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CXCL10 Induces Lytic Reactivation of EBV through *EXTL*1

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Abstract

Epstein-Barr virus (EBV) infects over 90% of the global population, establishing latent infections in most individuals. Under specific conditions like inflammation and immune suppression, EBV can be reactivated, leading to the initiation and progression of related diseases. While inflammation is known to induce EBV reactivation, the precise mechanisms underlying this phenomenon remain unclear. Chemokine (C-X-C motif) ligand (CXCL10), a key inflammatory factor, plays a significant role in various infectious diseases. In this study, we investigated how CXCL10 levels regulate the transition between the latent and lytic replication phases of the EBV lifecycle using cell culture, Western blot, fluorescent quantitative PCR, immunofluorescence, and flow cytometric apoptosis assays. Our findings indicate that CXCL10 induces EBV transition from latency to lytic replication through its receptor CXCR3 by regulating the downstream effector, exostosis-like glycosyltransferase 1. Additionally, CXCL10 activates the JAK2/STAT3 pathway. This study confirms the role of CXCL10 in promoting EBV lytic replication, providing crucial insights into the pathogenic mechanisms of inflammation-triggered EBV reactivation.

Keywords

Epstein-Barr Virus, Reactivation, Inflammation, Chemokine (C-X-C Motif) Ligand 10, EXTL1

1. Introduction

Epstein-Barr virus (EBV) or human herpesvirus 4, is a member of the gamma-herpesvirus family [1] discovered by Epstein and Barr in Burkitt lymphoma in 1964, marking the first identified human tumor virus. EBV has since been closely associated with various tumors, including nasopharyngeal carcinoma and

gastric cancer [2]. Besides malignancies, EBV is associated with various non-malignant diseases, including infectious mononucleosis, oral hairy leukoplakia, systemic lupus erythematosus, and multiple sclerosis [3] [4]. EBV infection is a very common infection primarily transmitted through saliva, but also through blood transfusion, organ transplantation, and other routes, with humans being its sole host [5]. EBV-induced diseases affect multiple organs and systems, presenting diverse clinical manifestations and posing significant health risks [6] [7].

EBV has a biphasic lifecycle, specifically infecting human oropharyngeal B lymphocytes through endocytosis or fusion with the plasma membrane, leading to two forms of infection: lytic replication and latent infection [8]. These two lifecycles can interconvert, reflecting a highly regulated interaction with the host, influenced by environmental factors or cell types [9]. The primary EBV infection is usually asymptomatic, leading to latent infections in most individuals. In immunocompetent individuals, the immune system effectively clears most infected cells and the virus, with only a small portion of EBV establishing lifelong latent infections in host lymphoid tissues [10]. In a normal immune environment, latent EBV maintains a balanced state with the immune system. However, under certain stimuli such as inflammation, stress, fever, immune suppression, emotions, or pressure, EBV reactivation can occur. As cell immunity weakens, EBV reenters the lytic replication phase, causing acute infectious symptoms [11]. Lytic replication of EBV is evident in EBV-associated diseases, with infectious viral particles produced during this phase being necessary for the spread between cells and hosts [12]. Furthermore, the lytic replication of EBV following reactivation replenishes the cellular reservoir of latent infection [13].

Inflammation is a crucial trigger for EBV reactivation and is closely linked to various diseases [14]. Furthermore, the reactivation of the latent virus contributes to inflammation initiation and development due to suppressed immune cells, leading to heightened inflammatory responses and an inflammatory storm where inflammatory factors play a crucial role [15].

The chemokine (C-X-C motif) ligand (CXCL) 10, also known as IP-10, is a 10 kDa peptide belonging to the CXC chemokine family. Its mRNA was first isolated by Luster *et al.* in 1985 from monocytes stimulated with interferon (IFN)-γ [16]. The secretion of CXCL10 is primarily driven by IFN-γ and other endogenous cytokines, with lipopolysaccharide (LPS) stimulation playing a secondary role [17]. Once secreted, CXCL10 binds to its receptor, C-X-C motif chemokine receptor 3 (CXCR3), a classic seven-transmembrane G protein-coupled receptor, and exerts its function [18]. The plasma levels of CXCL10 are elevated in individuals with EBV-related diseases compared to those with disease not related to EBV, highlighting the crucial role of CXCL10 in viral infection-associated diseases [19].

