Inhibition of IFN-γ Signaling by an Epstein-Barr Virus Immediate-Early Protein

Thomas E. Morrison,[1,2] Amy Mauser,[1]
Athena Wong,[1,4] Jenny P.-Y. Ting,[1,3,4]
and Shannon C. Kenney[1,2,3,5]
[1] Lineberger Comprehensive Cancer Center
[2] Department of Microbiology and Immunology
[3] Department of Medicine
[4] Curriculum in Genetics
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina 27599

Summary

Viruses have evolved elaborate mechanisms to target many aspects of the host's immune response. The cytokine IFN-γ plays a central role in resistance of the host to infection via direct antiviral effects as well as modulation of the immune response. In this study, we demonstrate that the Epstein-Barr virus (EBV) immediate-early protein, BZLF1, inhibits the IFN-γ signaling pathway. BZLF1 decreases the ability of IFN-γ to activate a variety of important downstream target genes, such as IRF-1, p48, and CIITA, and prevents IFN-γ-induced class II MHC surface expression. Additionally, BZLF1 inhibits IFN-γ-induced STAT1 tyrosine phosphorylation and nuclear translocation. Finally, we demonstrate that BZLF1 decreases expression of the IFN-γ receptor, suggesting a mechanism by which EBV may escape antiviral immune responses during primary infection.

Introduction

Epstein-Barr virus (EBV) is a ubiquitous human γ herpesvirus that is the causative agent of infectious mononucleosis (IM) (Kieff, 1996; Rickinson and Kieff, 1996). EBV is also associated with several B cell and epithelial cell malignancies, including Burkitt's lymphoma and nasopharyngeal carcinoma (Rickinson and Kieff, 1996). During primary infection, EBV initially undergoes lytic replication in the oropharyngeal epithelium (Cohen 2000; Kieff, 1996; Li et al., 1992; Sixby et al., 1984). The virus subsequently infects trafficking B cells where it converts to a latent infection. Lytic replication is initiated by the expression of two immediate-early (IE) genes, *BZLF1* and *BRLF1* (Chevallier-Greco et al., 1986; Countryman and Miller, 1985; Ragoczy et al., 1998; Rooney et al., 1989; Takada et al., 1986; Urier et al., 1989; Zalani et al., 1996). The protein products of *BZLF1* and *BRLF1* function as transcriptional transactivators and induce the lytic cascade of viral gene expression (Chang et al., 1990; Cox et al., 1985; Flemington and Speck, 1990, Flemington et al., 1992; Hardwick et al., 1988, 1992; Holley-Guthrie et al., 1990; Kenney et al., 1989; Lieberman et al., 1989; Quinlivan et al., 1993; Rooney et al., 1989). Infection by EBV results in both humoral and cellular

immune responses. However, cell-mediated responses are believed to be more important in the control of EBV infection (Cohen, 2000). Elevated levels of activated CD8+ cytotoxic T cells, natural killer (NK) cells, and CD4+ T cells have been observed during acute EBV infection (Tomkinson et al., 1987). The serum levels of IFN-γ are increased during the acute phase of infectious mononucleosis (IM) (Hornef et al., 1995; Linde et al., 1992), and increased IFN-γ and tumor necrosis factor (TNF) expression have been observed in tonsil sections from IM patients (Andersson et al., 1994). However, EBV establishes a life-long relationship with the infected person, maintaining a persistent latent infection within the B cell population, and undergoing periodic spontaneous reactivation resulting in lytic replication, reinfection of the oral epithelium, and shedding of virus into the saliva (Cohen, 2000: Kieff, 1996; Rickinson and Kieff, 1996). This suggests that the virus has evolved mechanisms to evade host immune system responses.

Primary infection by viruses is countered initially by the host's innate immune responses, followed by adaptive-immune responses. Components of the innate response include phagocytic cells, natural killer cells, and cytokines such as the interferons. Adaptive responses involve proliferation of antigen-specific T cells and B cells. As part of innate and adaptive immune responses, the type I (IFN-α/β) and type II (IFN-γ) interferons are major lines of defense against viral infection. IFN-α/β and IFN-γ induce expression of cellular proteins, such as protein kinase R and 2′-5′-oligoadenylate synthetase, which elicit direct antiviral effects (Boehm et al., 1997; Stark et al., 1998). IFN-α/β and IFN-γ both upregulate MHC class I expression. In addition, IFN-γ strongly induces MHC class II expression on non-APC cells such as endothelial cells and fibroblasts (Collins et al., 1984; Pober et al., 1983). IFN-α/β is produced by virally infected cells, whereas IFN-γ is produced by immune system cells that function in early immune responses such as natural killer cells, natural killer T cells, and γ/δ T cells, as well as immune system cells activated during adaptive responses such as Th1 cells and CD8+ cells (Biron, 1999).

Viruses have evolved elaborate strategies to evade detection and destruction by host immune responses, including targeting pathways for antigen presentation, programmed cell death, and cytokine- and chemokine-mediated signaling (Tortorella et al., 2000). There is increasing evidence that lytic cycle proteins of herpesviruses play a role in evading host immune responses, including disruption of interferon signaling. For example, cytomegalovirus (human and murine) (Heise et al., 1998; Miller et al., 1998; Sedmak et al., 1994) and Varicella-Zoster virus (VZV) (Abendroth et al., 2000) lytic cycle proteins inhibit IFN-γ-induced MHC class II expression. In addition, Kaposi's sarcoma-associated herpesvirus (KSHV) encodes lytic viral proteins which are viral interferon response factor (vIRF) homologs and interfere with interferon signal transduction and subsequent gene transactivation (Gao et al., 1997: Li et al., 1998; Zimring et al., 1998).

[5] Correspondence: shann@med.unc.edu

In the present study, we have examined the ability of the EBV immediate-early proteins to disrupt cell signaling pathways activated by IFN-γ. Binding of IFN-γ to its cell surface receptor initiates a signal transduction cascade which involves tyrosine phosphorylation of the receptor-associated Janus kinases (Jaks) Jak1 and Jak2, followed by tyrosine phosphorylation of STAT1 (Bach et al., 1997). Phosphorylated STAT1 molecules subsequently form homodimers, translocate to the nucleus, bind γ-activated sequence (GAS) elements, and upregulate the expression of IFN-γ-inducible genes. Here, we report that expression of BZLF1 inhibits IFN-γ induction of a variety of important downstream target genes including class II MHC surface expression. Expression of BZLF1 but not BRLF1 inhibits IFN-γ-induced STAT1 tyrosine phosphorylation. We demonstrate that the transactivation and the DNA binding domains of BZLF1 are required for inhibition of IFN-γ-induced STAT1 nuclear translocation. Finally, we show that BZLF1 abrogates cellular responses to IFN-γ by decreasing expression of the IFN-γ receptor. Our results suggest a mechanism by which EBV may escape early antiviral immune responses during primary infection.

Results

BZLF1 Inhibits IFN-γ-Induced Gene Expression

IFN-γ exerts a wide range of biological effects by activating a Jak-STAT signaling pathway and inducing expression of cellular genes (Bach et al., 1997; Boehm et al., 1997). To investigate whether BZLF1 disrupts IFN-γ-induced gene expression, we used a replication-deficient adenovirus vector (AdZ) to express the BZLF1 protein or the LacZ protein (AdlacZ) in HeLa cells and the lung carcinoma cell line A549 using a moi of 50 (Figure 1). HeLa cells infected with AdZ at a moi of 50 express BZLF1 at a similar level as the lytically infected population of EBV-positive AGS gastric carcinoma cells (Figure 1A). In mock-infected and AdlacZ-infected HeLa and A549 cells (moi of 50), IFN-γ treatment induced expression of both IRF-1 and p48 (Figure 1B), which are members of the Interferon Regulatory Factor (IRF) family of proteins (Nguyen et al., 1997). In contrast, IFN-γ-induced IRF-1 and p48 expression was significantly decreased in cells infected with AdZ. A control transcription factor (the p65 component of NF-κB) was not affected by IFN-γ or AdZ. These results suggest that BZLF1 expression interferes with some aspect of IFN-γ signaling.

BZLF1 Inhibits IFN-γ-Induced STAT1 Tyrosine Phosphorylation

IFN-γ induces tyrosine phosphorylation of latent STAT1 molecules in the cell cytoplasm (Decker et al., 1991; Shuai et al., 1992, 1993). Once phosphorylated on tyrosine, STAT1 molecules form homodimers, translocate to the nucleus, and activate transcription of cellular genes by binding to specific DNA elements known as γ-activated sequences (GAS) (Decker et al., 1991; Lew et al. 1991). We therefore investigated whether the ability of BZLF1 to disrupt IFN-γ-induced gene expression (Figure 1) is due to a defect in IFN-γ-induced STAT1 tyrosine phosphorylation in HeLa cells (Figures 2A and 2B) and A549 cells (Figure 2C). Using an antibody that specifi-

cally recognizes the tyrosine-phosphorylated form of STAT1, we observed by immunoblot analysis that IFN-γ treatment induces STAT1 tyrosine phosphorylation in mock-infected and AdlacZ-infected cells. In contrast, IFN-γ-induced STAT1 tyrosine phosphorylation is significantly decreased in AdZ-infected cells. However, BZLF1 expression in HeLa and A549 cells does not affect the total level of STAT1. Thus, the inhibition of IFN-γ-induced gene expression by BZLF1 is due to a defect in the IFN-γ signaling pathway that lies upstream of STAT1 tyrosine phosphorylation.

BZLF1 has been previously reported to inhibit STAT activation of the EBV BamH1-Q promoter in a p53-dependent manner (Chen et al., 1999). Therefore, we investigated whether BZLF1 inhibits IFN-γ-induced STAT1 tyrosine phosphorylation in p53-deleted Saos-2 cells (Figure 2D). IFN-γ-induced STAT1 tyrosine phosphorylation in AdZ-infected Saos-2 cells was inhibited, in contrast to mock-infected and AdlacZ-infected cells. The total levels of STAT1 were similar in all groups. Therefore, the ability of BZLF1 to inhibit STAT1 tyrosine phosphorylation induced by IFN-γ is a p53-independent process.

BZLF1 Does Not Inhibit IFN-α-Induced STAT1 Tyrosine Phosphorylation

IFN-α induces tyrosine phosphorylation of STAT1 by activating the Jak kinases Tyk2 and Jak1 associated with the cytoplasmic tails of the IFN-αR1 and IFN-αR2 subunits, respectively (Stark et al., 1998). We examined whether the inhibition of STAT1 tyrosine phosphorylation by BZLF1 (Figure 2) is specific to that induced by IFN-γ or if other signaling pathways leading to STAT1 tyrosine phosphorylation might also be affected. In contrast to its effect on IFN-γ signaling, expression of BZLF1 in HeLa cells does not significantly alter STAT1 tyrosine phosphorylation induced by IFN-α (Figure 3A).

