K92E) failed to prime the cells. These data suggest that induction of IRF-7 by LMP-1 may be an important step in priming cells for IFN production.

There are several viral genes expressed in EBV type III latency. However, because LMP-1 induces IRF-7 and other ISGs and has antiviral effect without inducing IFNs (20, 24, 30, 47) and because IRF-7 is a master gene for IFN production (50), LMP-1 apparently is a good candidate for the priming action. In addition, EBNA-2, the primary inducer of LMP-1 (51–54), might play a role for the priming of the latency cells. DG75 cells, which are EBV-negative Burkitt’s lymphoma cells, were used for the experiments because of transcription efficiency. Vector, LMP-1, or EBNA-2 and a CD4 expression plasmid were transfected into cells. Transfected cells were enriched and split into two wells, one of which was infected by viruses. As shown in Figure 3A, LMP-1 caused a marked increase in IFN-β RNA levels in DG75 cells; however, EBNA-2 did not enhance the expression. The expression of EBNA-2 is confirmed by Western blot (data not shown). Therefore, LMP-1, but not EBNA-2, is probably responsible for the priming effect of type III latency cells. In addition, if EBNA-2 is involved in the priming, it is likely to do so indirectly via induction of LMP-1 in EBV-infected cells.

It is of note that the LMP-1-mediated priming effect is similar to IFN priming (Figure 3B). However, due to the fact that LMP-1 did not induce IFN in DG75 cells in our system (30), the priming effect must be due to gene(s) regulated by LMP-1.

Signaling Derived from LMP-1 Is Required for the Priming Effect—LMP-1 is an integral membrane protein with two regions in the C terminus (CTARs) that have been shown to initiate signaling processes including the activation of NF-κB and IRF-7. LMP-DM is a mutant of LMP-1 in both CTARs that fails to activate NF-κB and IRF-7 (24). LMP-1 or LMP-DM and a CD4 expression plasmid were transfected into DG75 cells, and the priming effect of LMP-1 was examined. As shown in Figure 4, whereas LMP-1 caused a marked increase in IFN-β RNA, LMP-DM seemed to have no effect. The expression of LMP-1 proteins was confirmed (Figure 4B). Thus, signaling from LMP-1 CTARs is required for the priming action.

NF-κB Is Necessary and Sufficient for LMP-1-mediated Priming in DG75 Cells—Next, the roles of intracellular molecules involved in LMP-1-mediated priming action were examined. Because both LMP-1 CTARs can activate NF-κB and because NF-κB plays a pivotal role in IFN priming (Figure 3B), LMP-1-expressing cells can robust IFN production upon viral infection. DG75 cells were transfected with pcDNA3, LMP-1, or EBNA-2 expression plasmids. The transfected cells were isolated and equally split into two wells; one well of the cells was infected with Sendai virus for 6 h. Total RNA were isolated and used for RPA. Yeast RNA was used as negative control. Specific protections of IFN-β and GAPDH RNAs are indicated. +/−, with or without virus infection. B, IFN primes DG75 cells. DG75 were treated with IFN-α (200 IU/ml) overnight, and the cells were infected by Sendai virus for 6 h. Total RNA were isolated and used for RPA. +/−, with or without virus infection.
Discussion

It is well established that IFN primes cells for robust IFN production upon viral infection. In this report, we show that EBV type III latency cells are also primed for robust IFN production (Figures 1 and 2). Because current evidence suggests that EBV latency cells cannot produce type I IFNs, the priming effect of type III latency cells must be due to viral protein(s) expressed during latency. We further identify EBV LMP-1 as the gene to prime cells for robust IFN production in EBV-infected B lymphocytes.

The mechanisms of IFN production upon viral infection are under intensive investigation. However, the mechanism for IFN priming is not yet well understood. The stimulation of IFN production observed upon priming is known from an increase in the rate of IFN gene transcription and requires an IFN-inducible factor(s) (17, 18). It seems likely that IRF-7 is an important factor in priming action of IFN because IRF-7 is inducible by IFN and IRF-7 is responsible for the robust and wide variety of type I IFN production (23, 55). It is of note that there is a striking similarity between IFN-primed and LMP-1-expressing cells: the high expression levels of ISGs including IRF-7. It is interesting that although LMP-1 is able to activate IRF-7, NF-xB, and ATF-2, three critical factors for type I IFN production, LMP-1 apparently could not activate type I IFN production in viral latency but selectively induces ISGs (20, 24, 30).