Exostosin-like glycosyltransferase 1 (EXTL1), a member of the exostosin protein family, possesses glycosyltransferase activity crucial for the initiation and elongation of heparan sulfate proteoglycans (HSPG), which are essential for cell-cell interactions within the extracellular matrix. EXTL1 plays a significant

role in protein glycosylation, impacting cell adhesion, migration, and proliferation [20]. While the precise role of EXTL1 in immune responses and inflammation remains underexplored, emerging evidence suggests its involvement in regulating immune cell activation and inflammatory processes. Abnormal EXTL1 expression has been associated with various diseases, including cancers and genetic disorders, and it may influence viral entry, spread, and immune evasion mechanisms. In this study, we observed an upregulation of EXTL1 during the CXCL10-induced EBV reactivation process, indicating its potential role in modulating the EBV lifecycle. Understanding EXTL1's function could provide insights into the mechanisms linking inflammation and EBV reactivation, offering potential therapeutic targets for related diseases.

2. Materials and Methods

2.1. Reagents and Samples

Recombinant human CXCL10 (IP-10) was purchased from PeproTech (Catalog #: 300-12); CXCR3 inhibitor AMG487 was acquired from MCE (Catalog #: HY-15319); antibodies against BZLF1 and glycoprotein (gp) 350 were obtained from Santa Cruz (Catalog #: sc-53904, sc-56981); antibodies against BRLF1 were sourced from Boster Bio (Catalog #: bs-4542R); antibodies against Extl1 were procured from ImmunoWay Biotechnology (Catalog #: YN5348); antibodies against phosphorylated Janus kinase 2 (p-JAK2) and phosphorylated Signal Transducer and Activator of Transcription 3 (p-STAT3) were purchased from CST (Catalog #: 4406T; Catalog #: 9145T); antibodies against JAK2 were obtained from Affinity (Catalog #: AF6002); antibodies against STAT3 were sourced from Proteintech (Catalog #: 60199-1-Ig); JAK2/STAT3 inhibitor ruxolitinib was acquired from MCE (Catalog #: HY-50856); whole protein extraction kits were purchased from KeyGen Biotech (Catalog #: KGP250); RNA extraction kits were obtained from Shanghai Yishan Biotech (Catalog #: RN001).

2.2. Cell Culture

EBV-positive human Burkitt's lymphoma cell line Raji (Catalog No: CL-0189) and EBV-transformed human peripheral blood B lymphocyte immortalized cell line LCL (Catalog No: CL-0774) were purchased from Wuhan Pronas Life Technologies Co., Ltd. Raji cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (PM150110) supplemented with 10% fetal bovine serum (FBS; 164210-50) and 1% penicillin/streptomycin solution (P/S; PB180120). LCL cells were cultured in a specialized LCL medium (RPMI-1640 + 10% FBS + 1% P/S). The culture medium for Raji cells was replaced every two days.

When suspension cell density reached 80%, centrifugation was performed at 1200 rpm for 5 minutes to remove the supernatant. After adding fresh medium, passaging was carried out at a 1:3 ratio. Cells were cultured at 37˚C with 5% carbon dioxide and were used for the assessment of target protein levels. Following seeding into culture plates, cells were grouped for treatment: the control

group (solvent only), solvent + CXCL10 groups (trehalose-containing solvent + concentrations of CXCL10 in the medium of 0 ng/mL, 10 ng/mL, 100 ng/mL, or 200 ng/mL), solvent + CXCL10 + AMG487 groups (dimethyl sulfoxide [DMSO] as the solvent +100 ng/mL AMG487 + concentrations of CXCL10 in the medium of 0 ng/mL, 10 ng/mL, 100 ng/mL or 200 ng/mL), solvent + CXCL10 + ruxolitinib group (DMSO + 100 ng/mL ruxolitinib + concentrations of CXCL10 in the medium of 0 ng/mL, 10 ng/mL, 100 ng/mL, and 200 ng/mL), solvent + CXCL10 + siRNA Extl1 group (diethyl pyrocarbonate water as the solvent + siRNA Extl1 + concentrations of CXCL10 in the medium of 0 ng/mL, 10 ng/mL, 100 ng/mL, and 200 ng/mL).

2.3. Western Blot

Untreated and treated Raji and LCL cells were collected and centrifuged to remove the supernatant. The cells were sonicated after adding lysis buffer. After centrifuging at 4˚C and 12000 rpm for 15 minutes, the cleared cellular protein samples were separated from the upper layer, mixed with 2× SDS loading buffer and heated to 95˚C for 5 minutes.

