Suppression of type I and type II interferon production and signalling by Epstein-Barr virus BGLF2 protein: abridged secondary publication

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KEY MESSAGES

1. The Epstein-Barr virus (EBV) has to circumvent the production and action of interferons (IFNs) in order to replicate and maintain a high copy number of its DNA genome in infected cells.
2. EBV effectively suppresses induction of IFN-stimulated genes.
3. BGLF2 tegument protein is a potent suppressor of JAK-STAT signalling.
4. BGLF2 recruits SHP1 phosphatase to STAT1 and targets STAT2 for degradation.
5. BGLF2-defective EBV activates IFN signalling more robustly.
6. Our findings have important implications in understanding EBV-associated diseases and developing antivirals or vaccines.

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Introduction

Host innate immunity recognises pathogen-associated molecular patterns of Epstein-Barr virus (EBV) such as its DNA to counteract viral infection, replication, and reactivation. As countermeasures, EBV subverts innate immunity using some of its lytic proteins such as BGLF5, which targets TLR9 for degradation during lytic replication.[1] EBV also encodes a deubiquitinase and a late lytic tegument protein named BPLF1, which deubiquitinates and inactivates multiple signal transducers in innate immune signalling.[2,3] The outcome of EBV infection is dictated by the interplay between EBV and innate immunity, but the mechanistic details of this interplay are incompletely understood. Particularly, additional EBV-encoded suppressors of interferon (IFN) signalling remain to be identified and characterised.

JAK-STAT signalling activated upon binding of IFNs to their respective receptors mediates the downstream effects of all three types of IFNs.[4] For type I and type III IFNs, a ternary complex comprising pSTAT1, pSTAT2, and IRF9 binds to IFN-stimulated response elements (ISREs) in the promoter region of IFN-stimulated genes (ISGs) to activate transcription. For type II IFN, γ-activated factor is a dimeric p-STAT1, which recognises γ-activated sequence in the promoter region of a subset of unique ISGs. Protein tyrosine phosphatases such as SHP1 are negative regulators of JAK-STAT signalling. EBV is thought to subvert JAK-STAT signalling by use of multiple countermeasures. Identification and characterisation of novel EBV-encoded IFN antagonists may elucidate how EBV engages cellular negative regulators and cripples cellular activators to modulate critical events in JAK-STAT signalling.

Methods

We expressed BGLF2 in transfected lymphoid and epithelial cells to characterise its suppressive activity on type I and type II IFN production and signalling. We dissected its action points in the pathways of IFN production and signalling as well as the underlying molecular mechanism. Importantly, we used a BGLF2-deficient recombinant virus to analyse the relevance and biological significance of BGLF2-induced innate immunosuppression in EBV infection and pathogenesis.

Results

Inhibition of type I, type II, and type III IFN signalling by BGLF2

The negative regulatory effect of EBV BGLF2 on IFN signalling was validated. HEK293 cells were used for this experiment owing to its high transfection efficiency and full competence of IFN signalling. BGLF2 inhibited ISRE-Luc activity in a dose-dependent fashion. When levels of ISG15 and OAS1 mRNAs were quantified, BGLF2 was found to exert an inhibitory effect on expression of these two ISGs in the presence of IFN-β. Moreover, BGLF2 dampened IFN-β-stimulated expression of ISG15

protein. Enforced expression of BGLF2 in HEK293 cells resulted in the suppression of IFN-γ-dependent γ-activated sequence–Luc activity. In agreement with this, IFN-γ-activated expression of IP10 transcript was mitigated by BGLF2. A pronounced suppressive effect of BGLF2 on IFN-λ1-induced activation of ISRE-Luc activity, ISG15 mRNA expression, and OAS1 mRNA expression was observed.

Inhibition of STAT1 phosphorylation by BGLF2 through recruitment of SHP1 phosphatase

JAK-STAT signalling is intricately regulated by tyrosine phosphatases.[5] To determine whether tyrosine phosphatases were affected by BGLF2, BGLF2-overexpressing HEK293 cells were either treated or left untreated with Na_3VO_4, which inhibits a wide spectrum of tyrosine phosphatases. Interestingly, treatment with Na_3VO_4 prevented the reduction of p-STAT1 level in the presence of IFN-β and BGLF2. A series of RNA interference experiments were carried out to pinpoint which tyrosine phosphatase was involved. SHP1, which is known to dephosphorylate key transducers of JAK-STAT signalling, was a prime candidate. SHP1 was depleted in HEK293 cells by siRNA. The requirement of SHP1 tyrosine phosphatase for suppression of STAT1 phosphorylation and activation by BGLF2 was verified. Total SHP1 protein levels were not affected by BGLF2, but both SHP1 and BGLF2 were found to associate with STAT1. These findings are in general agreement with the concept that BGLF2 recruits SHP1 to dampen STAT1 signalling.

