Virology 450-451 (2014) 55–63

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Sphingosine kinase 1 regulates measles virus replication

Madhuvanthi Vijayan [a], Young-Jin Seo [a], Curtis John Pritzl [a], Sarah Angela Squires [a], Stephen Alexander [b], Bumsuk Hahm [a,*]

[a] Departments of Surgery & Molecular Microbiology and Immunology, University of Missouri-Columbia, Columbia, MO 65212, USA
[b] Division of Biological Sciences, University of Missouri-Columbia, Columbia, MO, USA

ARTICLE INFO

Article history:
Received 2 October 2013
Returned to author for revisions
21 October 2013
Accepted 26 November 2013
Available online 20 December 2013

Keywords:
Measles virus
Sphingosine kinase
NF-κB

ABSTRACT

Measles virus (MV) manipulates host factors to facilitate virus replication. Sphingosine kinase (SK) is an enzyme catalyzing the formation of sphingosine 1-phosphate and modulates multiple cellular processes including the host defense system. Here, we determined the role of SK1 in MV replication. Over-expression of SK1 enhanced MV replication. In contrast, inhibition of SK impaired viral protein expression and infectious virus production from cells expressing MV receptor, SLAM or Nectin-4. The inhibition of virus replication was observed when the cells were infected by vaccine strain or wild type MV or V/C gene-deficient MV. Importantly, SK inhibition suppressed MV-induced activation of NF-κB. The inhibitors specific to NF-κB signal pathway repressed the synthesis of MV proteins, revealing the importance of NF-κB activation for efficient MV replication. Therefore, SK inhibition restricts MV replication and modulates the NF-κB signal pathway, demonstrating that SK is a cellular factor critical for MV replication.

Introduction

Measles is a highly contagious disease that remains as one of the leading causes of pediatric morbidity and mortality worldwide (CDC, 2013). Despite the availability of a safe vaccine, there were approximately 158,000 measles-related deaths globally in 2011 (CDC, 2012). In the USA, 222 measles cases, which were associated with travel/importations, were reported in 2011 (CDC, 2012).

Measles virus (MV), the causative agent for measles, is an enveloped, negative stranded RNA virus that belongs to the order *Mononegavirales*, family *Paramyxoviridae,* and genus *Morbillivirus* (Griffin, 2001). Wild type MV uses the signaling lymphocyte activation molecule (SLAM)/CD150 (Tatsuo et al., 2000) and Nectin-4/PVRL4 as cellular receptors (Muhlebach et al., 2011; Noyce et al., 2011), while the attenuated vaccine strains of MV can interact with CD46 to enter cells in addition to being able to use SLAM and Nectin-4 (Dorig et al., 1993; Naniche et al., 1993). A profound immunosuppression is a hallmark characteristic of MV infection, however the exact mechanisms of this process are not clearly understood (Avota et al., 2010; Hahm, 2009). Transgenic mice bearing human CD46 (Oldstone et al., 1999; Rall et al., 1997; Sellin and Horvat, 2009) or human SLAM (Hahm et al., 2003; Hahm et al., 2004; Ohno et al., 2007; Welstead et al., 2005) have been generated to study MV-induced immune suppression and measles pathogenesis. These animal models have increased our understanding of measles biology (Oldstone et al., 2005), although they did not fully support MV replication to cause clinical symptoms of measles in the presence of the host immune system. However, transgenic mice harboring human Nectin-4 have not yet been established. Furthermore, there are no specific antivirals for treating measles (Moss and Griffin, 2012). Thus, it is important to identify cellular factors that are critically involved in MV replication and to define regulatory pathways of MV–host interaction.

MV is known to modulate host machinery and its signaling pathways to facilitate its own replication (Gerlier and Valentin, 2009; Kerdiles et al., 2006; Rima and Duprex, 2011). For example, MV proteins such as the non-structural V and C proteins inhibit type I interferon (IFN)-mediated anti-viral activity (Ramachandran and Horvath, 2009; Shaffer et al., 2003). Further, although MV was shown to induce the activation of NF-κB signaling (Helin et al., 2001), viral proteins suppress strong activation of NF-κB signaling pathway (Pfaller and Conzelmann, 2008; Schuhmann et al., 2011; Yokota et al., 2008).

Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid mediator and its level is tightly regulated by cellular enzymes (Gandy and Obeid, 2013; Rosen et al., 2013). Sphingosine kinase (SK) converts sphingosine to S1P via its kinase activity. SK/S1P pathway mediates a variety of crucial cellular processes such as cell growth/survival/differentiation, lymphocyte trafficking, and host immunity (Maceyka et al., 2012; Spiegel and Milstien, 2011). Intracellular S1P and SK1 bind TNF receptor-associated

* Correspondence to: One Hospital Drive, Medical Sciences Building, NW301C, University of Missouri-Columbia, Columbia, MO 65212, USA. Tel: +1 573 884 8838; fax: +1 573 882 4287.
E-mail address: hahmb@health.missouri.edu (B. Hahm).

0042-6822/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2013.11.039

factor 2 (TRAF2) to activate TNF-α-induced NF-κB signaling (Alvarez et al., 2010), which could be important for regulation of the inflammatory responses. Recently, SK was reported to affect virus replication. Bovine viral diarrhea virus inhibited SK1 for efficient viral replication (Yamane et al., 2009), whereas SK1 increased the propagation of influenza virus (Seo et al., 2010; Seo et al., 2013) and human cytomegalovirus (Machesky et al., 2008). Yet, the precise role of the sphingolipid system during virus replication has not been defined.

In this study, we determined if SK1 regulates MV replication. Our data demonstrate that SK1 exhibits a pro-viral function to enhance MV amplification. Further, MV activates NF-κB in an SK-dependent manner to promote virus replication.

Results

Overexpression of SK1, but not exogenous S1P addition, enhances MV replication

In order to investigate whether SK1 affects the replication of MV, we used HEK 293 cells (HEK cells) that were engineered to over-express SK1 (SK1 cells) (Min et al., 2007). SK1 cells or HEK cells were infected with the Edmonston strain of MV (MV-Ed) and at 1 day post-infection (dpi), the expression levels of measles viral nucleoprotein (N) and matrix (M) protein were compared between SK1 cells and HEK cells. As indicated by the Western blot result in Fig. 1A, the amounts of both N and M proteins were clearly increased in SK1 cells compared to HEK cells at both 0.1 and 0.5 multiplicity of infection (MOI) conditions, indicating that SK1 overexpression promotes the expression of MV proteins upon infection. This result directly correlated with the extent of viral cytopathic effect (CPE) that was observed on these cells by visual inspection under a phase contrast microscope, i.e., MV-infected SK1 cells exhibited more CPE compared to the infected HEK cells (data not shown). Since the enzymatic function of SK1 is to catalyze the formation of S1P, we determined if exogenously supplied S1P enhances MV protein synthesis in a manner similar to the effect of SK1 overexpression. However, exogenous addition of S1P did not alter the level of MV N protein (Fig. 1B). Collectively, these results indicate that overexpression of intracellular SK1 enhances MV replication, whereas exogenously added S1P that is known to trigger S1P receptor signaling (Rosen et al., 2013) does not alter MV replication.