Thirty micrograms of protein were loaded onto a polyacrylamide gel for electrophoresis and transferred to a nitrocellulose membrane. After blocking with 5% skimmed milk at room temperature for 1 - 2 hours, the membrane was incubated overnight at 4˚C with primary antibodies against BZLF1 (1:1000), BRLF1 (1:1000), gp350 (1:1000), JAK (1:1000), STAT (1:1000), p-JAK (1:1000), p-STAT (1:1000), Extl1 (1:1000), GAPDH (1:1000), or β-actin (1:1000). The next day, the membrane was washed three times with TBS-T at room temperature, followed by incubation with secondary antibodies (1:1000) for 1 hour.

2.4. Fluorescent Quantitative PCR Detection

Raji and LCL cells were seeded in a 6-well culture plate. After 24 hours of treatment, total RNA was extracted from the cells using a total RNA extraction kit and reverse transcribed into cDNA using the TAKARA reverse transcription kit. Takara fluorescent quantitative detection kit was used to assess BZLF1, BRLF1, JAK, STAT, p-JAK, p-STAT, and EXTL mRNA levels. Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

2.5. Immune of Fluorescence Detection of Cells

After passage, Raji cells were seeded in culture plates and treated. The control group was treated with the solvent only, and the solvent + CXCL10 group was treated with a solvent containing seaweed sugar and 100 μmol/L CXCL10. After 24 hours of culture, the supernatant was removed by centrifugation, and cells were resuspended. Following three washes with PBS, cells were fixed at room temperature with 4% paraformaldehyde for 30 minutes. Membrane permeabilization was achieved with 0.25% TritonX-100 for 15 minutes, followed by blocking with 5% bovine serum albumin for 1 hour at room temperature.

Anti-BZLF1 monoclonal antibody (1:500) and Anti-BRLF1 polyclonal antibody (1:500) were added separately and incubated overnight at 4˚C. After PBS washing, mouse and rabbit IgG fluorescent secondary antibodies were added and incubated at room temperature for 1 hour. Following another PBS wash, DAPI staining was conducted at room temperature for 10 minutes. Subsequently, fluorescent signals were observed under a fluorescence microscope.

2.6. Flow Cytometric Apoptosis Assay

Flow cytometric apoptosis assay was conducted using the annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit. Raji cell apoptosis was detected using an FACSCalibur flow cytometer. Raji cells (1×10^6 cells/well) were washed, resuspended in PBS buffer, and stained with annexin V-FITC and PI at 37˚C for 30 minutes.

2.7. Statistical Analysis

The statistical significance of differences between two groups was assessed using either an unpaired t-test or a two-tailed unpaired Mann-Whitney test. Differences among multiple groups were analyzed using one-way analysis of variance (ANOVA). A P-value < 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism software.