EBV infection and reactivation by BGLF2 through suppression of type I IFN signalling

The GFP-marked M81 strain of EBV enables identification of infected cells as GFP-positive cells. This facilitates investigation of how IFN-β and BGLF2 affect EBV infection. When HEK293 cells were pre-treated with IFN-β, EBV infectivity diminished, compared with the untreated group. Overexpression of BGLF2 led to reversal of this phenotype. This was generally in keeping with results obtained in flow cytometric analysis of GFP-positive cells. The EBV genome copy number was also diminished upon treatment with IFN-β. Pre-expression of BGLF2 prior to IFN-β treatment reversed the fall in the genome copy number. Furthermore, the expression of EBV lytic genes including BLLF1/gp350, BMRF1, and BRLF1/Rta was suppressed by IFN-β in M81-infected HEK293 cells. This suppression was relieved when BGLF2 was expressed. Hence, BGLF2 counteracts IFN-β in the inhibition of EBV infection.

Discussion

BGLF2 was identified to be a suppressor of JAK-STAT signalling through multiple mechanisms, independent of the modulatory effect on JNK, p38, or NF-κB. BGLF2 binds to STAT1, STAT2, and cullin 1. It recruits tyrosine phosphatase SHP1 to STAT1 and leads to STAT1 dephosphorylation and inactivation. It also recruits cullin 1 to STAT2 and results in STAT2 ubiquitination and proteolysis. Expression of lytic genes is less pronounced in a BGLF2-deficient EBV, concurrent with a more robust induction of ISG expression. Thus, BGLF2 serves as a suppressor of JAK-STAT signalling and a facilitator of EBV infection.

Conclusions of our study and those of another study[5] are generally consistent regarding the suppression of IFN signalling and promotion of EBV reactivation by BGLF2. We demonstrated the impact of BGLF2 on primary infection of EBV. Importantly, we constructed BGLF2-deficient EBV and provided the first evidence for its increased activity in IFN-β signalling.

Type III IFNs exhibit strong antiviral property against herpes simplex virus infection in vivo, but the roles of IFN-1 in EBV infection remain elusive. We demonstrated the inhibitory effect of EBV and its BGLF2 protein on type I and type III IFN signalling. Whether type III IFNs are induced by EBV and influential in EBV primary infection and reactivation warrant further investigations.

BGLF2 is a multifunctional tegument protein. BGLF2 exerts differential effects on STAT1 and STAT2. Particularly, BGLF2 does not target STAT1 for degradation or dephosphorylate STAT2. Further studies are warranted to elucidate the specificity of the effect of BGLF2 on STAT1 and STAT2 by differences in binding domains, nature of conformational changes, and/or partner selection. The SH2 domain present in all STATs for recognition, and binding to pTyr in other JAKs or STATs is highly conserved. In contrast, coiled-coil domain, transcriptional activation domain and other regions in STATs are more divergent. Further studies are needed to determine whether their binding with BGLF2 might be responsible for the differential effects. The conformational changes induced by the binding of BGLF2 with STAT1 and STAT2 may account for the differential effects. We revealed that SHP1 and cullin 1 were key mediators of the effect of BGLF2 on STAT1 and STAT2, respectively. Although no interaction between BGLF2 and SHP2 was detected, further investigations are required to clarify whether additional protein tyrosine phosphatases including SHP2 and additional cullin-type E3 ubiquitin ligases are involved in BGLF2-depenent suppression of STAT1 and STAT2.

Other cellular and viral proteins have been shown to recruit other E3 ubiquitin ligases (UBR4, PDLIM2, and Fbw7) to augment STAT2 ubiquitination and degradation. Further studies are

warranted to determine the mechanisms by which different E3s cooperate with each other to mediate STAT2 ubiquitination and degradation in different physiological and pathological settings. Cullin 1 is only one of the key components in the SCF complex, which represents a large family of cullin-RING E3 ubiquitin ligases. Our finding provides new support to the notion that SCF complex might be critical in the degradation of STAT2. Experimental validation of the role of SKP1 and cullin 1 in STAT2 ubiquitination and degradation is warranted.

BGLF2 is highly conserved among herpesviruses. Homologs of EBV BGLF2 include herpes simplex virus 1 UL16, murine gammaherpesvirus 68 ORF33, cytomegalovirus UL94, varicella zoster virus ORF44, and Kaposi's sarcoma-associated herpesvirus ORF33. Some of these homologs are also known to be capable of inducing cell cycle arrest. It is important to investigate if they can also recruit SHP1 and cullin 1 to suppress JAK-STAT signalling.

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Disclosure

The results of this research have been previously published in:

1. Jangra S, Bharti A, Lui WY, et al. Suppression of JAK-STAT signaling by Epstein-Barr virus tegument protein BGLF2 through recruitment of SHP1 phosphatase and promotion of STAT2 degradation. J Virol 2021;95:e0102721.

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