Analysis of the entry mechanism of Crimean-Congo hemorrhagic fever virus, using a vesicular stomatitis virus pseudotyping system

Yuto Suda¹,² • Shuetsu Fukushi² • Hideki Tani² • Shin Murakami¹ • Masayuki Saijo² • Taisuke Horimoto¹ • Masayuki Shimojima²

Received: 24 September 2015 / Accepted: 21 February 2016 / Published online: 3 March 2016
© Springer-Verlag Wien 2016

Abstract    Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne disease causing severe hemorrhagic symptoms with a nearly 30 % case-fatality rate in humans. The experimental use of CCHF virus (CCHFV), which causes CCHF, requires high-biosafety-level (BSL) containment. In contrast, pseudotyping of various viral glycoproteins (GPs) onto vesicular stomatitis virus (VSV) can be used in facilities with lower BSL containment, and this has facilitated studies on the viral entry mechanism and the measurement of neutralizing activity, especially for highly pathogenic viruses. In the present study, we generated high titers of pseudotyped VSV bearing the CCHFV envelope GP and analyzed the mechanisms involved in CCHFV infection. A partial deletion of the CCHFV GP cytoplasmic domain increased the titer of the pseudotyped VSV bearing the CCHFV envelope GP. Using the pseudotype virus, DC-SIGN (a calcium-dependent [C-type] lectin cell-surface molecule) was revealed to enhance viral infection and act as an entry factor for CCHFV.

Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a potentially fatal tick-borne infectious disease that has been reported in over 30 countries in parts of Africa, Eastern Europe, and Asia [1–5]. Human infection can occur through the bite of an infected tick or through contact with the tissue or blood of viremic animals or CCHF patients. The case-fatality rate is nearly 30 % [1–4, 6]. Both the incidence and geographic range of confirmed CCHF cases have increased [1, 5]. In Turkey, the first cases of CCHF were identified in 2002, after which the number of patients increased, and the number of identified cases is currently more than 9,000 [4]. In Iran, 1,017 cases occurred between 2000 and 2014 [4]. In China, 286 cases were identified between 1965 and 1997 [7]. In Kazakhstan, during 2000 to 2013, 212 cases were reported [8]. At present, there are no established countermeasures to combat CCHF.

CCHF is caused by infection with CCHF virus (CCHFV), which is a member of the genus *Nairovirus* in the family *Bunyaviridae* [9]. In most countries, experimental use of CCHFV requires biosafety level (BSL) 4 containment. The virus has a tri-segmented, negative-sense, single-stranded RNA genome and forms enveloped virions. The L, M, and S segments encode the RNA-dependent RNA polymerase, the envelope glycoprotein (GP), and the nucleocapsid protein, respectively. The precursor of GP is cleaved and modified to generate the structural proteins Gn and Gc and the non-structural proteins GP38 and NSm [10–12]. As the structural proteins form complexes on the envelope and interact with host receptors to initiate infection, they are the primary targets of neutralizing antibodies [13]. It was reported previously that the early stage of CCHFV infection is dependent on pH and cholesterol [14, 15]. Furthermore, it was previously shown that the Gc cytoplasmic tail of some viruses of the family *Bunyaviridae*, such as Rift Valley fever virus, Uukuniemi virus and Bunyamwera virus, is crucial for the trafficking and localization of not only Gc but also Gn [16–18].

¹ Department of Veterinary Microbiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
² Special Pathogens Laboratory, Department of Virology I, National Institute of Infectious Diseases, Gakuen 4-7-1, Musashimurayama, Tokyo 208-0011, Japan

✉ Masayuki Shimojima
shimoji-@nih.go.jp
Vesicular stomatitis Indiana virus (VSV), a member of the family *Rhabdoviridae*, is a non-segmented, negative-sense, single-stranded RNA virus that can be handled in facilities with BSL-2 containment. Pseudotyping of viral glycoproteins onto VSV has facilitated studies of viral entry and measurement of virus neutralizing activity under BSL-2 containment [19–26]. As there are fewer than 50 BSL-4 containment facilities in the world, these pseudo-type viruses are valuable tools, especially for the study and diagnosis of highly pathogenic viruses.