3. Results

3.1. CXCL10 Induces the Reactivation of Latent EBV in Infected Cells

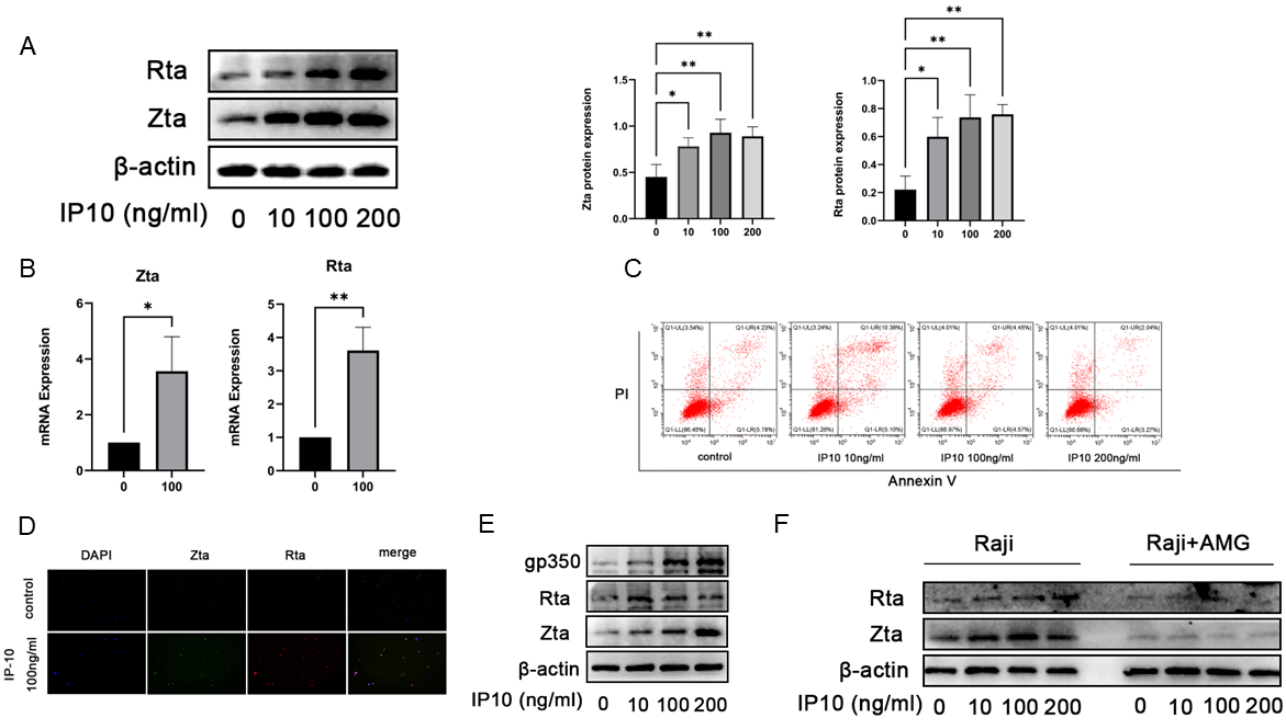
To investigate the impact of CXCL10 on the interconversion of EBV lifecycle, we treated EBV latent infection Burkitt's lymphoma Raji cells and EBV-transformed human peripheral blood B lymphocytes LCL cells with purified CXCL10 at concentrations of 10 ng/mL, 100 ng/mL, and 200 ng/mL. After 24 h, we assessed the expression of lytic replication proteins BZLF1, and BRLF1.

The experimental results revealed that CXCL10-induced Raji cells exhibited significantly higher levels of lytic replication proteins than the non-induced control group (**Figure 1(A)**). These results were also confirmed at the transcriptional level (**Figure 1(B)**). Flow cytometry analysis revealed no statistical differences in cell apoptosis among different CXCL10 concentrations (**Figure 1(C)**).

Immunofluorescence analysis revealed a significant increase in BZLF1 and BRLF1 expression at protein levels in the experimental group treated with CXCL10 at 100 ng/mL compared to the control group (**Figure 1(D)**). This trend was consistent in both LCL and Raji cell lines (**Figure 1(E)**). To further confirm the role of CXCL10 in EBV lytic reactivation, we employed AMG487, a specific antagonist of CXCR3.

Raji cells treated with CXCL10 were harvested 24 h post-treatment with or without the inhibitor, and protein levels of BZLF1 and BRLF1 were assessed. As shown in **Figure 1(F)**, AMG487 significantly reduced the expression levels of

EBV lytic replication proteins in Raji cells, indicating that CXCL10 primarily induces EBV lytic reactivation through CXCR3 binding. Blocking this interaction effectively suppresses EBV reactivation.



(A) Western blot analysis of EBV lytic replication proteins BZLF1 and BRLF1 in Raji cells treated with different concentrations of CXCL10. The experiment was conducted in triplicate, and statistical analysis was carried out using one-way ANOVA. Results with $P < 0.05$ were considered statistically significant. (B) Relative mRNA expression of BZLF1 and BRLF1 in Raji cells treated with CXCL10 at 100 ng/mL. The experiment was conducted in triplicate, and statistical significance was considered for P-values < 0.05. (C) Assessment of cell apoptosis after exposure to different concentrations of CXCL10 assessed using flow cytometry. (D) Detection of BZLF1 and BRLF1 expression using immunofluorescence. (E) Western blot analysis of EBV lytic phase proteins BZLF1, BRLF1, and gp350 in LCL cells treated with different concentrations of CXCL10. (F) Inhibition of CXCL10 receptor CXCR3 with AMG487 and its effect on EBV lytic replication phase BZLF1 and BRLF1 protein expression.