We also investigated the ability of the other EBV immediate-early protein, BRLF1, to inhibit IFN-α- or IFN-γ-induced STAT1 tyrosine phosphorylation. IFN-α and IFN-γ both effectively induced STAT1 tyrosine phosphorylation in HeLa cells infected with an adenovirus vector expressing the BRLF1 protein (Figure 3B). In addition, expression of BRLF1 did not affect IFN-γ induction of IRF-1 (Figure 3B).

Inhibition of IFN-γ Signaling by BZLF1 Requires the Transactivation and DNA Binding Domains

The BZLF1 protein has transcriptional transactivation, DNA binding, and dimerization domains (Flemington et al., 1992; Kouzarides et al., 1991; Packham et al., 1990). In order to determine the domains of BZLF1 required for inhibition of IFN-γ signaling, we expressed various BZLF1 wild-type and mutant plasmids (Figure 4A) in HeLa cells and examined the effect on STAT1 nuclear translocation after treatment with IFN-γ (Figure 4B). STAT1 staining of untreated cells transfected with the vector control is distinctly cytoplasmic (Figure 4B, part A). Following treatment of these cells with IFN-γ, as expected, STAT1 translocates into the nucleus (Figure 4B, part F). The expression of wild-type BZLF1 (Figure 4B, part B) inhibits STAT1 nuclear translocation induced by IFN-γ (Figure 4B, part G, arrows). Deletion of the first
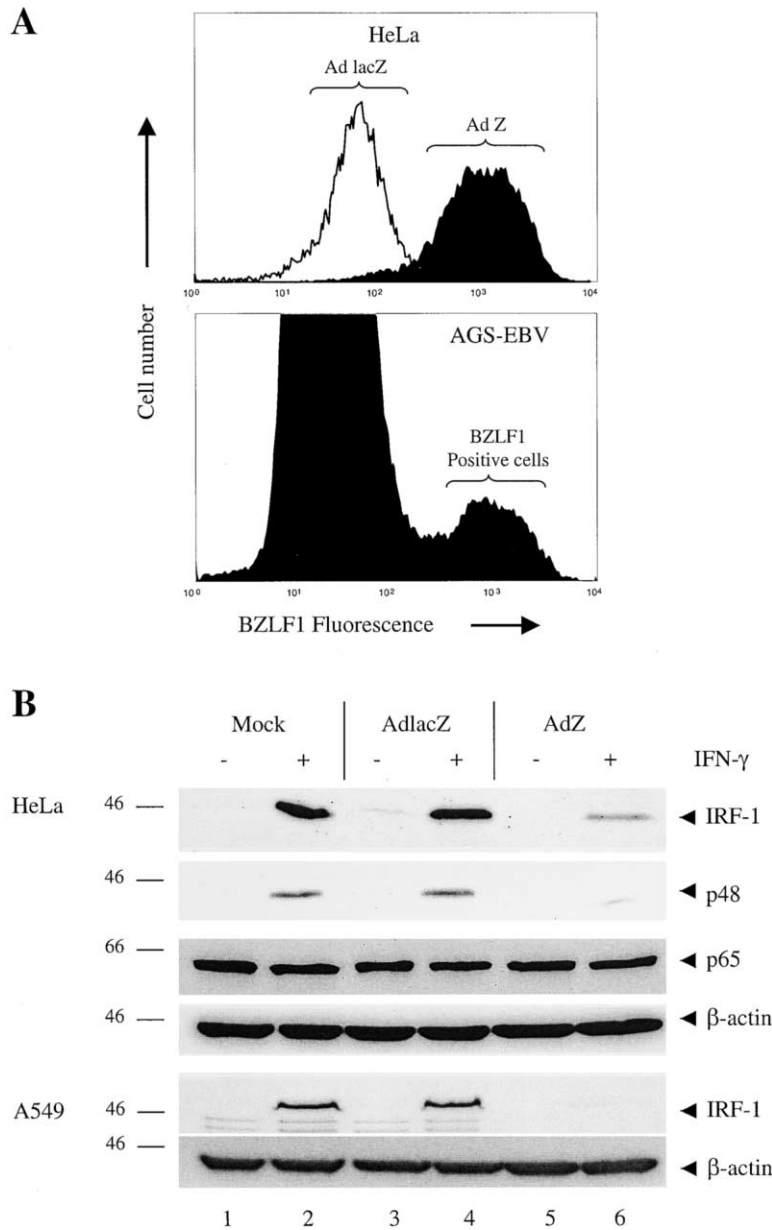
A

Figure 1. BZLF1 Expression Inhibits IFN-γ-Induced IRF-1 and p48 Expression

HeLa cells and A549 cells were mock infected or infected with an adenovirus (moi of 50) expressing either LacZ or BZLF1.

(A) The level of cellular BZLF1 expression was determined by FACS analysis in HeLa cells infected with AdlacZ or AdZ, and EBV-positive gastric carcinoma cells (AGS-EBV), which have approximately 5% of cells expressing BZLF1.

(B) Forty-eight hours postinfection, cells were untreated or treated with 100 IU/ml IFN-γ for 24 hr. Immunoblot analyses were performed with anti-IRF-1, anti-p65, or anti-p48 antibodies. Membranes were reprobed with anti-β-actin to control for protein loading

B

24 amino acids of BZLF1, previously shown to have a replication function (Sarisky et al., 1996) and contain the site for SUMO-1 modification (Adamson and Kenney, 2001), does not affect its ability to inhibit IFN-γ-induced STAT1 nuclear translocation (Figure 4B, part C and 4B, part H). However, deletion of amino acids 1–86, which include the transactivation domain, renders BZLF1 unable to inhibit STAT1 nuclear translocation induced by IFN-γ (Figure 4B, part D and 4B, part I). A BZLF1 mutant unable to bind DNA due to a single amino acid change at residue 185 in the DNA binding domain is also unable to inhibit IFN-γ-induced STAT1 nuclear translocation (Figure 4B, part E and 4B, part J). Therefore, both the transactivation domain and the DNA binding domain are necessary for BZLF1 to inhibit IFN-γ-induced STAT1 nuclear translocation. These results indicate that BZLF1 inhibition of IFN-γ signaling may be secondary to a transcriptional effect.

BZLF1 Reduces Tyrosine Phosphorylation of Jak1 and Jak2

Due to our observations that BZLF1 inhibits both STAT1 tyrosine phosphorylation and nuclear translocation induced by IFN-γ, we examined the upstream components of the signaling pathway: Jak1 and Jak2 (Silvennoinen, et al. 1993). The Jak1 and Jak2 tyrosine kinases are associated with the cytoplasmic tails of the IFN-γRα and the IFN-γRβ subunits, respectively (Bach et al., 1997). Once activated, the Jak kinases phosphorylate STAT1 molecules bound to a single phosphotyrosine on the IFN-γRα subunit. We performed immunoprecipitation of Jak1 (Figure 5A) and Jak2 (Figure 5B) with HeLa cell extracts that had been mock infected or infected with either AdlacZ or AdZ. The total levels of Jak1 in AdZ infected cells was similar to Jak1 levels in both mock-infected and AdlacZ-infected cells. The levels of Jak2 in AdZ-infected cells also remained unchanged
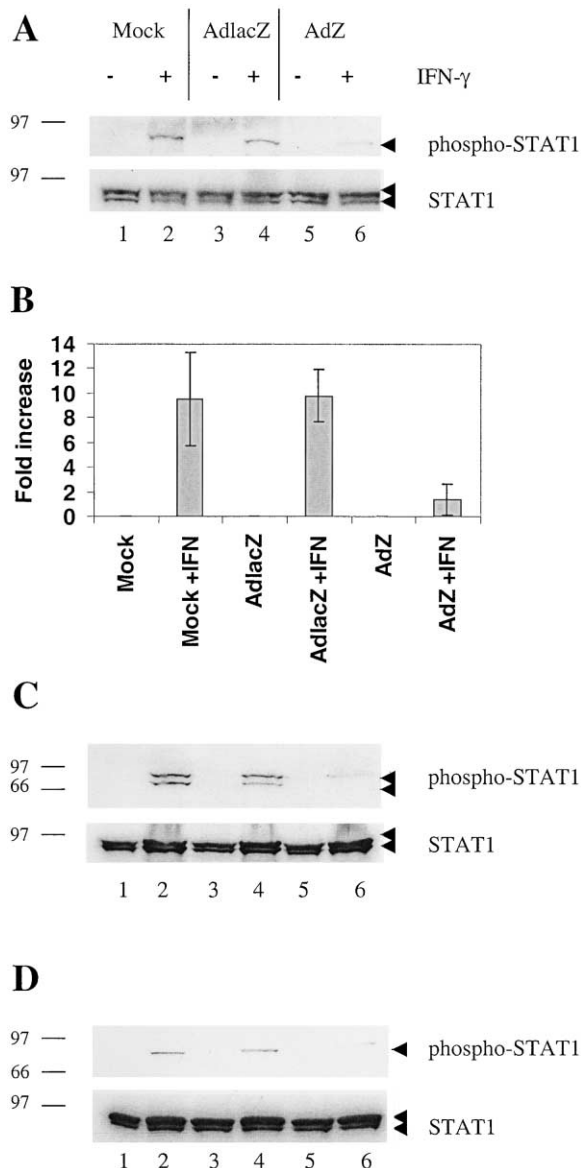
Figure 2. BZLF1 Expression Inhibits IFN-γ-Induced STAT1 Tyrosine Phosphorylation

HeLa cells (A and B), A549 cells (C), and Saos-2 cells (D) were mock infected or infected with an adenovirus expressing either LacZ or BZLF1. Forty-eight hours postinfection, cells were untreated or treated with 100 IU/ml IFN-γ for 30 min. Immunoblot analysis was performed with an antibody that specifically recognizes only STAT1 phosphorylated on tyrosine 701 (upper panels) or an antibody that recognizes total STAT1 (lower panels). The IFN-γ-induced increase in STAT1 tyrosine phosphorylation was quantitated from three separate experiments in HeLa cells (B) using laser densitometry analysis.

relative to mock-infected and AdlacZ-infected cells. However, IFN-γ induction of the activated (tyrosine phosphorylated) forms of Jak1 and Jak2 was reduced in the AdZ-infected cells in comparison to AdlacZ-infected cells (Figures 5C and 5D).

