Virology 510 (2017) 234–241

Contents lists available at ScienceDirect

Virology

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

Cholesterol is required for stability and infectivity of influenza A and respiratory syncytial viruses

Shringkhala Bajimaya, Tünde Frankl, Tsuyoshi Hayashi, Toru Takimoto*

Department of Microbiology and Immunology, University of Rochester Medical Center, Box 672, 601 Elmwood Avenue, Rochester, NY 14642, USA

ARTICLE INFO

Keywords:
Respiratory viruses
Cholesterol
Lipid rafts
Virus stability
Antiviral therapy

ABSTRACT

Cholesterol-rich lipid raft microdomains in the plasma membrane are considered to play a major role in the enveloped virus lifecycle. However, the functional role of cholesterol in assembly, infectivity and stability of respiratory RNA viruses is not fully understood. We previously reported that depletion of cellular cholesterol by cholesterol-reducing agents decreased production of human parainfluenza virus type 1 (hPIV1) particles by inhibiting virus assembly. In this study, we analyzed the role of cholesterol on influenza A virus (IAV) and respiratory syncytial virus (RSV) production. Unlike hPIV1, treatment of human airway cells with the agents did not decrease virus particle production. However, the released virions were less homogeneous in density and unstable. Addition of exogenous cholesterol to the released virions restored virus stability and infectivity. Collectively, these data indicate a critical role of cholesterol in maintaining IAV and RSV membrane structure that is essential for sustaining viral stability and infectivity.

1. Introduction

Influenza A virus (IAV), respiratory syncytial virus (RSV), and human parainfluenza viruses (hPIVs) are the major causes of viral respiratory infections (Gasparini et al., 2014; Griffiths et al., 2017; Henrickson, 2003). These respiratory RNA viruses are enveloped with membrane derived from the host plasma membrane. Viral envelope and associated glycoproteins play multiple functions such as protecting the viral core, virus attachment and entry to the cells, as well as packaging and maintaining stability of newly formed infectious virus. These enveloped viruses utilize sphingolipids and cholesterol-rich microdomains, called lipid rafts in the plasma membrane, for infectious virus production. Newly synthesized viral components are transported to the lipid rafts, where assembly and budding of virus particles take place (Nayak et al., 2004; Rossman and Lamb, 2011; Schmitt and Lamb, 2005).

Cholesterol contributes to maintaining the integrity of lipid raft domains by binding to long saturated acyl chains of sphingolipids (Brown and London, 1998, 2000; Brown and Rose, 1992). Ordered lipid rafts serve as a platform for specific viral protein interactions during assembly (Veit and Thaa, 2011). Extensive studies on IAV have shown that transmembrane residues of IAV HA and NA are required for partitioning with lipid raft membrane (Barman et al., 2004; Barman and Nayak, 2000; Melkonian et al., 1999; Veit and Thaa, 2011). IAV M1 has been found to associate with rafts through the interactions with cytoplasmic tail domains of HA and NA (Ali et al., 2000; Carrasco et al., 2004; Nayak et al., 2009; Zhang et al., 2000). Similarly, residues in the transmembrane domains of RSV F intrinsically target the protein to the lipid rafts and facilitate binding of M protein (Fleming et al., 2006; Henderson et al., 2002; Marty et al., 2004; Oomens et al., 2006). Additionally, RSV G and SH proteins have been found to co-localize with raft-specific host proteins (Brown et al., 2004, 2002; McDonald et al., 2004; Rixon et al., 2004). Extraction of cholesterol by cholesterol-extracting agent methyl-β-cyclodextrin (MβCD) has been shown to disrupt lipid raft microdomains and decrease infectious RSV production, suggesting that cholesterol in the raft domain is required for coordinated viral protein interactions essential for virus assembly and infectious virus production (Chang et al., 2012).

We previously showed that FDA-approved cholesterol reducing agents, gemfibrozil (Gem) and lovastatin (Lov), efficiently reduce the cellular cholesterol level in human airway A549 cells, especially when cells were treated with both agents (Bajimaya et al., 2017). Gem is a lipid lowering fibrate, which causes intracellular cholesterol efflux decreasing storage of cellular cholesterol, and Lov inhibits cholesterol biosynthesis (Maron et al., 2000; Roy and Pahan, 2009). Depletion of cholesterol by Gem and Lov disrupted lipid raft integrity and decreased hPIV1 protein association with the lipid raft domains, inhibiting virus assembly and virus particle production (Bajimaya et al., 2017). However, a previous study on

* Corresponding author.
E-mail address: toru_takimoto@urmc.rochester.edu (T. Takimoto).

http://dx.doi.org/10.1016/j.virol.2017.07.024
Received 30 May 2017; Received in revised form 12 July 2017; Accepted 19 July 2017
Available online 25 July 2017
0042-6822/