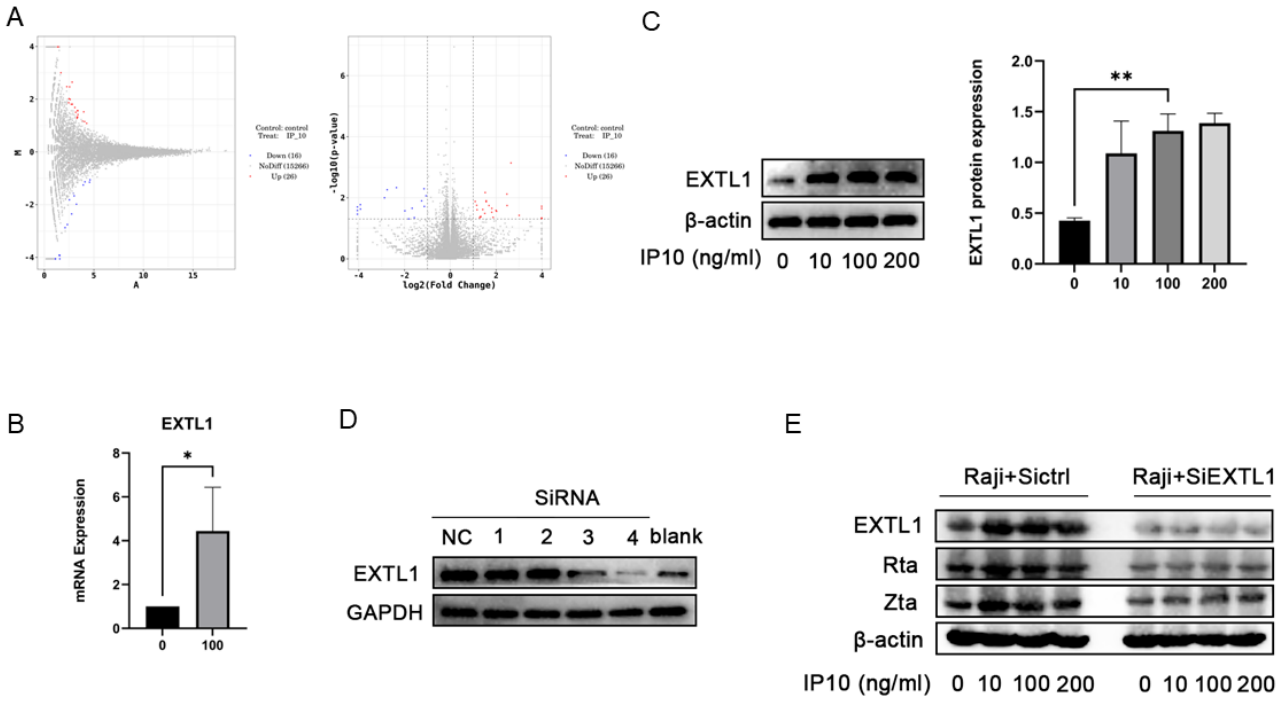
Figure 1. CXCL10 induces EBV lytic reactivation and upregulates Zta, Rta, and gp350 expression, counteracted by the CXCR3 Inhibitor AMG487.

3.2. EXTL1 Regulates CXCL10-Induced EBV Reactivation

To further explore the molecular mechanisms of CXCL10-induced EBV reactivation, Raji cells were treated with 100 ng/mL CXCL10 for 24 hours. Transcriptomic sequencing was employed, and genes differentially expressed between the treated and control groups were determined (**Figure 2(A)**). In total, 42 differentially expressed genes were identified, with 26 upregulated and 16 downregulated genes. Analysis of these differentially expressed genes revealed *EXTL*1 as a key gene with significant expression changes before and after CXCL10-induced EBV reactivation, prompting further investigation into its role.

Our study revealed that CXCL10 treatment upregulated the expression of Extl1 in Raji cells at both transcriptional (**Figure 2(B)**) and protein (**Figure 2(C)**) levels. To confirm whether CXCL10-induced EBV reactivation is mediated via

*EXTL*1, we specifically silenced it using siRNA (Figure 2(D)). Experimental and control groups were transfected with si*EXTL*1 or non-targeting siRNA, respectively. Cells were treated with CXCL10 at concentrations ranging from 0 ng/mL to 200 ng/mL for 24 hours. As shown in Figure 2(E), knocking down *EXTL*1 significantly inhibited CXCL10-mediated EBV reactivation.