BZLF1 Decreases Expression of the IFN-γRα Subunit
IFN-γ initiates signaling by binding high-affinity sites on the extracellular domain of two IFN-γRα subunits (Bach,

et al., 1997). We therefore examined the effect of BZLF1 on expression of the IFN-γRα subunit. We performed immunoprecipitation of IFN-γRα with HeLa cell extracts followed by immunoblot with anti-IFN-γRα antibody (Figure 6A). Cells infected with AdZ had lower levels of immunoprecipitated IFN-γRα than both mock-infected and AdlacZ-infected cells. We also observed decreases of IFN-γRα by direct immunoblot of AdZ-infected A549 cells (Figure 6B) as well as AdZ-infected normal human fibroblasts (Figure 6C). From these experiments, we conclude that BZLF1 inhibits IFN-γ-induced signal transduction and resultant gene expression by decreasing the level of the α subunit of the IFN-γ receptor, the site of both ligand binding and STAT1 docking.

IFN-γRα RNA Level Is Decreased by BZLF1
To further investigate the mechanism by which BZLF1 decreases the level of IFN-γRα, we examined whether BZLF1 decreases the RNA level of the receptor subunit. We expressed BZLF1 in HeLa cells and performed Northern analysis on total RNA using a probe for the IFN-γRα subunit (Figure 6D). The level of IFN-γRα RNA in cells that expressed BZLF1 was greatly decreased in comparison to cells that expressed the control protein LacZ- and mock-infected cells. In contrast, the level of GAPDH RNA was unaffected.

**IFN-γ-Induced MHC Class II Expression
Is Inhibited by BZLF1**
A key downstream effect of IFN-γ signaling is the induction of class II expression in cells not normally expressing class II, thereby allowing cells to present viral antigens to CD4^{+} cells (Boehm et al., 1997). To determine if BZLF1 prevents this critical aspect of IFN-γ signaling, normal human fibroblasts were either mock infected, AdlacZ infected, or AdZ infected. Two days after infection, a portion of the cells were treated with IFN-γ for 48 hr. Fibroblasts do not constitutively express MHC class II but can be induced by IFN-γ to express MHC class II (Collins et al., 1984; Pober et al., 1983). Flow cytometry analysis of HLA-DR surface expression demonstrated that IFN-γ induced MHC class II expression on both mock-infected and AdlacZ-infected fibroblasts (Figure 7A). In contrast, the ability of IFN-γ to stimulate MHC class II expression was almost completely abrogated in cells containing BZLF1. These results demonstrate that BZLF1 expression inhibits IFN-γ induction of MHC class II surface expression, a major immunomodulatory function.

The expression of class II MHC is regulated by the class II transactivator (CIITA) (Steimle et al., 1993). Specifically, the IFN-γ induction of CIITA occurs through the activation of two CIITA promoters, III and IV (Harton and Ting, 2000). RT-PCR analysis of CIITA transcripts derived from the IFN-γ-inducible promoters of CIITA demonstrated that expression of BZLF1 inhibits the ability of IFN-γ to upregulate CIITA expression (Figure 7B). In contrast, IFN-γ-induced CIITA expression from promoters III and IV in cells expressing LacZ. CIITA promoter I is not expressed in fibroblasts and is not IFN-γ responsive (Figure 7B). Thus, expression of BZLF1 inhibits IFN-γ induction of the class II master regulator CIITA, which is essential for MHC class II gene expression.
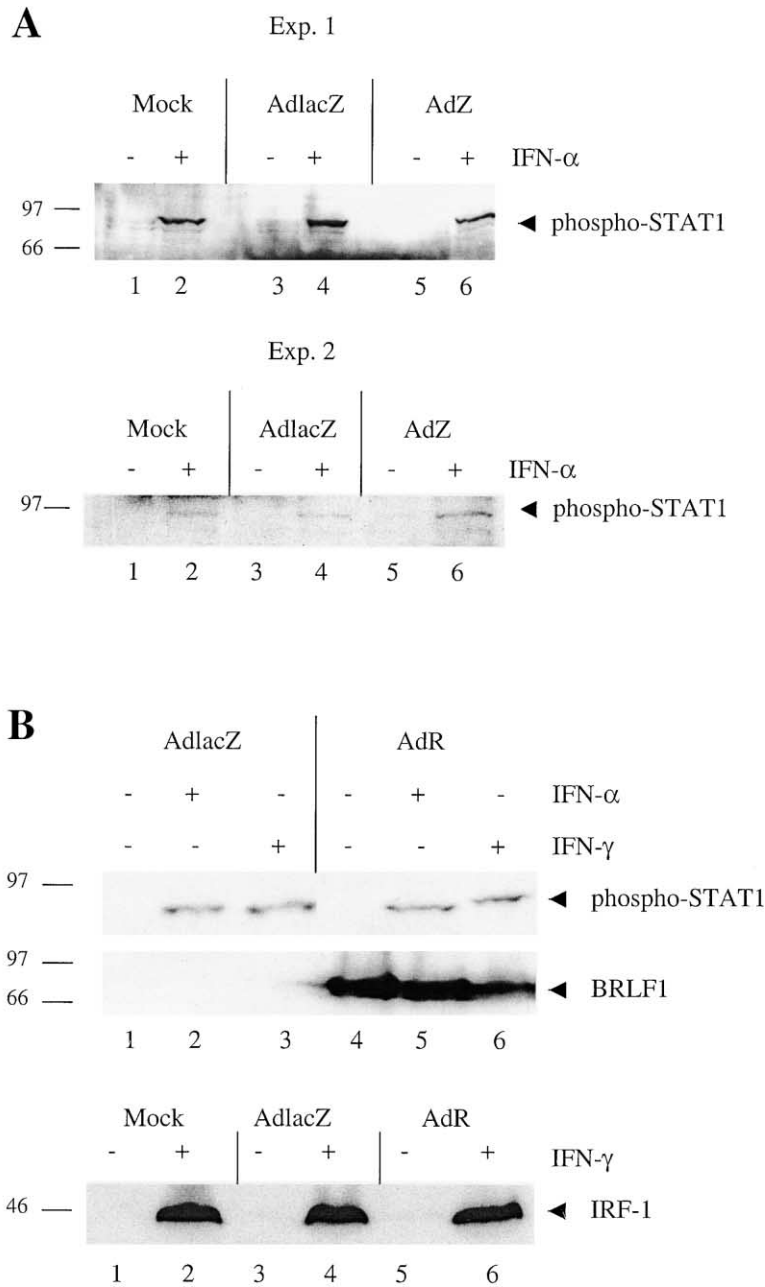
A

Exp. 1

Mock   AdlacZ   AdZ
 -   +   -   +   -   +   IFN-α

97 —
66 —   ◄ phospho-STAT1

1 2   3 4   5 6

Exp. 2

Mock   AdlacZ   AdZ
 -   +   -   +   -   +   IFN-α

97—   ◄ phospho-STAT1

1   2   3   4   5   6

B

AdlacZ   AdR
 -   +   -   -   +   -   IFN-α
 -   -   +   -   -   +   IFN-γ

97 —   ◄ phospho-STAT1

97 —
66 —   ◄ BRLF1

1   2   3   4   5   6

Mock   AdlacZ   AdR
 -   +   -   +   -   +   IFN-γ

46 —   ◄ IRF-1

1   2   3   4   5   6

Figure 3. IFN-γ-Induced STAT1 Tyrosine Phosphorylation Is Specifically Inhibited by BZLF1

(A) HeLa cells were mock infected or infected with an adenovirus expressing either LacZ or BZLF1. Forty-eight hours postinfection, cells were treated with 500 IU/ml IFN-α for 30 min. Immunoblot analysis was performed with an anti-phopsho-STAT1 (tyrosine 701) antibody. (B) HeLa cells were infected with an adenovirus expressing either LacZ or BRLF1. In the upper panel, cells were untreated or treated with either 500 IU/ml IFN-α or 100 IU/ml IFN-γ for 30 min. In the lower panel, cells were treated with IFN-γ (100 IU/ml) for 24 hr. Immunoblot analyses were performed using anti-phospho-STAT1 (upper panel) or anti-IRF-1 (lower panel) antibodies. The blot was reprobed with an antibody that recognizes the BRLF1 protein in the upper panel.

Discussion

IFN-γ plays an essential role in the regulation of the immune system and the control of many different infectious diseases, including herpesvirus infections. In vivo studies have clearly established a protective role for IFN-γ in controlling acute murine cytomegalovirus (MCMV) infection (Gribaudo et al., 1993; Lucin et al., 1994; Orange et al., 1995). In addition, *IFN-γ*$^{-/-}$ mice and *IFN-γR*$^{-/-}$ mice are more susceptible to MCMV infection (Presti et al., 1998) as well as infection with herpes simplex virus Type I (HSV-1) (Bouley et al., 1995; Cantin et al., 1999). In this paper, we demonstrate that the EBV immediate-early protein, BZLF1, inhibits IFN-γ-induced MHC class II expression as well as the induction of other

important IFN-γ-responsive genes such as CIITA, IRF-1, and p48, by decreasing the cellular level of the ligand binding subunit of the IFN-γ receptor (IFN-γRα). Our findings, together with previous reports documenting the inhibition of IFN-γ-induced MHC class II expression by lytic viral proteins of Varicella-Zoster virus (VZV) (Abendroth et al., 2000) and Cytomegalovirus (CMV) (Heise et al., 1998; Miller et al., 1998; Sedmak et al., 1994), suggest that inhibition of MHC class II expression may be universally important for successful herpesvirus infection.