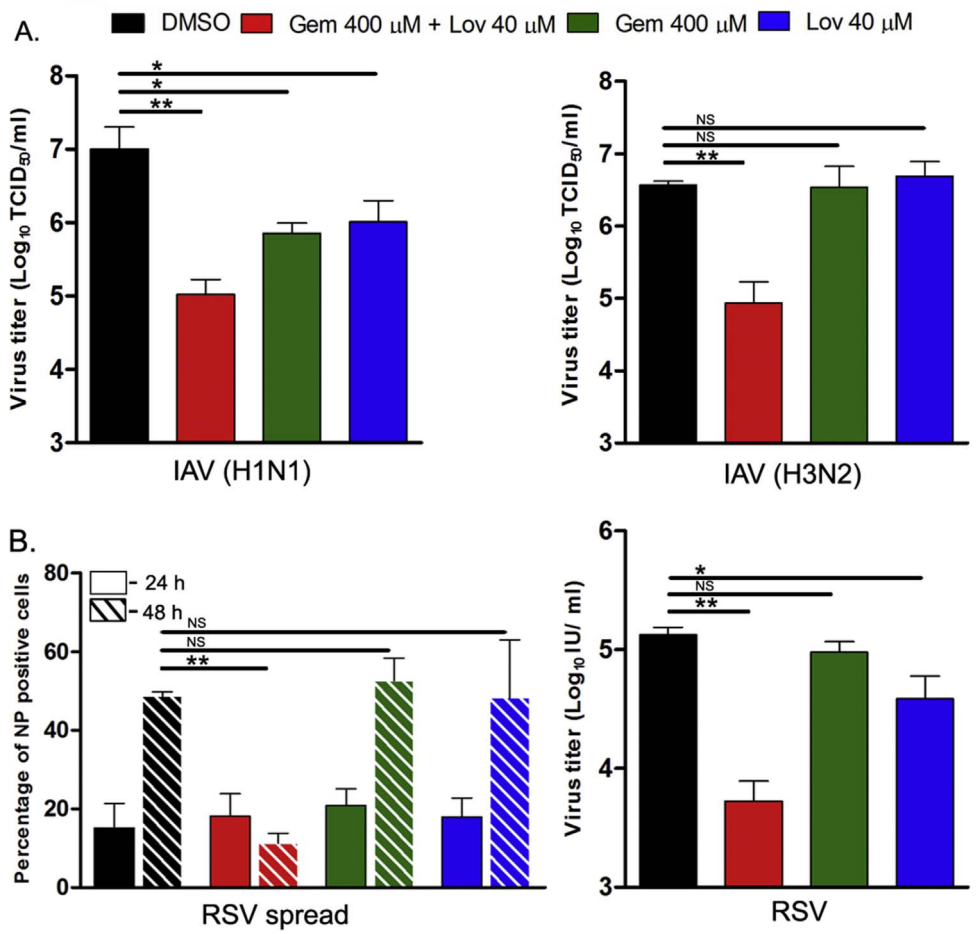

Fig. 1. Cholesterol is required for infectious IAV and RSV production. Cells untreated or treated with the drugs for 24 h were infected with H1N1 (A, left), H3N2 IAV (A, right) or RSV (B, right) at MOI of 3, and cultured in presence of drugs for an additional 24 h. Harvested viruses were saved in −80 °C freezer, and virus titers were measured by $TCID_{50}$ or IF assays ($n = 3$). (B, left) Cells either untreated or treated with the drugs were infected with RSV at MOI of 0.1 and cultured in presence or absence of the drugs for an additional 24 h and 48 h. Infected cells were detected by IF analysis using anti-NP mAb ($n = 3$). **; $P < 0.01$, *; $P < 0.05$ (Student t-test).

IAV reported that depletion of cholesterol by MβCD enhanced IAV budding from the infected cells (Barman and Nayak, 2007), suggesting that the requirement of cholesterol for virus assembly and release may vary among respiratory RNA viruses.

To understand the role of cholesterol in the life cycle of various respiratory RNA viruses, we analyzed the effect of cholesterol depletion on infection, replication, and assembly of IAV and RSV using cholesterol-reducing agents, Gem and Lov. Depletion of cellular cholesterol significantly decreased infectious virus production without affecting virus entry or protein synthesis in the cells. However, in contrast to hPIV, cholesterol depletion did not inhibit IAV and RSV assembly or release, although released virions were less homogeneous in density. Strikingly, released virus particles were less stable due to decreased cholesterol content in the viral membrane, which was rescued by adding exogenous cholesterol. Collectively, these results suggest that cholesterol is a critical component for maintaining IAV and RSV membrane structure, which is required for sustaining virus stability and infectivity.

2. Results

2.1. Depletion of cellular cholesterol decreases infectious IAV and RSV production

To determine the role of cholesterol on infectious IAV and RSV production, we utilized cholesterol reducing agents, Gem and Lov, to deplete cellular cholesterol in human airway epithelial A549 cells. We previously reported that treatment of A549 cells with Gem (400 μM) and Lov (40 μM), individually or in combination did not affect cell viability, but significantly reduced cellular cholesterol. When the cells were treated with both drugs, cellular cholesterol was depleted by 20% and 44% at 24 h and 48 h, respectively (Bajimaya et al., 2017). First, we determined the production of infectious IAV A/California/04/2009 (H1N1) and A/Victoria/361/2011 (H3N2), which are currently circulating IAV subtypes. We found that treatment of cells with Gem and Lov alone reduced infectious H1N1 production by 14.2 fold and 9.9 fold, respectively (Fig. 1A). Treatment with both drugs significantly reduced virus production by 97.2 fold (Fig. 1A). Similarly, treatment of cells with both drugs resulted in a 42.3 fold reduction in H3N2 virus production, while there was subtle effect on virus production when cells were treated with Gem or Lov, individually (Fig. 1A).

We next analyzed the effect of cholesterol depletion on RSV spread and infectious virus production. Cells pre-treated with the agents were infected at MOI of 0.1 and cultured for 24 and 48 h in the presence of drugs. In untreated cells, RSV infection spread from 15.1% (at 24 h post infection, hpi) to 48.7% (at 48 hpi) (Fig. 1B). In cells treated with both agents, RSV spread was completely blocked, although individual treatment did not inhibit virus spread in the cultures. Similarly, in the presence of both drugs, release of infectious RSV into the culture medium was reduced by 25 fold (Fig. 1B). These results indicate that cholesterol reducing agents are effective in blocking infectious virus production of IAV and RSV similar to hPIV1, as we previously reported (Bajimaya et al., 2017).