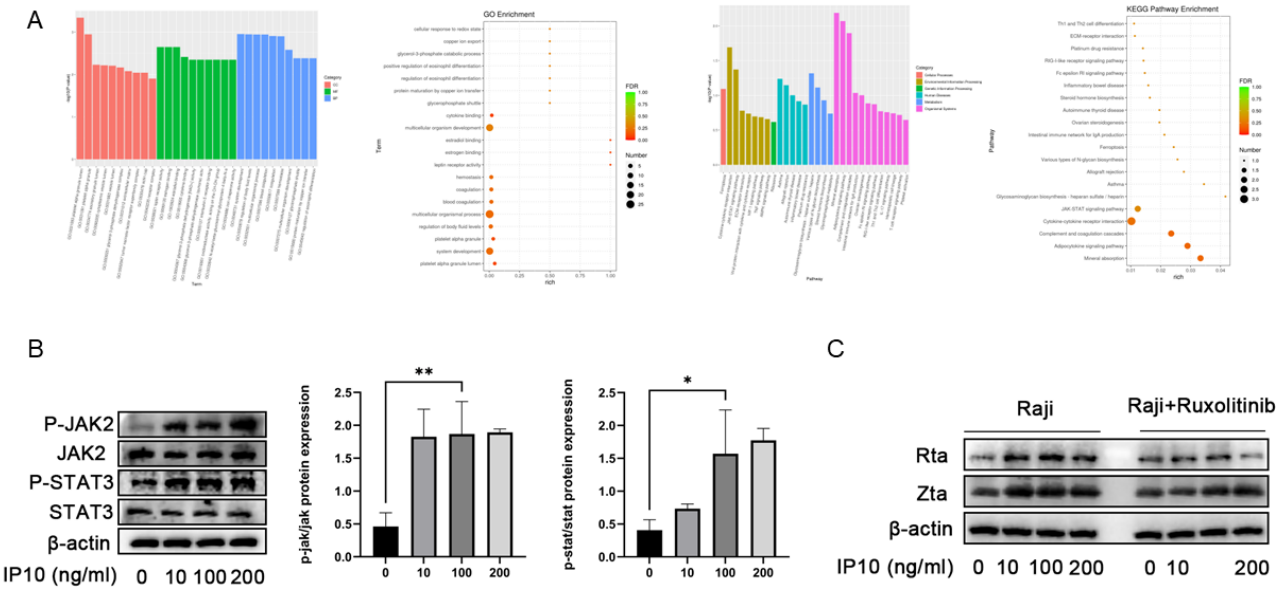
(A) Raji cells were divided into experimental and control groups before and after induction by CXCL10 for transcriptome sequencing analysis to detect changes in gene expression. (B) Relative mRNA expression of EXTL1 was evaluated in Raji cells treated with CXCL10 at 100 ng/mL. The experiment was conducted in triplicate, and statistical significance was considered for P-values < 0.05. (C) Expression of Extl1 protein in CXCL10-induced Raji cells assessed using Western blot. The experiment was conducted in triplicate, and statistical significance was determined with $P < 0.05$. (D) Specific knockdown of EXTL1 using siRNA (E) The impact of different concentrations of CXCL10 on the expression of EBV lytic replication phase proteins BZLF1 and BRLF1 assessed using Western blot.

Figure 2. CXCL10 upregulates *EXTL*1 expression in Raji Cells, and *EXTL*1 siRNA inhibits CXCL10-induced EBV lytic reactivation in latently infected cells.

3.3. The JAK2/STAT3 Signaling Pathway Is Reactivated during the CXCL10-Induced EBV Lytic Replication Process

In the transcriptomic analysis, we conducted functional gene analysis including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses to understand the roles of the identified genes and their associated pathways (Figure 3(A)). Our KEGG analysis highlighted an enrichment of differentially expressed genes involved in the JAK/STAT signaling pathway. To delve deeper into the mechanisms underlying CXCL10-induced EBV lytic reactivation, Raji cell lines were treated with varying concentrations of CXCL10 (0 ng/mL, 10 ng/mL, 100 ng/mL, and 200 ng/mL). Subsequently, the expression of key proteins in the JAK/STAT signaling pathway was examined.

Protein levels of p-JAK2 and p-STAT3 in Raji cell lines were significantly higher in the treated groups than in the control group (Figure 3(B)). Ruxolitinib specifically inhibits JAK2, blocking the JAK/STAT signaling pathway. Its addition to Raji cells treated with CXCL10 significantly decreased the expression of lytic replication genes BZLF1 and BRLF1-encoding gene in the genomic DNA (Figure 3(C)).



(A) Functional and GO and KEGG enrichment analyses (B) Impact of different concentrations of CXCL10 on the JAK/STAT signaling pathway in Raji cells assessed using Western blot. The experiment was conducted in triplicate, and statistical analysis was carried out using one-way ANOVA. Results with $P < 0.05$ were considered statistically significant. (C) JAK2 inhibitor ruxolitinib impacts the protein expression of EBV lytic replication phase proteins BZLF1 and BRLF1, as revealed by Western blot.