It is noteworthy that one of the downstream targets of BZLF1 is the CIITA protein, which is a master regulator of all class II MHC genes (Steimle et al., 1993) in addition to other antigen processing molecules such as the In-
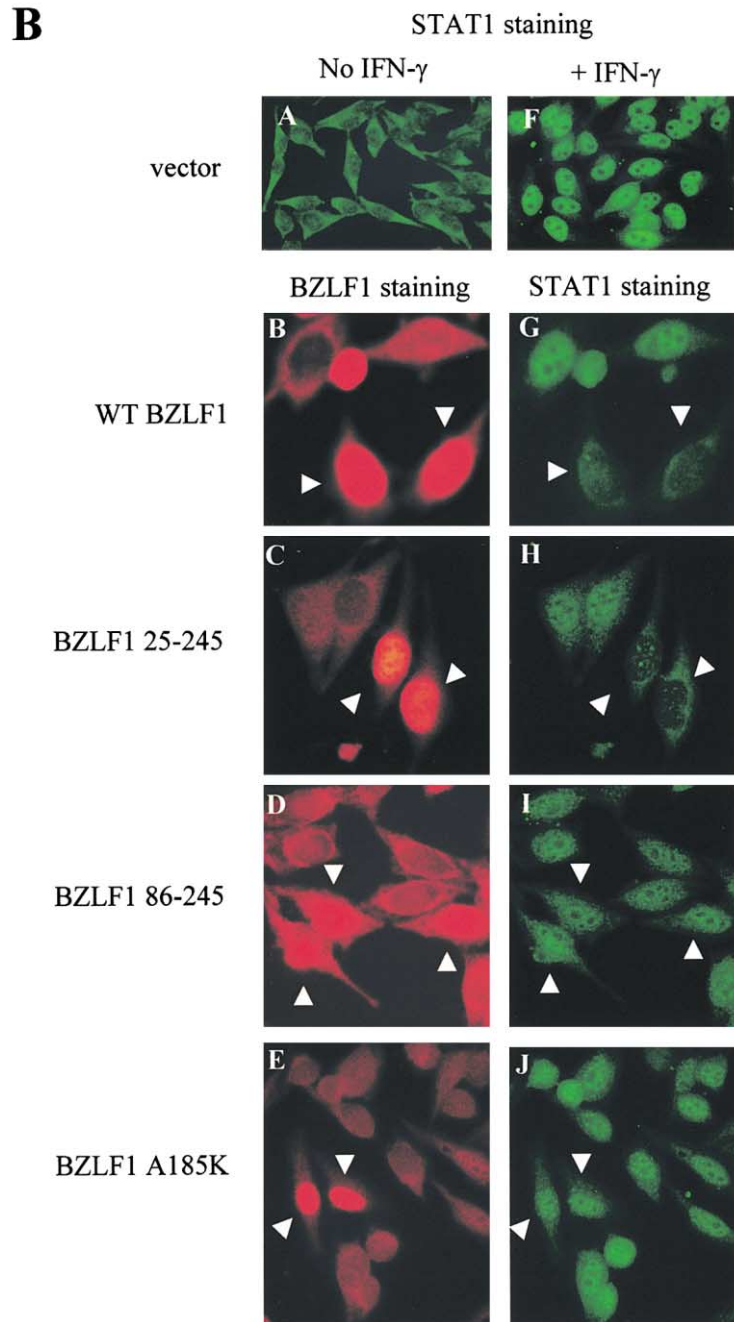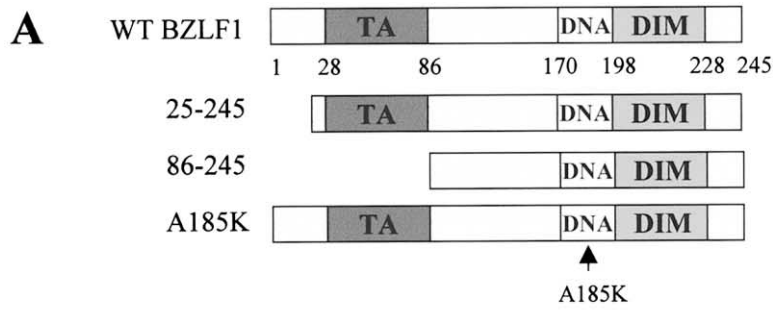
A

WT BZLF1

25-245

86-245

A185K

A185K

B

STAT1 staining

No IFN-γ   + IFN-γ

vector

BZLF1 staining   STAT1 staining

WT BZLF1

BZLF1 25-245

BZLF1 86-245

BZLF1 A185K

Figure 4. BZLF1 Domains Required for Inhibition of IFN-γ-Induced STAT1 Nuclear Translocation

(A) Structures of the wild-type and mutant BZLF1 proteins. The transactivator domain (TA), DNA binding domain (DNA), and dimerization domain (DIM) are indicated along with the respective amino acid numbers.

(B) HeLa cells were transfected with vector control (A and F), wild-type BZLF1 (B and G), BZLF1 25-245 (C and H), BZLF1 86-245 (D and I), or BZLF1 A185K (E and J) plasmid DNA. Forty-eight hours posttransfection, cells were treated with 200 IU/ml IFN-γ for 30 min. Cells were stained with anti-BZLF1 (mouse monoclonal) and anti-STAT1 (rabbit polyclonal) antibodies. BZLF1 staining is shown in (B–E). STAT1 staining is shown in (A and F–J). The arrows indicate the BZLF1-transfected cells.
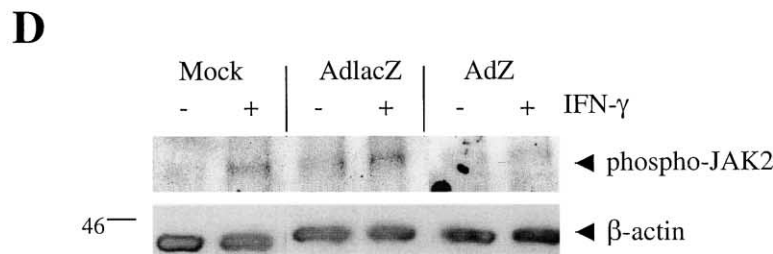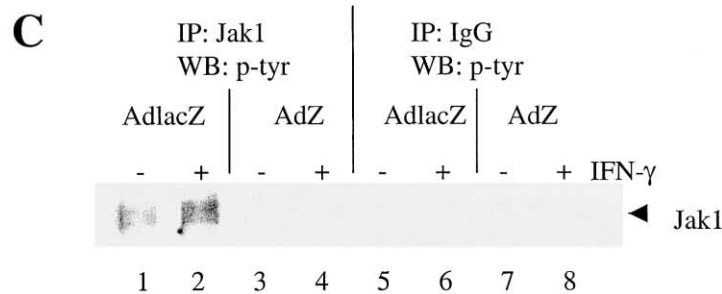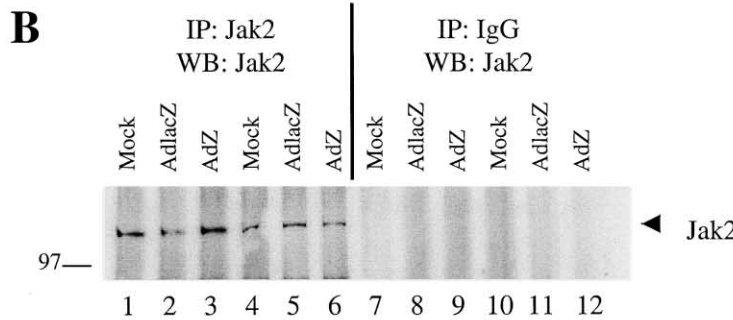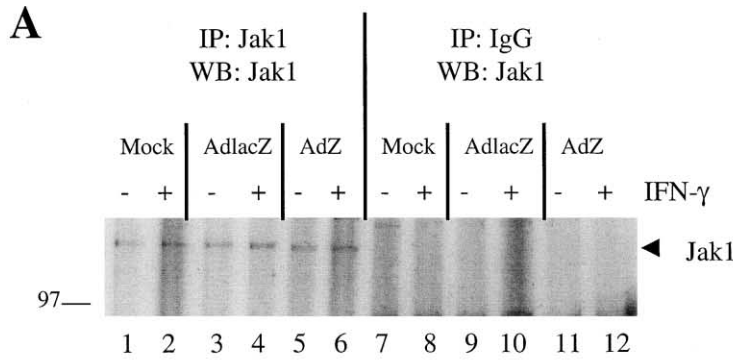
A

IP: Jak1   IP: IgG
WB: Jak1   WB: Jak1

Mock AdlacZ AdZ   Mock AdlacZ AdZ
- + | - + | - + | - + | - + | - +   IFN-γ

◀ Jak1

97—

1 2 3 4 5 6 7 8 9 10 11 12

B

IP: Jak2   IP: IgG
WB: Jak2   WB: Jak2

Mock AdlacZ AdZ Mock AdlacZ AdZ Mock AdlacZ AdZ Mock AdlacZ AdZ

◀ Jak2

97—

1 2 3 4 5 6 7 8 9 10 11 12

C

IP: Jak1   IP: IgG
WB: p-tyr   WB: p-tyr

AdlacZ   AdZ   AdlacZ   AdZ
- + | - + | - + | - +   IFN-γ

◀ Jak1

1 2 3 4 5 6 7 8

D

Mock   AdlacZ   AdZ
- + | - + | - +   IFN-γ

◀ phospho-JAK2

46—

◀ β-actin

Figure 5. BZLF1 Reduces Tyrosine Kinase Phosphorylation of Jak1 and Jak2

(A) HeLa cells were mock infected or infected with an adenovirus expressing either LacZ or BZLF1. Forty-eight hours postinfection, cells were treated with 100 IU/ml IFN-γ for 30 min and harvested, and immunoprecipitation was performed with either anti-Jak1 (rabbit polyclonal) antibody or rabbit IgG control antibody. Immunoblot analysis was performed with anti-Jak1 antibody.

(B) HeLa cells were infected as in (A). Forty-eight hours postinfection, cells were harvested and immunoprecipitation performed with anti-Jak2 (rabbit polyclonal) antibody or rabbit IgG control antibody. Immunoblot analysis was performed with anti-Jak2 antibody.

(C) HeLa cells were infected as in (A), immunoprecipitated using anti-Jak1 or rabbit control antibody, then probed with an anti-phosphotyrosine antibody.

(D) HeLa cells were infected as in (A). Forty-eight hours postinfection, cells were treated with 1500 IU/ml IFN-γ for 5 min and harvested, and immunoblot analysis was performed with anti-phospho-Jak2 antibody (upper panel) or anti-β-actin antibody (lower panel).

variant chain and DM molecules (Harton and Ting, 2000). By affecting both promoters III and IV of CIITA, BZLF1 essentially shuts down the transcription of all IFN-γ-inducible class II MHC expression, and hence the activation of T helper cells required to initiate an immune response. This provides a clever and significant circumvention of the immune response.

BZLF1 is a transcriptional transactivator that binds AP-1-like sites (Farrell et al., 1989, Urier et al., 1989).