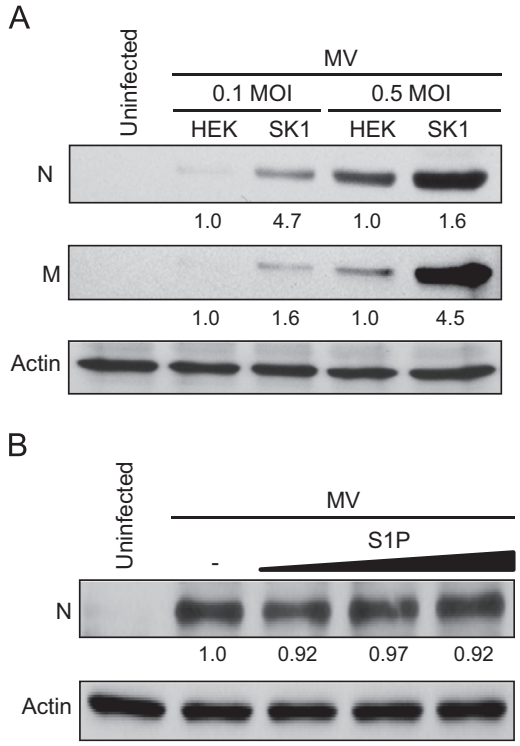

Fig. 1. Overexpression of SK1, but not exogenous S1P addition, enhances MV replication. (A) HEK 293 (HEK) cells or sphingosine kinase 1 (SK1) overexpressing HEK cells were infected with the Edmonston strain of measles virus (MV) either at 0.1 MOI or 0.5 MOI. At 1 day post-infection (dpi) Western blotting was performed to detect the nucleoprotein (N) or the matrix (M) protein of MV. The level of viral protein expressed by the control HEK cells was set to 1.0 at both MOIs. (B) NCI-H358 cells (H358) were pre-treated with 50, 100, or 500 nM of S1P for 10 min followed by infection with MV at 0.1 MOI. At 1 dpi, cell lysates were harvested for Western blot analysis to detect N protein of MV. The relative intensities for each band of N protein are shown.

Inhibition of SK impairs the replication of MV

To further investigate the role of SK in MV replication, we employed a pharmacological approach by using inhibitors that are known to impair SK activity such as N,N-dimethylsphingosine (DMS) (Edsall et al., 1998; Orr Gandy and Obeid, 2013; Yatomi et al., 1997) and 4-[[4-(4-chlorophenyl)-2-thiazolyl]amino]phenol (SKI-II) (French et al., 2003; Orr Gandy and Obeid, 2013). As shown in Fig. 2A, inhibition of SK with these inhibitors led to a marked decrease in the expression level of MV N protein in H358 cells, which express Nectin-4, the epithelial cell receptor for MV (Muhlebach et al., 2011; Noyce et al., 2011). To exclude the possibility that SK inhibitors exhibit cytotoxic effects, which would eventually interfere with virus propagation, we performed a trypan blue exclusion assay to compare the percentage of viable cells between SKI-II-treated and untreated cells in the presence or absence of MV infection. As shown by the percentage of viability of H358 cells in Fig. S1, SKI-II did not exhibit any significant cytotoxicity in our experimental condition, indicating that the impaired virus replication is not due to the altered cell viability. Since MV is able to use SLAM as well as Nectin-4 as a receptor to infect cells, SLAM-expressing B95-8 cells were infected with MV and incubated with the inhibitor SKI-II. The SK inhibitor displayed a similar inhibitory effect on virus replication in B95-8 cells (Fig. 2B). This result suggests that SK acts as a pro-viral factor in MV replication and importantly, this phenomenon occurs irrespective of the cellular receptor usage by MV, as we observed the SKI-II-mediated inhibition of viral replication in cells expressing either SLAM or Nectin-4. Next, we performed flow cytometric analysis to assess the level of MV proteins expressed on the surface of infected cells on a single cell basis. SKI-II treatment decreased the expression level of MV proteins on the surface of infected cells compared to the untreated cells (Fig. 2C), as indicated by the decrease of mean fluorescence intensity (MFI) (309–193). Also, SK inhibition suppressed the replication of JW strain of wild type MV (Fig. 2D), demonstrating the importance of SK in promoting the replication of both wild type and the attenuated strains of MV.

MV non-structural proteins such as V and C proteins are involved in modulating host defense mechanisms including type I IFN response (Ramachandran and Horvath, 2009; Shaffer et al., 2003). In order to determine if there is a role of MV V or C protein in SK-mediated modulation of MV replication, we used recombinant viruses that lack the V or C gene namely the V^- or C^- viruses (Patterson et al., 2000). The replication of V or C-deficient viruses was diminished by SK inhibition similar to the V and C sufficient MV (Fig. 2E). This result indicates that both MV V^- and MV C^- are sensitive to SK inhibition and that V and C proteins do not play a key role in SK-mediated regulation of MV replication. Furthermore, we used a small interfering RNA (si-RNA) approach to knockdown SK1 levels to further confirm our results. Knockdown of SK1 also reduced the level of pSK1 and resulted in a strong