Figure 3. Activation of the JAK2/STAT3 signaling pathway in CXCL10-induced EBV lytic reactivation is inhibited by JAK2 inhibitor ruxolitinib

4. Conclusions

Significantly elevated plasma levels of CXCL10, alongside abundant CXCL10 and CXCR3 cells, were previously reported in patients with herpesvirus reactivation [21]. Recently, in EBV-positive diffuse large B-cell lymphoma (DLBCL) with chronic inflammation, prolonged inflammation was shown to contribute to disease development. EBV-positive pyothorax-associated lymphoma (PAL) serves as a valuable model for studying this disease category. Various types of PAL cells exhibit high expression and secretion of CXCL10, the ligand of CXCR3, while EBV-negative DLBCL cell lines do not. The expression of CXCL10 was detected in patient PAL tumor biopsy samples, along with abundant CXCR3-positive lymphocytes in tissue samples [22]. Further investigation into the role of CXCL10/CXCR3 in EBV is warranted.

The inflammatory response to viral infection seems to be related to the pathogenesis of virus-associated diseases, promoting cell transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis [23]. Persistent vi-

ruses such as herpesviruses are not cleared from the host, but alternate between lytic and latent states throughout their entire lifecycle [24]. The direct role of inflammation in regulating the lifecycle of infectious viral factors is still unclear. The transition between the latent and lytic replication phases during the virus lifecycle and the crucial role of inflammation in this process necessitates further exploration.

Cytokines play a dual role in many viral infections. They serve as key mediators in communication, initiation, activation, and immune balance for the host. Simultaneously, viruses manipulate host cytokine production to support their survival, replication, and infection [25]. This mutually reinforcing cycle of inflammation and viral infection poses a significant threat and can trigger various severe diseases, including malignant tumors. The association between inflammation, cytokines, and herpesvirus reactivation has been previously established [26].

In this study, we investigated the relationship between the pro-inflammatory factor CXCL10 and the Th1-related chemokine IFN-γ-induced CXCL10 in inducing EBV lytic reactivation. Using latent EBV-positive Raji and LCL cells as a model system, we found that CXCL10 addition to latent EBV-positive cells resulted in elevated expression of EBV immediate-early lytic genes BZLF1 and BRLF1.

In EBV, BZLF1 (encoding Zta) and BRLF1 (encoding Rta) expression can trigger lytic replication [27], while the late lytic gene encoding gp350 is expressed during active infection or lytic reactivation [28]. CXCL10 acts either in a paracrine or autocrine manner, potentially inducing the transition of EBV from the latent to the lytic activation phase by binding to CXCR3 [29]. CXCL10/ CXCR3 interaction regulates essential biological functions in several viral infections, such as those with lymphotropic neurotropic virus, enterovirus, and hepatitis C virus [30].

AMG487 specifically blocks CXCR3, reducing the expression of immediate early lytic genes *BZLF*1 and *BRLF*1. This indicates that CXCL10-mediated EBV transition from latency to lytic replication is primarily CXCR3-mediated. Simultaneous addition of AMG487 and CXCL10 effectively inhibits EBV reactivation, potentially impairing downstream signal transduction functions, emphasizing the crucial role of CXCL10 and CXCR3 in regulating the EBV infection cycle. Additionally, the group treated solely with AMG487, without CXCL10, exhibited a lower expression of lytic replication phase EBV proteins than the blank control group. The degree of EBV reactivation was partially inhibited by AMG487. We posit this inhibition is likely due to AMG487 acting as a specific inhibitor of CXCR3, not only blocking CXCL10 but also suppressing other ligands such as CXCL9 and CXCL11 [31], leading to a comprehensive blockade of all ligands of CXCR3. This results in impairments in all CXCR3-related signal transduction pathways within the cells.

The CXCL9, -10, -11/CXCR3 axis primarily regulates the migration, differen-

tiation, and activation of immune cells, with Th1 polarization activating immune cells in response to IFN-γ. This axis also plays a role in immune reactivity within the tumor environment, using both paracrine and autocrine signaling to recruit immune cells [32]. Therefore, other members of the CXC chemokine family may also regulate the EBV lifecycle. After CXCL10 induction and reactivation of EBV-positive Raji cells, further transcriptomic analysis revealed a significant increase in the transcriptional expression of EXTL1. Moreover, assessment at the protein level led to similar results. EXTL1 is known as a tumor suppressor gene [33], with recent research also revealing its significant role in infection and autoimmunity [34].