Although nucleolin has been reported to be a candidate receptor for CCHFV [27], it is still unclear which factors are required for CCHFV entry factors. Calcium-dependent (C-type) lectins recognize glycans, which exist as GPs and/or glycolipids, and some lectins such as DC-SIGN, LSECtin, MGL, and CLEC5A are known to be entry factors for several viruses, including human immunodeficiency virus 1 [28], measles virus [29], dengue virus [30], severe acute respiratory syndrome coronavirus (SARS-CoV) [31], filoviruses [31], and Lassa virus [32]. It has been shown that some members of the family *Bunyaviridae*, including the phleboviruses Rift Valley fever virus, Uukuniemi virus [33], and severe fever with thrombocytopenia syndrome virus (SFTSV) [34] use DC-SIGN as a receptor, and Uukuniemi virus was shown to bind directly to DC-SIGN [33]. Recognition of glycans on virions by C-type lectins results in the enhancement of viral endocytosis, after which the cellular and viral membranes fuse. CCHFV Gn and Gc have some N-glycosylated sites [10, 35] and are likely to bind to C-type lectins.

In the present study, in facilities with BSL-2 containment, we generated high titers of pseudotyped VSV bearing CCHFV envelope GP on its surface (CCHFVpv) and analyzed the mechanisms of CCHFV cell entry using the pseudotype virus.

Materials and methods

Cells

293T cells (ATCC CRL-3216), Vero cells (ATCC CCL81), and VeroE6 cells (ATCC CRL-1586) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Sigma) supplemented with 10 % (v/v) fetal bovine serum (FBS) (Gibco). Jurkat cells (ATCC TIB-152) were cultured in RPMI-1640 (Sigma) medium supplemented with 10 % (v/v) FBS. Jurkat cells stably expressing ICD2ΔCT, DC-SIGN, and LSECtin were prepared as described previously [32].

Plasmids

cDNAs encoding the open reading frame of CCHFV IbAr 10200 strain GP (NP_950235) were cloned into pCAGGS using a Rapid DNA Ligation Kit (Roche) to generate pC-CCHFV GP. For constructing plasmids encoding mutant GPs in which the carboxyl terminal region was partially truncated, stop codons were introduced in pC-CCHFV GP by PCR-based mutagenesis to express the mutant GPs listed in Table 1. All plasmid constructions were confirmed by sequencing, using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

Production of pseudotyped VSV bearing CCHFV envelope GP

Pseudotyped VSV bearing CCHFV envelope GP was generated as described previously [25, 26]. Briefly, 293T cells transfected with CCHFV GP plasmids using TransIT-LT1 (Mirus) were infected with VSV G/GFP-*G or VSV ΔG/Luc-*G, in which the G gene was replaced with the green fluorescent protein (GFP) or the luciferase gene, respectively, at a multiplicity of infection of 0.1-1. The virus was adsorbed for 2 h at 37 °C and then removed by extensively washing four times with serum-free DMEM. After 24 h of incubation at 37 °C with the culture medium, the culture supernatants were centrifuged to remove cell debris and stored until use at -80 °C. To produce a pseudotype virus (with the GFP gene or the luciferase gene) bearing no viral envelope proteins, empty plasmid pCAGGS or GFP expression plasmid pC-GFP was used. VSVpv/Luc (pseudotyped VSV with the luciferase gene bearing VSV G), MLVpv/Luc (pseudotyped VSV with murine leukemia virus envelope proteins), Lassa/GFP (pseudotyped VSV with the GFP gene bearing Lassa virus envelope protein), and EBOVpv/GFP (pseudotyped VSV with the GFP gene bearing Ebola

| Variant | Amino acid sequence of the cytoplasmic region | Titer (IU/ml) |
|---------|---------------------------------------------|--------------|
| Full-length | CFKCCRRTRGFLKRYRHLKDEETGYRRIIEKLNKKGKKNKLDGERLADRRIELFSTK | 200 |
| del4 | CFKCCRRTRGLKRYRHLKDEETGYRRIIEKLNKKGKKNKLDGERLADRRIE | 1,150 |
| del9 | CFKCCRRTRGLKRYRHLKDEETG | 1,700 |
| del10 | CFKCCRRTRG | 6,250 |
| del14 | C | 1,225 |
virus envelope protein) were generated as described previously [22].

