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Mapping regions of Epstein–Barr virus (EBV) glycoprotein B (gB) important for fusion function with gH/gL

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ABSTRACT

Glycoproteins gB and gH/gL are required for entry of Epstein–Barr virus (EBV) into cells, but the role of each glycoprotein and how they function together to mediate fusion is unclear. Analysis of the functional homology of gB from the closely related primate gammaherpesvirus, rhesus lymphocryptovirus (Rh-LCV), showed that EBV gB could not complement Rh gB due to a species-specific dependence between gB and gL. To map domains of gB required for this interaction, we constructed a panel of EBV/Rh gB chimeric proteins. Analysis showed that insertion of Rh gB from residues 456 to 807 restored fusion function of EBV gB with Rh gH/gL, suggesting this region of gB is important for interaction with gH/gL. Split YFP bimolecular complementation (BiFC) provided evidence of an interaction between EBV gB and gH/gL. Together, our results suggest the importance of a gB–gH/gL interaction in EBV-mediated fusion with B cells requiring the region of EBV gB from 456 to 807.

Introduction

Epstein–Barr Virus (EBV) is a gammaherpesvirus that has a high prevalence in humans, with more than 90% of the population latently infected with the virus (Rickinson, 2007). Primary infection during childhood is typically asymptomatic, but infection in adolescents can result in the development of infectious mononucleosis. Virions are transmitted through the saliva into epithelial cells of the oral pharynx, and subsequently to resting B cells. Following this initial infection, EBV persists in a latent state in memory B lymphocytes where it can remain indefinitely (Babcock et al., 1998; Thorley-Lawson and Babcock, 1999). The expansion and proliferation of these cells can lead to the development of a number of EBV-related malignancies including Hodgkin's disease, Burkitt's lymphoma, some T-cell lymphomas, and tumors associated with epithelial tissues such as nasopharyngeal carcinoma and gastric carcinoma (Rickinson, 2007). EBV is also associated with the lymphoproliferative disorders in immunocompromised patients, such as oral hairy leukoplakia and post-transplant lymphoproliferative disorders (Rickinson, 2007).

Entry of herpesviruses requires the concerted efforts of multiple glycoproteins in a multi-step process that results in binding of the virus to the cell surface, interaction with cellular entry receptors, membrane-virion fusion, and internalization of the virion (Spear and Longnecker, 2003). For Epstein–Barr virus (EBV), the viral glycoproteins required for fusion are glycoprotein B (gB), the complex of gH and gL (gH/gL), and gp42 (Spear and Longnecker, 2003). Infection of B cells is initiated by an interaction between glycoprotein gp350/220 and the CD21/CR2 cellular receptor (Fingeroth et al., 1984; Nemerow et al., 1985; Tanner et al., 1987; Speck et al., 2000). Following initial binding, viral glycoprotein gp42 binds to human leukocyte antigen (HLA) class II molecules and triggers fusion, which is mediated by glycoproteins gB, gH, and gL (Li et al., 1997; Speck et al., 2000). The mechanism of infection of epithelial cells by EBV is less clear, though it has recently been found that fusion is triggered upon binding of gH/gL with cellular integrins αvβ6 or αvβ8 (Chesnokova et al., 2009). Glycoprotein gB and the heterodimer gH/gL constitute the conserved fusion machinery that is required for fusion of the virion and cell membrane across all members of the herpesviridae (Spear and Longnecker, 2003; McShane and Longnecker, 2004). However, the mechanism of how these glycoproteins function to mediate fusion is still unclear and the subject of much investigation.