A detailed investigation into the role of EXTL1 in EBV lytic reactivation was carried out. Raji cells were transfected with siRNA targeting EXTL1. Under CXCL10 induction, the experimental group with siRNA transfection exhibited a significant reduction in the expression of EBV early lytic genes BZLF1 and BRLF1 compared to the control group without siRNA transfection. This suppression of EBV reactivation under CXCL10 induction indicates that the loss of EXTL1 stabilizes the latent state of EBV and weakens the response induced by CXCL10, emphasizing the crucial role of EXTL1 in the CXCL10-induced reactivation of EBV.

In the transcriptomic analysis, functional gene analysis was performed using GO and KEGG pathway enrichment analyses to determine gene functions and associated pathways. The study revealed the enrichment of multiple differentially expressed genes in the JAK/STAT signaling pathway. STAT3, serving as the executive molecule of this pathway, is a transcription factor activated through various signaling transduction pathways or EBV infection [35]. STAT3 has been described as a critical switch between the latent and lytic phases of EBV, inhibiting the lytic reactivation of EBV in B lymphocytes through PCBP2 [36].

To better understand EBV lytic reactivation, the protein levels of total and phosphorylated JAK2 and STAT3 were assessed in a cellular model before and after CXCL10 induction. While total protein levels of JAK2 and STAT3 remained unchanged after CXCL10 induction, p-JAK2 and p-STAT3 significantly increased, indicating the activation of the JAK2/STAT3 signaling pathway. Studies suggest over 50% of latent EBV may be refractory, resisting entering the cell lytic cycle. The mechanism underlying this process remains unclear but it seems closely associated with high levels of unphosphorylated STAT3 [37]. Simultaneously, dimerized p-STAT3 activation promotes the transcription of unphosphorylated STAT3, creating a positive auto-regulatory loop [38]. We hypothesize that conflicting decisions governing the EBV life cycle—CXCL10-induced reactivation and STAT3 maintaining latency—are controlled by various factors.

Ruxolitinib, which inhibits JAK/STAT3 pathway activation, significantly decreased the CXCL10-induced expression of immediate early lytic genes BZLF1 and BRLF1. Ruxolitinib is a selective JAK1/2 inhibitor that reduces inflammation and has immunosuppressive effects [39] [40]. Additionally, in a clinical study,

pediatric patients with EBV-related hemophagocytic lymphohistiocytosis were more sensitive to ruxolitinib treatment than patients with a disease not related to EBV. Most of the patients with EBV-related hemophagocytic lymphohistiocytosis had eruptive primary EBV infection [41]. Ruxolitinib also serves as an effective alternative treatment for patients who require avoidance of long-term corticosteroid use, which may induce EBV activation and hemophagocytic lymphohistiocytosis recurrence [42].

Therefore, ruxolitinib effectively inhibits the JAK1/2/STAT3 pathway, reducing STAT3 phosphorylation, inhibiting the expression of downstream proinflammatory cytokines, and suppressing factors that induce EBV lytic reactivation. Furthermore, it significantly reduces EBV viral load [43] [44], which may also contribute to the inhibition of EBV lytic reactivation. Our study led to similar results.

However, some studies suggest that ruxolitinib, in combination with other immunosuppressive factors like splenectomy and long-term steroid therapy, may trigger EBV reactivation [45]. Therefore, further research is needed to validate the relationship between EBV and ruxolitinib.

This study provides evidence for the impact of inflammation on the transition of the EBV life cycle. We observed a significant increase in Extl1 protein level after CXCL10 induction, indicating its crucial role in viral transitioning to the lytic reactivation state. This protein acts as a positive regulatory factor induced during the lytic cycle. The study also revealed that STAT3, a critical switch regulating the EBV latent state, is influenced by various induction factors. Understanding these key roles in the EBV life cycle is essential for future treatments of EBV-related diseases and for elucidating the immune control mechanisms of various cellular factors.

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Ethics Approval and Consent to Participate

Not applicable.

Data Availability Statement

The data that support the findings of this study are not publicly available.

Consent for Publication

Not applicable.

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Author Contributions

DBN and LYG conceived and designed the experiments. DBN, WYL, ZYY performed the experiments. DBN analyzed the data. DBN wrote the paper. LYG reviewed the manuscript. All authors contributed to this paper and approved the submitted version.

Conflicts of Interest

The authors declare no commercial or financial relationships and no known conflicts of interest.

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