Expression of BZLF1 is sufficient to induce the lytic form of EBV replication, and the BZLF1 transactivation function is required for expression of many EBV early genes. The protein also plays an essential role in replication by binding the lytic origin of replication (oriLyt) (Chang et al., 1990; Fixman et al., 1992, 1995; Schepers et al., 1993). However, increasing evidence demonstrates that BZLF1 has a variety of other functions that alter the host cell environment, presumably to promote

A

IP: Jak2
WB: IFN-γRα

IP: IFN-γRα
WB: IFN-γRα

Mock   AdlacZ   AdZ   Mock   AdlacZ   AdZ

− + | − + | − + | − + | − + | − +   IFN-γ

97 —

}IFN-γRα

66 —

◄ HC

46 —

1 2   3   4   5   6   7   8   9 10 11 12

B

Mock   AdlacZ   AdZ   Mock   AdlacZ   AdZ

97 —

}IFN-γRα

66 —

46 —

◄ β-actin

1   2   3   4   5   6

C

AdlacZ | AdZ | AdlacZ | AdZ

− + | − + | − + | − +   IFN-γ

97 —

} IFN-γRα

66 —

46 —

◄ β-actin

1   2   3   4   5   6   7   8

D

Exp. 1   Exp. 2

Mock AdlacZ AdZ   Mock AdlacZ AdZ

2.37kb —

◄ IFN-γRα

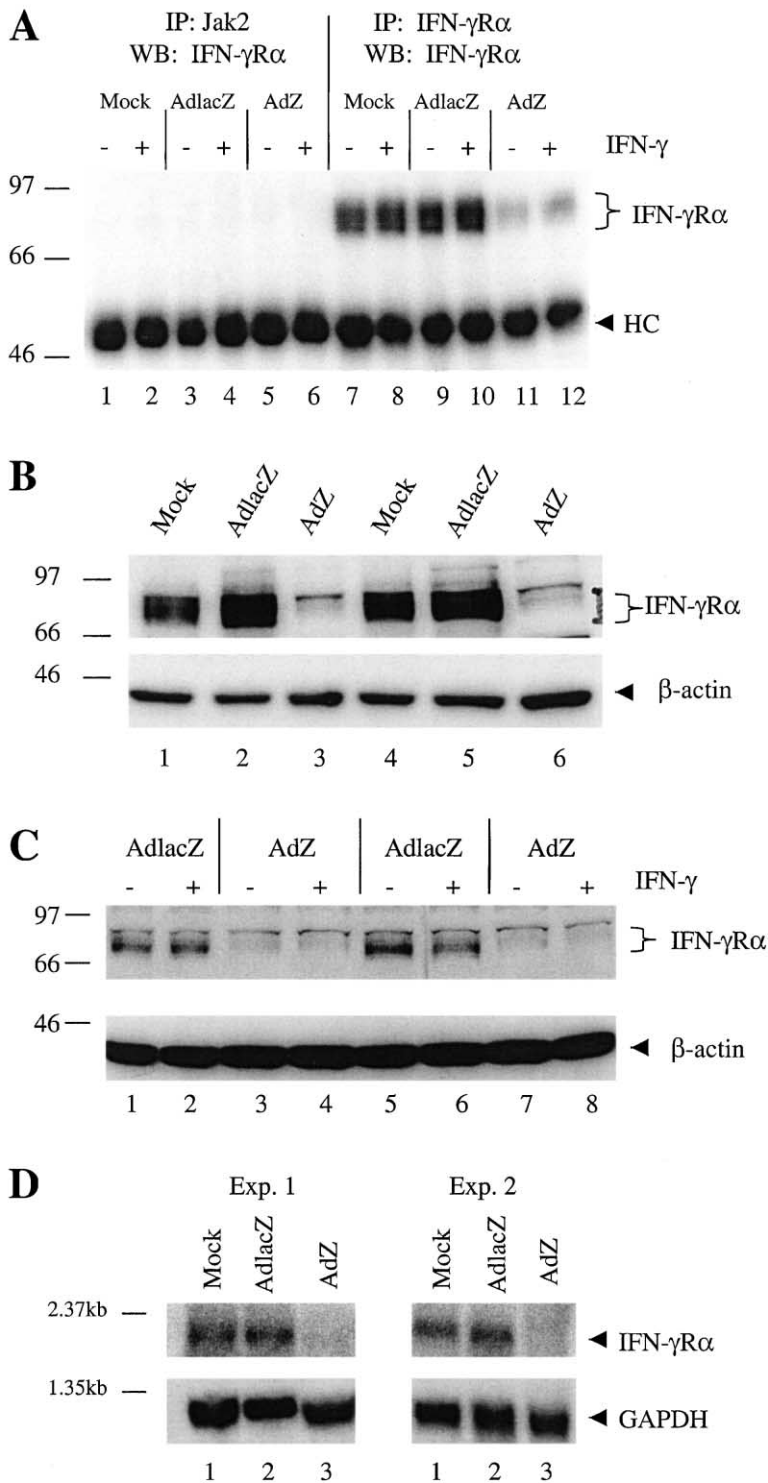1.35kb —

◄ GAPDH

1   2   3   1   2   3

Figure 6. BZLF1 Expression Decreases IFN-γRα Protein Levels and RNA Levels

HeLa cells (A) were mock infected or infected with an adenovirus expressing either LacZ or BZLF1. Forty-eight hours postinfection, the cells were treated with 100 IU/ml IFN-γ for 30 min and harvested, and immunoprecipitation performed with anti-IFN-γRα antibody (rabbit polyclonal) or anti-Jak2 antibody (rabbit polyclonal) as a control. Immunoblot analysis was performed with anti-IFN-γRα antibody.

A549 cells (B) and normal human fibroblasts (C) were infected in two separate experiments as described in (A). Forty-eight hours postinfection, a portion of the cells were treated with 200 IU/ml IFN-γ for 6 hr and harvested, and immunoblot analysis performed with anti-IFN-γRα antibody or anti-β-actin antibody.

(D) Total RNA was extracted from HeLa cells, and Northern blot analysis was performed with a probe specific for IFN-γRα mRNA (2.3 kb). Membranes were rehybridized with a probe specific for GAPDH to control for equal RNA loading.

the lytic replication cycle. For example, BZLF1 mediates cellular growth arrest (Cayrol and Flemington, 1996), interacts directly with p65 (Gutsch et al., 1994) and p53 (Zhang et al., 1994), alters CBP function (Adamson and Kenney, 1999), and activates the p38 kinase and c-Jun N-terminal kinase signaling pathways (Adamson et al., 2000). Additionally, BZLF1 induces the expression of the immunosuppressive cytokine TGFβ-1 (Cayrol and Flemington, 1995) and has recently been shown to induce dispersion of nuclear PML bodies which are believed to have antiviral functions (Adamson and Kenney, 2001). Here, we report that BZLF1 inhibits the IFN-γ signaling cascade, providing further evidence that the protein plays a major role in viral subversion of host immune responses.

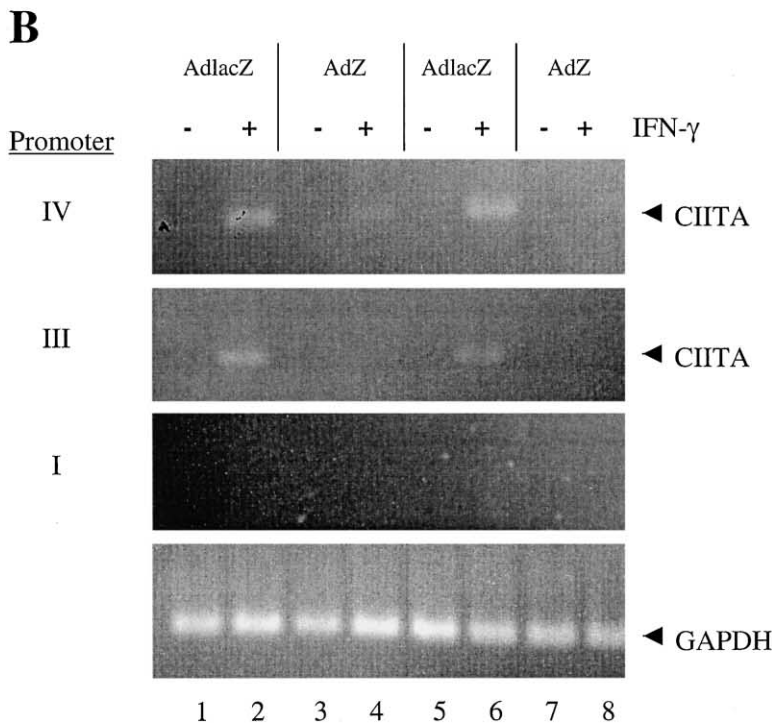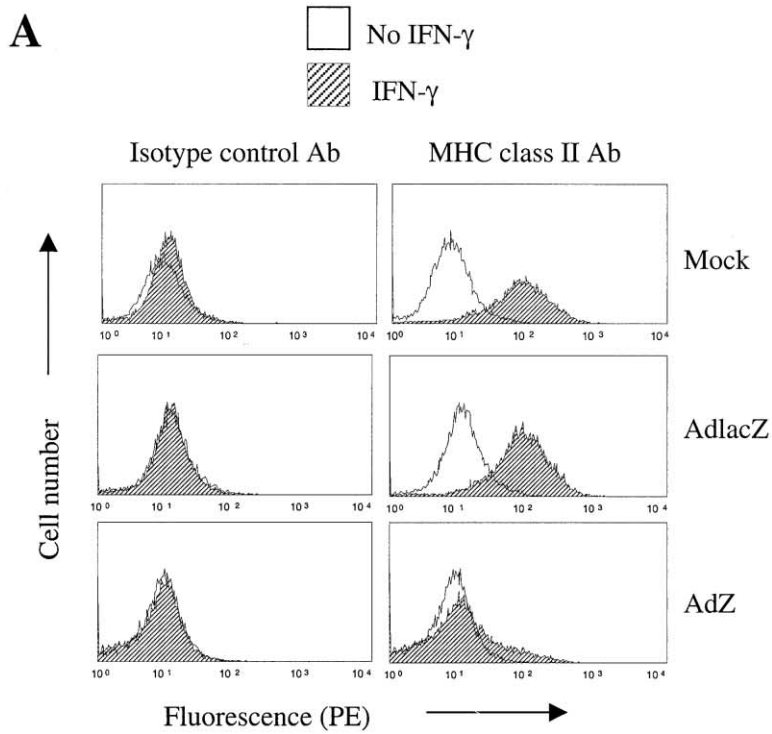Our findings reveal a new strategy among the herpes-

A

B

Figure 7. IFN-γ-Stimulated MHC Class II Expression Is Inhibited by BZLF1

(A) Normal human fibroblasts were mock infected or infected with an adenovirus expressing either LacZ or BZLF1. Forty-eight hours postinfection, cells were untreated or treated with 200 IU/ml IFN-γ for 48 hr. Cells were stained with PE-labeled anti-HLA-DR antibody or an isotype IgG$_{2a}$-PE conjugate and analyzed by fluorescence-activated flow cytometry.