Glycoprotein B is highly conserved throughout the herpesvirus family, and has been shown to be an essential component of virus-cell fusion (Spear and Longnecker, 2003). The crystal structure of EBV gB in a presumed postfusion trimer conformation was recently reported and

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has provided valuable information on the structural domains important for mediating fusion (Backovic et al., 2009). Based on the distinct structural properties of the glycoprotein, EBV gB was added to the class III group of viral fusion proteins that previously included glycoprotein G of Vesicular Stomatatis virus (VSV) and glycoprotein B of Herpes Simplex virus 1 (HSV-1) (Heldwein et al., 2006; Roche et al., 2006; Backovic and Jardetzky, 2009). The fusion domain of gB contains the two putative fusion loops that are thought to insert into the target membrane through a conformational change that brings about fusion between the two membranes. Mutagenic analysis of the hydrophobic residues within the putative fusion loops of EBV and HSV-1 gB significantly reduced fusion function, demonstrating that the putative fusion loops are essential for the ability of gB to mediate fusion (Backovic et al., 2007; Hannah et al., 2007, 2009). These studies, as well as others, have demonstrated that gB is likely the major fusogen that mediates fusion of the virion and cell membrane. However, a distinct deviation from other class III fusion proteins is that EBV and HSV-1 gB require the gH/gL complex for virus entry.

A central focus in understanding the entry of herpesviruses is deciphering the interactions that occur between viral glycoproteins and how these associations drive fusion. Much of our present knowledge on this matter has come from studies of HSV-1. In addition to interactions between the HSV-1 viral glycoproteins gD and gH/gL and gD and gB, a direct interaction between glycoproteins gB and gH/gL was detected using split fluorescence bimolecular fluorescence complementation (BiFC) assays (Atanasiu et al., 2007; Avitabile et al., 2007). For EBV, evidence for an interaction between gB and gH/gL first came from a study of gL from the closely related rhesus lymphocryptovirus (Rh-LCV) that suggested the role of gL in fusion involves the activation or recruitment of gB with the gH/gL complex (Omerovic and Longnecker, 2007; Plate et al., 2009). The Rh-LCV glycoproteins share a high sequence similarity with EBV but Rh gL could not mediate fusion with B cells when paired with EBV gB. However, a complete restoration of the fusion function of Rh gL was observed when paired with Rh gB, demonstrating a species-specific reliance between these two glycoproteins that are essential for fusion. The essential dependence between gB and gL was used to map the regions and specific amino acid residues of gL that are necessary for mediating fusion function with gB. Subsequent studies found that EBV gB was unable to mediate B cell fusion when expressed with the Rh-LCV glycoproteins necessary for fusion (gp42, gH, and gL) while expression of all Rh-LCV glycoproteins together mediated fusion with B cells very efficiently. Given that the sequence similarity between EBV and Rh gB is near 86%, we hypothesized that regions of gB essential for mediating fusion function with gH/gL could be identified using chimeric EBV/Rh gB proteins.

The objective of this study was to further analyze the interaction between gB and gH/gL in EBV and Rh-LCV and map regions of gB that are involved in the interaction with gH/gL. Analysis of the EBV/Rh gB chimeras in the cell–cell fusion assay identified the region from residues 456 to 807 as being important for a functional interaction with gH/gL, with evidence that sites from both regions 456–628 and 628–807 are required. Further analysis of chimeras using BiFC confirmed that insertion of these regions of Rh gB into EBV gB could modestly enhance the ability of EBV gB to interact with Rh gH/gL. These results provide further evidence of an interaction between gB and gH/gL and highlights that there are likely multiple domains of gB required for an interaction with gH/gL.

Results

Rh-LCV gB does not complement EBV gB for fusion function

The glycoproteins of the closely related EBV and Rh-LCV share a high degree of sequence homology and have proven to be a useful tool in studying the required domains for glycoprotein fusion function. We previously found that Rh gL can only mediate fusion with EBV glycoproteins when expressed together with Rh gB, suggesting that there is a species-specific dependence between gL and gB that is necessary for these two glycoproteins to mediate fusion together (Omerovic and Longnecker, 2007; Plate et al., 2009).