**Reporter assay**

To quantitatively measure the infectivity of CCHFVpv, the reporter activity in inoculated cells was assayed at 1 day postinfection as follows: For the pseudotype virus with the luciferase gene, luciferase activity was measured using a Bright-Glo luciferase assay system (Promega) in accordance with the manufacturer’s protocol with GloMax (Promega). For the pseudotype virus with the GFP gene, the supernatant was replaced with PBS (−), and GFP-positive cells were counted under a fluorescence microscope (BZ-X710, KEYENCE). The infectious units (IU) were determined as the number of the GFP-positive cells.

**Effects of ammonium chloride (NH₄Cl) and methyl-β-cyclodextrin (MβCD) on infectivity**

Cells were treated with the indicated concentrations of NH₄Cl for 60 min or MβCD for 30 min at 37 °C and then inoculated with the pseudotype viruses in DMEM supplemented with 2 % (v/v) FBS. Infectivity was determined as described above. To restore cholesterol, cells were treated with 200 μM exogenous cholesterol for 30 min after cholesterol removal.

**Serum samples**

The CCHF patient serum sample was obtained from the Center for Disease Control and Prevention (Atlanta, Georgia, USA). Two serum samples, which were collected from healthy Japanese adults, were used as controls. The usage of the sera was approved by the Research and Ethics Committee of the National Institute of Infectious Diseases, Tokyo, Japan (reference no. 439).

**Neutralization assay**

CCHFVpv was incubated with diluted human serum samples for 30 min at 37 °C and inoculated onto VeroE6 cells. At 1 day after inoculation, CCHFVpv infectivity was measured as described above.

**Flow cytometry**

Antibody staining and analysis were performed as described previously [32, 36]. The antibodies used to detect lectins were as follows: normal mouse IgG antibody (Mouse IgG Isotype Control) (R&D systems), anti-ICD2 antibody [37], anti-DC-SIGN antibody (Clone DC28, R&D systems), and anti-LSECtin antibody (SOTO-1, Santa Cruz Biotechnology, Inc.).
Statistical analysis

The differences in the infectivity were compared using Student’s t-test.

Results

Effect of deletions in the GP carboxyl terminal region on the titer of the pseudotype virus

To generate pseudotyped VSV bearing the CCHFV envelope GP, we first used the full-length GP as an envelope protein and VSVΔG/GFP-ΔG, the reporter gene of which is GFP, as a seed virus. VeroE6 cells were inoculated with the resultant pseudotype virus and the cells expressing GFP were counted. The titer of the pseudotype virus with full-length GP was 200 IU/ml (Table 1). Next, we examined the effects of deletions within the carboxyl terminal region of GP on the titers of the pseudotype virus, because deletion of the Gc cytoplasmic tail might change the trafficking of the envelope protein, which could affect pseudotype virus production. Furthermore, it was reported previously that a pseudotyped VSV bearing the envelope protein of SARS-CoV with a truncation in the cytoplasmic domain was more efficiently incorporated in the viral particle than the full-length protein [20]. We used four mutant GPs (Table 1) as envelope proteins to generate the pseudotype virus. All of the cytoplasmic-region-deleted GPs that we examined produced higher titers of the pseudotype virus than the full-length GP (Table 1). The highest titer, which was approximately 30 times that of the full-length GP, was obtained with the GP del10 mutant. The pseudotype virus with GP del10, named CCHFVpv, was used for subsequent experiments. Consistent results were obtained when a luciferase reporter was used instead of a GFP reporter (Fig. 1). The luciferase activity obtained with CCHFVpv/Luc was approximately 30 times higher than in the cells incubated with the pseudotype virus without the envelope protein.