(B) Normal human fibroblasts were infected in two separate experiments with an adenovirus expressing either LacZ or BZLF1. Forty-eight hours postinfection, cells were untreated or treated with 200 IU/ml IFN-γ for 6 hr. RT-PCR analysis was performed with primers specific for CIITA transcripts from CIITA promoters I, III, and IV or with primers for GAPDH.

viruses for inhibition of IFN-γ-stimulated signal transduction. VZV infection reduces the protein levels of STAT1 and Jak2 (Abendroth et al., 2000), whereas HCMV infection induces increased degradation of Jak1 (Miller et al., 1998). In contrast, MCMV inhibition of IFN-γ-induced signal transduction occurs downstream of STAT1 phosphorylation, nuclear translocation, and transactivation (Heise et al., 1998). The specific VZV or CMV protein(s) responsible for the inhibition of IFN-γ signaling have not been identified, although the inhibition was found to occur during the lytic replication cycle of these viruses and, in the case of HCMV, was found to be due to the expression of immediate-early or early viral gene products (Miller et al., 1998). In contrast to

the effects of HCMV and VZV, we found no effect of BZLF1 expression on the protein levels of STAT1, Jak1, or Jak2. However, both IFN-γ-induced STAT1 tyrosine phosphorylation and nuclear translocation are inhibited by BZLF1, indicating a loss of signal upstream in the pathway. Consistent with this, we found that BZLF1 expression decreases both the RNA and protein levels of the IFN-γRα subunit, resulting in reduced Jak1 and Jak2 tyrosine phosphorylation. Interestingly, *IFN-γR*^{−/−} mice are more susceptible to HSV-1 infection than *IFN-γ*^{−/−} mice (Cantin et al., 1999), suggesting that an additional ligand may exist for the IFN-γR which also mediates antiviral effects. Thus, the loss of IFN-γRα induced by BZLF1 may be an efficient mechanism by which the virus can escape multiple antiviral responses of the host.

Mutational analysis of BZLF1 revealed that the first 85 amino acids (which contain the transactivation domain) as well as the DNA binding domain are required for the inhibition of IFN-γ-induced STAT1 nuclear translocation. The amino-terminal half of BZLF1 (containing the transactivation domain) has previously been shown to be required for the interaction of BZLF1 with the histone acetylase CREB binding protein (CBP) (Adamson and Kenney, 1999). CBP regulates transcription by conformational alteration of chromatin and BZLF1 interaction with CBP inhibits transcriptional activation by CBP in promoters that do not contain BZLF1 binding sites (Adamson and Kenney, 1999). The interaction between BZLF1 and CBP might thus explain the decrease in IFN-γRα RNA levels found in cells expressing BZLF1. However, DNA binding mutants of BZLF1 are able to associate with CBP, whereas we have found that a BZLF1 DNA binding mutant (ZA185K) is unable to inhibit IFN-γ-induced STAT1 nuclear translocation. We hypothesize instead that BZLF1 may bind to and activate the promoter of a gene that encodes a repressor of IFN-γRα gene expression. However, direct binding of BZLF1 to the IFN-γRα promoter and prevention of expression cannot be ruled out as a potential mechanism.

Interestingly, expression of BZLF1 has no consistent effect on STAT1 tyrosine phosphorylation induced by IFN-α, indicating that the upstream components of this pathway (Tyk2, Jak1, and the IFN-αR subunits) (Stark et al., 1998) remain intact in cells expressing BZLF1. However, these results do not rule out the possibility that BZLF1 may inhibit IFN-α responses downstream of STAT1 activation. Indeed, our findings that BZLF1 inhibits IFN-α induction of p48 (Boehm et al., 1997) (a key component of the IFN-α-induced DNA binding complex) indicates that at least some IFN-α-responsive genes are also likely inhibited by BZLF1. Additionally, expression of another EBV immediate-early protein, BRLF1, had no effect on STAT1 tyrosine phosphorylation induced by either IFN-α or IFN-γ. Taken together, these results suggest BZLF1 has evolved a mechanism to specifically inhibit the ability of host cells to respond to IFN-γ.

In summary, we have documented a unique strategy among the herpesviruses to inhibit the cellular responses to IFN-γ and have identified the specific viral protein responsible. This study adds BZLF1 to a growing list of EBV proteins that modulate the immune response during the lytic form of infection. The EBV glycoprotein, BZLF2, binds to the HLA-DRβ-chain of class II MHC and may disrupt its function (Spriggs et al., 1996). Addition-

ally, EBV encodes an IL-10 homolog (BCRF1) that inhibits production of IFN-γ by lymphocytes and IL-12 production by macrophages (Cohen, 1999), as well as a soluble receptor for colony-stimulating factor 1 (CSF-1), BARF1, that inhibits macrophage proliferation (Strockbine et al., 1998). These proteins likely function synergistically with BZLF1 to subvert the host immune response and enhance viral replication. The *BARF1* gene product is expressed early in EBV replication (Strockbine et al., 1998), whereas both the *BZLF2* and *BCRF1* gene products are expressed only late during EBV replication (Kieff, 1996). In contrast, *BZLF1* is an immediate-early gene whose expression may provide the virus with initial protection from antiviral defenses. We speculate that further investigation of the role of EBV immediate-early and early proteins in virus-immune system interactions will continue to reveal additional strategies for EBV evasion of the host immune response.

Experimental Procedures

Cell Culture

HeLa, a cervical carcinoma cell line, and A549, a lung carcinoma cell line, were maintained in Dulbecco's modified Eagle's medium H supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. Saos-2 is an osteosarcoma cell line that was maintained in McCoy's 5A medium supplemented with 10% FBS. Normal human foreskin fibroblasts (NHF5-neo), a gift from William Kaufmann at UNC Chapel Hill, were maintained in Eagle's minimal essential media (GIBCO-BRL) supplemented with 10% FBS and nonessential amino acids. The AGS-EBV cell line (a gift from Lindsey Hutt-Fletcher) was obtained by G418 selection of AGS cells (a gastric carcinoma line) that were infected with a recombinant Akata virus in which a neomycin resistance cassette had been inserted into the nonessential BDLF3 open reading frame and was maintained in Ham's F-12 medium with 10% FBS and 400 μg/ml G418. All cell lines were cultured at 37°C in a 5% CO_2 incubator.

Adenovirus Construction and Infection

The EBV IE genes *BZLF1* and *BRLF1* and the control *lacZ* gene, under control of the IE CMV promoter, were inserted via cre-loxP-mediated recombination into an adenovirus type 5 derivative lacking the E1 and E3 genes to create adenovirus-LacZ (AdlacZ), adenovirus-BZLF1 (AdZ), and adenovirus-BRLF1 (AdR), as previously described (Westphal et al., 1999). Virus stocks were grown on the 293 cell line and purified by double cesium chloride gradient followed by dialysis. HeLa, A549, and Saos-2 cell lines were plated at a density of 3.0×10^6 cells per 150 mm plate 24 hr prior to infection. Cells were infected with no adenovirus (mock infection), AdlacZ, or AdZ at a multiplicity of infection of 50.

Transfection and Plasmids

Cells were transfected with 5 μg of plasmid DNA by electroporation with 1500 V from a Zapper electroporation unit (Medical Electronics Shop, University of Wisconsin). Wild-type BZLF1 expression vectors contain either the BZLF1 genomic sequence inserted in the pSG5 expression vector (Stratagene) under the control of the simian virus 40 (SV40) promoter (a gift from Diane Hayward), or a BZLF1 cDNA inserted into the pHD1013 under the control of the CMV IE promoter as previously described (Kenney et al., 1989). The ZA185K vector contains a mutation in the DNA binding domain of BZLF1 in which amino acid 185 has been altered from alanine to lysine (A185K), abolishing DNA binding activity (Giot, et al., 1991). BZLF1 86-245 (previously referred to as RAZΔR) has a deletion of amino acids 2–85 (a gift of Joseph Pagano) (Furnari et al., 1994), and BZLF1 25-245 has a deletion of the codons for the first 24 amino acids of BZLF1 (Askovic and Baumann, 1997). ZA185K and BZLF1 86–245 are in the pHD1013 vector, whereas BZLF1 25-245 is in the SG5 vector. Appropriate vector controls and wild-type BZLF1 controls were included in each experiment.

Immunoblot Analysis

Immunoblot analysis of total cell extracts or immunoprecipitates was performed as previously described (Adamson and Kenney, 1999) with the following antibodies: anti-p48 (1:200), anti-IRF1 (1:200), anti-STAT1 (1:500), anti-phosphoSTAT1 (Tyr701) (1:200), anti-Jak1 (1:200), anti-Jak2 (1:200), anti-IFNγRα (1:200), anti-p65 (1:500) (all from Santa Cruz), anti-phospho-Jak2 (Y1007, Y1008) (1:400; UBI), anti-β-actin mouse monoclonal (1:5000; Sigma), anti-EBV BZLF1 (1:100; Argene), and anti-EBV BRLF1 (1:100; Argene). Quantification of immunoblot results was performed by laser densitometry using the ultroscan XL machine (LKB Broma).

Immunoprecipitation

Cells were harvested and lysed in IP lysis buffer (1% NP-40, 150 mM NaCl, 50 mM Tris [pH 8.0], 50 mM NaF, 1 mM sodium orthovanadate, 20 mM β-glycerophosphate, and complete protease inhibitors). 500–1000 μg of total protein was incubated with 2.0 μg of anti-Jak1, anti-Jak2, anti-IFNγRα, anti-phosphotyrosine PY20 (Santa Cruz), control rabbit IgG, or an isotype control antibody overnight at 4°C. Immune complexes were collected by incubation for 1 hr at 4°C with a 50% slurry of protein A/G sepharose (Santa Cruz).

Immunofluorescence

48 hr posttransfection, cells were treated with 200 IU/ml IFN-γ (R&D Systems) for 30 min. Cells were fixed in 100% MeOH, blocked in PBS/0.3% BSA/5% donkey serum, and stained with anti-BZLF1 monoclonal antibody (either Argene anti-BZLF1 at 1:50 or DAKO BZ.1 anti-BZLF1 at 1:40) and anti-STAT1 antibody (1:50; Santa Cruz) in PBS/0.3% BSA/5% donkey serum for 60 min at 37°C. Cells were washed with PBS/0.5% BSA/0.1% tween and counterstained with donkey anti-rabbit IgG fluorescein isothiocyanate (FITC) conjugate (1:100; Jackson ImmunoResearch) and donkey anti-mouse IgG Rhodamine Red-X (RRX) conjugate (1:100; Jackson ImmunoResearch) for 45 min at 37°C.

Northern Blot Analysis

20 μg of total RNA, purified using the Qiagen RNeasy Mini kit as specified by the manufacturer, was separated on a 1% agarose/formaldehyde gel and transferred to nylon membranes (Schleicher and Schuell). Membranes were incubated for 25 min at 68°C in QuikHyb hybridization solution (Stratagene), and radiolabeled probes were melted and mixed with 100 μl of 10 mg/ml calf thymus DNA (Sigma) and hybridized to membranes overnight at 68°C. After hybridization, membranes were washed twice in 2× SSC/0.1% SDS at room temperature, and a final wash was carried out in 0.2× SSC/ 0.1% SDS for 30 min at 60°C. The IFN-γRα probe was generated by RT-PCR of 1 μg total RNA using the following primers: IFN-γRα sense 5′-GTCCTCAGTGCCTACACCAACTAA-3′ and IFN-γRα antisense 5′-CCACACATGTAAGACTCCTCCTGC-3″. Random priming (Oligolabeling kit; Amersham) of a glyceraldehyde phosphate dehydreogenase (GAPDH) probe (Ambion) was performed.