To examine the dependence between gB and gL in mediating fusion with B cells further, we examined the functional homology of gBs from EBV to the closely related Rh-LCV. Similar to what we previously found (Omerovic and Longnecker, 2007; Plate et al., 2009) but somewhat different from others (Wu and Hutt-Fletcher, 2007), fusion with EBV gp42, gB, and gH and Rh gL was near background levels while fusion with EBV gp42, gH, and Rh gL and gB was restored to wild-type EBV fusion activity (Fig. 1A). Rh-LCV has been shown to infect human B cells in vitro (Moghaddam et al., 1998) so it was not surprising that expression of Rh gp42, gB, gH, and gL can also mediate fusion with B cells in our cell-based fusion assay (Fig. 1B, column 1). However, the ability of the Rh-LCV glycoproteins to mediate fusion was significantly reduced when EBV gB was expressed in place of Rh gB (Fig. 1B, column 2). This observation is in agreement with our previous conclusion that there is a species-specific dependence between gB and gL, and the inability to mediate fusion is specifically due to the expression of EBV gB with Rh gL. This is further supported by the finding that fusion of the Rh-LCV glycoproteins with EBV gB is enhanced when EBV gL is expressed with EBV gB (Fig. 1B, column 3).

Interestingly, we found that Rh gB is functional in mediating fusion with EBV glycoproteins gp42, gH, and gL (Fig. 1A, column 4). This finding would suggest that Rh gB can interact with EBV gH/gL while the converse, an interaction between EBV gB with Rh gH/gL, does not occur.

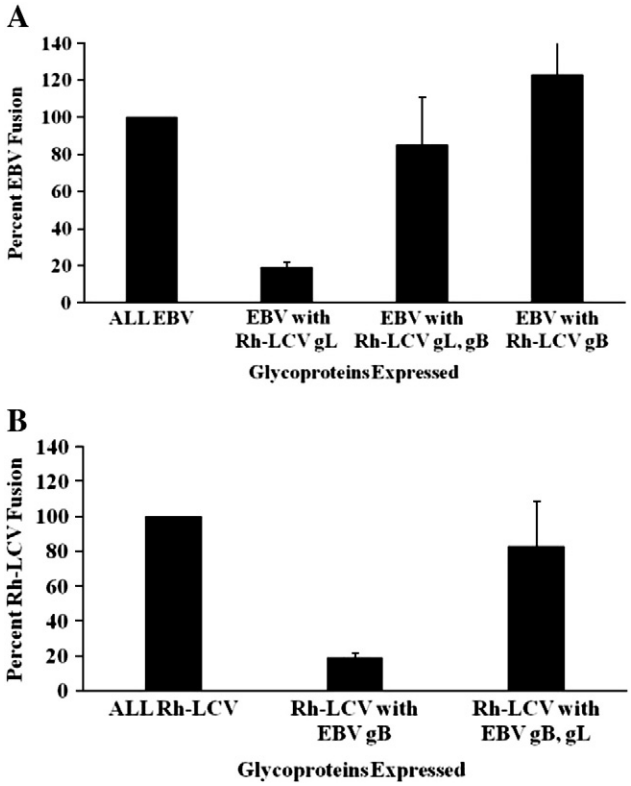

Fig. 1. Fusion is blocked by EBV gB and Rh gL co-expression. Fusion function of EBV and Rh-LCV glycoproteins with B cells. (A) CHO-K1 cells were transiently transfected with all of the EBV glycoproteins necessary for fusion (gp42, gB, gH, and gL) or the indicated Rh-LCV glycoprotein. Effector CHO-K1 cells were overlaid with target Daudi B cells and luciferase activity was measured. Luciferase activity was normalized to wild-type EBV levels, which was set to 100%. (B) CHO-K1 cells were transiently transfected with all of the Rh-LCV glycoproteins necessary for fusion (gp42, gB, gH, and gL) or the indicated EBV glycoprotein. Effector CHO-K1 cells were overlaid with target Daudi B cells and luciferase activity was measured. Luciferase activity was normalized to wild-type Rh-LCV levels, which was set to 100%.