It is well known that NF-xB is an essential factor in type I IFN production (10, 11). The activation of NF-xB by LMP-1 is clearly involved in LMP-1-mediated priming action, at least in DG75 cells. LMP-DM failed to activate NF-xB and failed to prime cells. Blocking LMP-1-mediated NF-xB activation by IκB expression eliminated the priming function. NF-κB alone was able to induce the priming state. Thus, available data strongly suggest that NF-xB is necessary and sufficient for LMP-1-mediated priming effect in DG75 cells (Figure 5).

The induction of ISGs, or IRF-7 in particular, may also be one of the molecular bases for the priming property of EBV latency cells. First, there is a great deal of correlative data to suggest that high IRF-7 is associated with primed latency cells. IRF-7 is highly expressed in EBV type III latency cells, such as in BL41-EBV and Sav III (19, 20). The high levels of IRF-7 in viral latency is primarily due to the expression of LMP-1 (20, 24). In DG75 cells, LMP-1 is able to induce IRF-7 and is able to prime cells for IFN production; however, LMP-DM, which failed to induce the expression of IRF-7 (24), was unable to significantly induce IFN-β (Figure 4). Also, in the human 293 fibroblast cell line, LMP-1 failed to induce the expression of IRF-7 and failed to enhance IFN production upon viral infection (data not shown). Second, we have data to suggest that NF-xB is necessary and sufficient to induce priming action in DG75 cells (Figure 5). However, NF-xB alone is able to induce IRF-7 in DG75 cells, and LMP-1-mediated induction of IRF-7 also requires NF-xB (24). Thus, it is reasonable to suspect that IRF-7 is also involved in NF-xB-mediated priming processes. Third, ectopic expression of IRF-7 alone is sufficient to achieve the higher levels of IFN production (Figure 5).
EBV has both latency and lytic replication in its life cycle. There is a constant battle between host and EBV in native environments. The success of EBV is likely dependent on its ability to keep latencies under a variety of environments in vivo. By establishing latencies, EBV can apparently escape host immune surveillance under various conditions. EBV latency can be disrupted into lytic replication by some chemical or physiological factors, including viral superinfection (30). It is well known that IFNs inhibit viral replications, including herpesviruses (61–68). Thus, the inhibition of EBV lytic replication by IFNs is anticipated, and we did find that IFN-α was capable of inhibiting lytic replication of EBV (data not shown). EBV latency cells in vivo may also be under attacks from other viruses, the process of which may lead to lytic replications and disruption of EBV latency. Although we have used Sendai virus in this report, the robust induction of IFN by Sendai would suggest that other viruses may also behave similarly. IFN would be among the first genes of EBV latency. Although we have used Sendai virus in this report, the robust induction of IFN by Sendai would suggest that other viruses may also behave similarly. IFN would be among the first genes to be synthesized and released upon viral superinfection. However, EBV lytic replication may take 24–48 h to complete. Therefore, high levels of IFNs from LMP-1-mediated priming would have opportunities to preserve EBV latency in two fronts: 1) to prevent the viral superinfection and 2) to inhibit EBV lytic replication itself. However, if IFNs fail to preserve latency, EBV enters lytic replication and is able to use its lytic genes to block IFN functions. For example, EBV BZLF1 is able to block the functions of IFR-7 as well as STAT1 (69, 70). Finally, it is interesting to point out that EBV latency cells seem to be insensitive to the anti-proliferation effect of IFN, possibly due to the expression of EBNA-2 (71). Thus, IFN might inhibit EBV lytic replication without affecting the growth properties of EBV type III latency.

In summary, our results indicate that LMP-1 primes viral latency cells for the production of type I IFNs upon viral superinfection and facilitates the antiviral effect of LMP-1. This report and our previous one (30) strongly suggest that LMP-1-mediated antiviral effect is likely to be an intrinsic property of EBV latency program, and this antiviral property may assist the establishment and/or maintenance of EBV latency.

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