RT-PCR

Reverse transcriptase polymerase chain reaction was performed with the Access RT-PCR System (Promega) with the following oligonucleotides: CIITA promoter I, sense, 5′-TACCACTGCACTCTGCT CCATGAG-3″; promoter III, sense, 5′-CCTGGCTCCACGCCCTG-3″; promoter IV, sense, 5′-GAGCTGGCGGGAGGGAGA-3″; promoter I, III, IV, antisense, 5′-GAACTGGTCGCAGTTGATG-3″; GAPDH, sense, 5′-CAAAAGGGTCATCATCTCTGC; and GAPDH, antisense, 5′-GAG GGGCCATCCACAGTCTTC-3′. Reactions were run for 35 cycles of 94°C for 30 s, 60°C for 1 min, and 68°C for 2 min. The final extension was 10 min at 68°C.

FACS Analysis

To quantitate MHC class II expression, normal human fibroblasts were mock infected or infected with either adenovirus-lacZ or adenovirus-BZLF1. 2 days after infection, cells were untreated or treated with 200 IU/ml IFN-γ for 48 hr. Cells were harvested with 0.1 M EDTA/PBS and stained with phycoerythrin (PE)-labeled anti-HLA-DR antibody from Becton Dickinson, catalog #347367 (1:100), or an isotype IgG₂ₐ-PE conjugate (1:100) (Becton Dickinson) in PBS-2% FBS for 30 min on ice. Cells were washed three times in PBS-2%

FBS and resuspended in 1 ml PBS. Stained cells were analyzed using a FACScan analytical flow cytometer (Becton Dickinson). The level of cellular BZLF1 expression was determined by fixing cells in 60% acetone, then incubating with Argene anti-BZLF1 antibody (1:100; Argene), followed by FITC-conjugated anti-mouse antibody (1:100; Sigma).

References

Abendroth, A., Slobedman, B., Lee, E., Mellins, E., Wallace, M., and Arvin, A.M. (2000). Modulation of major histocompatibility class II protein expression by varicella-zoster virus. J. Virol. *74*, 1900–1907.

Adamson, A.L., and Kenney, S. (1999). The Epstein-Barr virus BZLF1 protein interacts physically and functionally with the histone acetylase CREB-binding protein. J. Virol. *73*, 6551–6558.

Adamson, A.L., and Kenney, S. (2001). The Epstein-Barr virus immediate-early protein BZLF1 is SUMO-1 modified and disrupts promyelocytic leukemia (PML) bodies. J. Virol. *75*, 2388–2399.

Adamson, A.L., Darr, D., Holley-Guthrie, E., Johnson, R.A., Mauser, A., Swenson, J., and Kenney, S. (2000). Epstein-Barr virus immediate-early proteins BZLF1 and BRLF1 activate the ATF2 transcription factor by increasing the levels of phosphorylated p38 and c-Jun N-terminal kinases. J. Virol. *74*, 1224–1233.

Andersson, J., Abrams, J., Bjork, L., Funa, K., Litton, M., Agren, K., and Andersson, U. (1994). Concomitant in vivo production of 19 different cytokines in human tonsils. Immunology *83*, 16–24.

Askovic, S., and Baumann, R. (1997). Activation domain requirements for disruption of Epstein-Barr virus latency by ZEBRA. J. Virol. *73*, 10458–10471.

Bach, E.A., Aguet, M., and Schreiber, R.D. (1997). The IFN-γ receptor: a paradigm for cytokine receptor signaling. Annu. Rev. Immunol. *15*, 563–591.

Biron, C.A. (1999). Initial and innate responses to viral infections-pattern setting in immunity or disease. Curr. Opin. Micro. *2*, 374–381.

Boehm, U., Klamp, T., Groot, M., and Howard, J.C. (1997). Cellular responses to interferon-γ. Annu. Rev. Immunol. *15*, 749–795.

Bouley, D.M., Kanangat, S., Wire, W., and Rouse, B.T. (1995). Characterization of herpes simplex virus type-I infection and herpetic stromal keratitis development in IFN-gamma knockout mice. J. Immunol. *155*, 3964–3971.

Cantin, E., Tanamachi, B., Openshaw, H., Mann, J., and Clarke, K. (1999). Gamma interferon (IFN-γ) receptor null-mutant mice are more susceptible to Herpes Simplex virus type I infection then IFN-γ ligand null-mutant mice. J. Virol. *73*, 5196–5200.

Cayrol, C., and Flemington, E. (1995). Identification of cellular target genes of the Epstein-Barr virus transactivator Zta: Activation of transforming growth factor βigh3 (TGF-βigh) and TGF-β1. J. Virol. *69*, 4206–4212.

Cayrol, C., and Flemington, E. (1996). The Epstein-Barr virus bZip transcription factor Zta causes G0/G1 cell cycle arrest through induction of cyclin-dependent kinase inhibitors. EMBO J. *15*, 2748–2759.

Chang, Y., Dong, D., Hayward, G., and Hayward, S.D. (1990). The Epstein-Barr virus Zta transactivator: a member of the bZip family with unique DNA-binding specificity and dimerization domain that lacks the characteristic heptad leucine zipper motif. J. Virol *64*, 3358–3369.

Chen, H., Lee, J.M., Wang, Y., Huang, D.P., Ambinder, R.F., and Hayward, S.D. (1999). The Epstein-Barr virus latency *Bam*HI-Q pro-

moter is positively regulated by STATs and Zta interference with JAK/STAT activation leads to a loss of *Bam*HI-Q promoter activity. Proc. Natl. Acad. Sci. USA *96*, 9339–9344.

Chevallier-Greco, A., Manet, E., Chavrier, P., Mosnier, C., Dallie, J., and Sergeant, A. (1986). Both Epstein-Barr virus (EBV)-encoded trans-acting factors, EB1 and EB2, are required to activate transcription from an EBV early promoter. EMBO J. *5*, 3243–3249.

Cohen, J.I. (1999). The biology of Epstein-Barr virus: lessons learned from the virus and the host. Curr. Opin. Immunol. *11*, 365–370.

Cohen, J.I. (2000). Epstein-Barr virus infection. New. Eng. J. Med. *343*, 481–492.

Collins, T., Korman, A.J., Wake, C.T., Boss, J.M., Kappes, D.J., Fiers, W., Ault, K.A., Gimbobrone, M.A., Jr., Strominger, J.L., and Pober, J.S. (1984). Immune interferon activates multiple class II major histocompatibility complex genes and the associated invariant chain gene in human endothelial cells and dermal fibroblasts. Proc. Natl. Acad. Sci. USA *81*, 4917–4921.

Countryman, J., and Miller, G. (1985). Activation of expression of latent Epstein-Barr virus after gene transfer with a small cloned fragment of heterogeneous viral DNA. Proc. Natl. Acad. Sci. USA *82*, 4085–4089.

Cox, M., Leahy, J., and Hardwick, J.M. (1985). An enhancer within the divergent promoter of Epstein-Barr virus responds synergistically to the R and Z transactivators. J. Virol. *64*, 313–321.

Decker, T., Lew, D.J., Mirkovitch, J., and Darnell, J.E., Jr. (1991). Cytoplasmic activation of GAF, an IFN-gamma-regulated DNA-binding factor. EMBO J. *10*, 927–932.

Farrell, P., Rowe, D., Rooney, C., and Kouozarides, T. (1989). Epstein-Barr virus BZLF1 transactivator specifically binds to consensus AP1 sites and is related to c-fos. EMBO J. *8*, 127–132.

Fixman, E., Hayward, G., and Hayward, S.D. (1992). trans-acting requirements for replication of Epstein-Barr virus oriLyt. J. Virol. *66*, 5030–5039.

Fixman, E., Hayward, G., and Hayward, S.D. (1995). Replication of Epstein-Barr virus oriLyt: lack of a dedicated virally encoded origin-binding protein and dependence on Zta in cotransfection assays. J. Virol. *69*, 2998–3006.

Flemington, E., and Speck, S. (1990). Autoregulation of Epstein-Barr virus putative lytic switch gene BZLF1. J. Virol. *64*, 1227–1232.

Flemington, E., Borras, K., Lytle, J., and Speck, S. (1992). Characterization of the Epstein-Barr virus BZLF1 protein transactivator domain. J. Virol. *66*, 922–929.

Furnari, F.B., Zacny, V., Quinlivin, E.B., Kenney, S., and Pagano, J.S. (1994). RAZ, an Epstein-Barr virus transdominant repressor that modulates the viral reactivation mechanism. J. Virol. *68*, 1827–1836.

Gao, S.-J., Bishoff, C., Jayachandra, S., Weiss, R.A., Chang, Y., and Moore, P.S. (1997). KSHV ORF K9 (vIRF) is an oncogene which inhibits the interferon signaling pathway. Oncogene *15*, 1979–1985.

Giot, J.-F., Mikaelian, I., Buisson, M., Manet, E., Joab, I., Nicolas, J.-C., and Sergeant, A. (1991). Transcriptional synergy and interference between the EBV transcription factors EB1 and R require both the basic region and the activation domains of EB1. Nucleic Acids Res. *19*, 1251–1258.

Gribaudo, G., Ravaglia, S., Calendo, A., Cavallo, R., Gariglio, M., Martinotti, M.G., and Landolfo, S. (1993). Interferons inhibit onset of murine cytomegalovirus immediate-early gene transcription. Virology *197*, 303–311.

Gutsch, D., Holley-Guthrie, E., Zhang, Q., Stein, B., Blanar, M., Baldwin, A., and Kenney, S. (1994). The bZip transactivator, BZLF1, of Epstein-Barr virus functionally and physically interacts with the p65 subunit of NFκB. Mol. Cell. Biol. *14*, 139–149.

Hardwick, J.M., Lieberman, P., and Hayward, S.D. (1988). A new Epstein-Barr virus transactivator, R, induces expression of a cytoplasmic early antigen. J. Virol. *62*, 2274–2284.

Hardwick, J.M., Tse, L., Applegren, N., Nicholas, J., and Veliuona, M.A. (1992). The Epstein-Barr virus R transactivator (Rta) contains a complex, potent activation domain with properties different from those of VP16. J. Virol. *66*, 5500–5508.

Harton, J.A., and Ting, J.P.-Y. (2000). Class II transactivator: mas-

tering the art of major histocompatibility expression. Mol. Cell. Biol. *20*, 6185–6194.

Heise, M.T., Connick, M., and Virgin, H.W., IV. (1998). Murine cytomegalovirus inhibits interferon-γ-induced antigen presentation to CD4 T cells by macrophages via regulation of expression of major histocompatibility complex class II-associated genes. J. Exp. Med. *187*, 1037–1046.

Holley-Guthrie, E.A., Quinlivan, E.B., Mar, E.C., and Kenney, S. (1990). The Epstein-Barr virus (EBV) BMRF1 promoter for early antigen (EA-D) is regulated by the EBV transactivators, BRLF1 and BZLF1, in a cell-specific manner. J. Virol. *64*, 3753–3759.

Hornef, M.W., Wagner, H.J., Kruse, A., and Kirschner, H. (1995). Cytokine production in a whole-blood assay after Epstein-Barr virus infection in vivo. Clin. Diag. Lab. Immunol. *2*, 209–213.

Kenney, S., Holley-Guthrie, E., Mar, E.C., and Smith, M. (1989). The Epstein-Barr virus BMLF1 promoter contains an enhancer element that is responsive to the BZLF1 and BRLF1 transactivators. J. Virol. *63*, 3878–3883.

Kieff, E. (1996). Epstein-Barr virus and its replication. In Fields Virology, 3rd ed., B.N. Fields, D.M. Knipe, and P.M. Howley, et al., eds. (Philadelphia, PA: Lippincott-Raven), pp. 2343–2396.

Kouzarides, T., Packham, G., Cook, A., and Farrell, P.J. (1991). The BZLF1 protein of EBV has a coiled coil dimerization domain without a heptad leucine repeat but homology to the C/EBP leucine zipper. Oncogene *6*, 195–204.

Lew, D.J., Decker, T., Strehlow, I., and Darnell, J.E., Jr. (1991). Overlapping elements in the guanylate-binding protein gene promoter mediate transcriptional induction by alpha and gamma interferons. Mol. Cell. Biol. *11*, 182–191.

Li, M., Lee, H., Guo, J., Neipel, F., Fleckenstein, B., Ozato, K., and Jung, J.U. (1998). Kaposi's sarcoma-associated herpesvirus viral interferon regulatory factor. J. Virol. *72*, 5433–5440.

Li, Q.X., Young, L.S., Niedobitek, G., Dawson, C.W., Birkenbach, M., Wang, F., and Rickinson, A.B. (1992). Epstein-Barr virus infection and replication in a human epithelial cell system. Nature *356*, 347–350.

Lieberman, P., Hardwick, J.M., and Hayward, S.D. (1989). Responsiveness of the Epstein-Barr virus mediated Not I repeat promoter to the Z transactivator is mediated in a cell-type specific manner by two independent signal regions. J. Virol. *63*, 3040–3050.

Linde, A., Andersson, B., Svenson, S.B., Ahrne, H., Carlsson, M., Forsberg, P., Hugo, H., Karstorp, A., Lenkei, R., Lindwall, A., et al. (1992). Serum levels of lymphokines and soluble cellular receptors in primary Epstein-Barr virus infection and in patients with chronic fatigue syndrome. J. Infect. Dis. *165*, 994–1000.

Lucin, P., Jonjic, S., Messerle, M., Polic, B., Hengel, H., and Kosinowski, U.H. (1994). Late phase inhibition of murine cytomegalovirus replication by synergistic interaction of interferon-gamma and tumor necrosis factor. J. Gen. Virol. *75*, 101–110.

Miller, D.M., Rahill, B.M., Boss, J.M., Lairmore, M.D., Durbin, J.E., Waldman, W.J., and Sedmak, D.D. (1998). Human cytomegalovirus inhibits major histocompatibility complex class II expression by disruption of the Jak/Stat pathway. J. Exp. Med. *187*, 675–683.

Nguyen, H., Hiscott, J., and Pitha, P.M. (1997). The growing family of interferon regulatory factors. Cytokine Growth Factor Rev. *8*, 293–312.

Orange, J.S., Wang, B., Terhost, C., and Biron, C.A. (1995). Requirement for natural killer cell-produced interferon-γ in defense against murine cytomegalovirus infection and enhancement of this pathway by interleukin 12 administration. J. Exp. Med. *182*, 1045–1056.

Packham, G., Economou, A., Rooney, C.M., Rowe, D.T., and Farrell, P.J. (1990). Structure and function of the Epstein-Barr virus BZLF1 protein. J. Virol. *64*, 2110–2116.

Pober, J.S., Collins, T., Grimbrone, M.A., Jr., Cotran, R.S., Gitlin, J.D., Fiers, W., Clayberger, C., Krensky, A.M., Burakoff, S.J., and Reiss, C.S. (1983). Lymphocytes recognize human vascular endothelial and dermal fibroblast Ia antigens induced by recombinant immune interferon. Nature *305*, 726–729.

Presti, R.M., Pollock, J.L., Dal Canto, A.L., O'Guin, A.K., and Virgin

H.W., IV. (1998). Interferon γ regulates acute and latent murine cyto-megalovirus infection and chronic diseases of the great vessels. J. Exp. Med. *188*, 577–588.

Quinlivan, E.B., Holley-Guthrie, E.A., Norris, M., Gutsch, D., Bachenheimer, S.L., and Kenney, S.C. (1993). Direct BRLF1 binding is required for cooperative BZLF1/BRLF1 activation of the Epstein-Barr virus early promoter, BMRF1. Nucleic Acids Res. *25*, 1999–2007.

Ragoczy, T., Heston, L., and Miller, G. (1998). The Epstein-Barr virus Rta protein activates lytic cycle genes and can disrupt latency in B lymphocytes. J. Virol. *72*, 7978–7984.

Rickinson, A.B., and Kieff, E. (1996). Epstein-Barr virus. In Fields Virology, 3rd ed., B.N. Fields, D.M. Knipe, and P.M. Howley, et al., eds. (Philadelphia, PA: Lippincott-Raven), pp. 2397–2446.

Rooney, C.M., Rowe, D.T., Ragot, T., and Farrell, P.J. (1989). The spliced BZLF1 gene of Epstein-Barr virus (EBV) transactivates an early EBV promoter and induces the virus productive cycle. J. Virol. *63*, 3109–3116.

Sarisky, R.T., Gao, Z., Lieberman, P.M., Fixman, E.D., Hayward, G.S., and Hayward, S.D. (1996). A replication function associated with the activation domain of the Epstein-Barr virus Zta transactivator. J. Virol. *70*, 8340–8347.

Schepers, A., Pich, D., and Hammerschmidt, W. (1993). A transcription factor with homology to the Ap1 family links RNA transcription and DNA replication in the lytic cycle of Epstein-Barr virus. EMBO J. *12*, 3921–3929.

Sedmak, D.D., Guglielmo, A.M., Knight, D.A., Birmingham, D.J., Huang, E.H., and Waldman, W.J. (1994). Cytomegalovirus inhibits major histocompatibility class II expression on infected endothelial cells. Am. J. Pathol. *144*, 683–692.

Shuai, K., Schindler, C., Prezioso, V.R., and Darnell, J.E., Jr. (1992). Activation of transcription by IFN-γ: tyrosine phosphorylation of a 91-kD DNA binding protein. Science *258*, 1808–1812.

Shuai, K., Stark, G.R., Kerr, I.M., and Darnell, J.E., Jr. (1993). A single phosphotyrosine residue of Stat91 required for gene activation by interferon-γ. Science *261*, 1744–1746.

Silvennoinen, O., Ihle, J.N., Schlessinger, J., and Levy, D.E. (1993). Interferon-induced nuclear signaling by Jak protein tyrosine kinases. Nature *366*, 583–585.

Sixby, J.W., Nedrud, J.G., Raab-Traub, N., Hanes, R.A., and Pagano, J.S. (1984). Epstein-Barr virus replication in oropharyngeal epithelial cells. New Eng. J. Med. *310*, 1225–1230.

Spriggs, M.K., Armitage, R.J., Comeau, M.R., Strockbine, L., Farrah, T., Macduff, B., Ulrich, D., Alderson, M.R., Mullberg, J., and Cohen, J.I. (1996). The extracellular domain of the Epstein-Barr virus BZLF2 protein binds the HLA-DRβ chain and inhibits antigen presentation. J. Virol. *70*, 5557–5563.

Stark, G.R., Kerr, I.M., Williams, B.R.G., Silverman, R.H., and Schreiber, R.D. (1998). How cells respond to interferons. Annu. Rev. Biochem. *67*, 227–264.

Steimle, V., Otten, L.A., Zufferey, M., and Mach, B. (1993). Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or bare lymphocyte syndrome). Cell *75*, 135–146.

Strockbine, L.D., Cohen, J.I., Farrah, T., Lyman, S.D., Wagener, F., DuBose, R.F., Armitage, R.J., and Spriggs, M.K. (1998). The Epstein-Barr virus BARF1 gene encodes a novel, soluble colony-stimulating factor-1 receptor. J. Virol. *72*, 4015–4021.

Takada, K., Shimuzu, N., Sakuma, S., and Ono, Y. (1986). Transactivation of the latent Epstein-Barr virus (EBV) genome after transfection of the EB DNA fragment. J. Virol *57*, 1016–1022.

Tomkinson, B.E., Wagner, D.K., Nelson, D.L., and Sullivan, J.L. (1987). Activated lymphocytes during acute Epstein-Barr virus infection. J. Immunol. *139*, 3802–3807.

Tortorella, D., Gewurz, B.E., Furman, M.H., Schust, D.J., and Ploegh, H.L. (2000). Viral subversion of the immune system. Annu. Rev. Immunol. *18*, 861–926.

Urier, G., Buisson, M., Chambard, P., and Sergeant, A. (1989). The Epstein-Barr virus immediate-early protein EB1 activates transcrip-tion from different responsive elements including AP-1 binding sites. EMBO J. *8*, 1447–1453.

Westphal, E.-M., Mauser, A., Swenson, J., Davis, M.G., Talarico, C.L., and Kenney, S.C. (1999). Induction of lytic Epstein-Barr virus infection in EBV-associated malignancies using adenovirus vectors in vitro and in vivo. Cancer Res. *59*, 1485–1491.

Zalani, S., Holley-Guthrie, E., and Kenney, S. (1996). Epstein-Barr viral latency is disrupted by the immediate-early BRLF1 protein through a cell-specific mechanism. Proc. Natl. Acad. Sci. USA *93*, 9194–9199.

Zhang, Q., Gutsch, D., and Kenney, S. (1994). Functional and physical interaction between p53 and BZLF1: implications for Epstein-Barr virus latency. Mol. Cell. Biol. *14*, 1929–1938.

Zimring, J.C., Goodbourn, S., and Offerman, M.K. (1998). Human herpesvirus 8 encodes an interferon regulatory factor (IRF) homolog that represses IRF-1-mediated transcription. J. Virol. *72*, 701–707.