Characteristics of CCHFVpv infection

We examined whether CCHFVpv infection had the characteristics that were recognized in authentic CCHFV infection. Authentic CCHFV infection has been reported to be dependent on pH and cholesterol [14, 15]. First, we examined the effects of NH4Cl, which increases the pH of intracellular compartments, on CCHFVpv infection. As shown in Fig. 2, treatment with NH4Cl at concentrations of 12.5 mM and 25 mM decreased CCHFVpv/Luc infection in a dose-dependent manner. NH4Cl treatment had a similar effect on infection with VSVpv/Luc, which requires a low-pH step for the entry [15, 38]. In contrast, the treatment did not affect the infectivity of MLVpv/Luc, which is independent of pH for the entry [22]. This indicated that CCHFVpv infection was pH dependent. Next, the effects of MβCD, which removes cholesterol from the cell membranes, were investigated. As shown in Fig. 3a, treatment with MβCD at concentrations of 2.5 mM and 5 mM decreased CCHFVpv/Luc infection in a dose-dependent manner. MβCD treatment had a similar effect on infection with VSVpv/Luc, which requires a low-pH step for the entry [15, 38]. In contrast, the treatment did not affect the infectivity of MLVpv/Luc, which is independent of pH for the entry [22]. This indicated that CCHFVpv infection was pH dependent. Next, the effects of MβCD, which removes cholesterol from the cell membranes, were investigated. As shown in Fig. 3a, treatment with MβCD at concentrations of 2.5 mM and 5 mM decreased CCHFVpv/Luc infection in a dose-dependent manner. MβCD treatment had a similar effect on infection with VSVpv/Luc, which requires a low-pH step for the entry [15, 38]. In contrast, the treatment did not affect the infectivity of MLVpv/Luc, which is independent of pH for the entry [22].
Cell-surface molecules involved in CCHFV infection

Because the CCHFVpv titer measured in Jurkat cells was found to be quite low (5 IU/mL) in comparison to that in VeroE6 cells (6250 IU/mL) (Table 1), Jurkat cells were used to investigate the involvement of C-type lectins, cell-surface molecules that have been well investigated in studies of various viral entry mechanisms and shown to enhance the entry of Ebola virus and Lassa virus entry into cells [32, 36]. Expression of a control molecule (ICD2ΔCT) and C-type lectins was confirmed by flow cytometry (Fig. 5a). As shown in Fig. 5b, CCHFVpv/GFP and Lassapv/GFP infections in Jurkat cells were enhanced by the expression of DC-SIGN. Whereas LSECtin expression enhanced Lassapv/GFP and EBOVpv/GFP infection (Fig. 5b), the lectin did not affect the CCHFVpv/GFP infection. The results showed that DC-SIGN, but not LSECtin, was involved in CCHFVpv infection. It was reported previously that the binding of Lassapv to DC-SIGN, which mainly recognizes high-mannose-type glycans, was blocked by mannan, a polymer of mannose [32]. CCHFVpv/GFP infection in DC-SIGN-expressing Jurkat cells was inhibited by pretreatment with mannan, but not with GlcNAcβ1-2Man, one of the high-affinity ligands of LSECtin [32, 39] (Fig. 5c). Furthermore, CCHFVpv/GFP infection was also inhibited by pretreatment with the antibody to DC-SIGN (Fig. 5d). These results show that CCHFVpv may preferentially infect cells that express DC-SIGN as a result of binding between carbohydrates on the envelope GP and DC-SIGN.

Discussion

Quantitative measurement of infection with a pseudotyped VSV in which the viral envelope protein has been replaced with that of a different virus is highly sensitive, rapid, and easy. Here, we report the creation of a VSV pseudotype bearing the envelope protein of CCHFV, which requires high BSL containment for experimental use. Although a pseudotyped VSV bearing a full-length CCHFV envelope GP has been reported recently [40], the intensity of luminescence (the expression of the reporter used in the experiment) in infected cells was only eight times higher than the background. Therefore, a pseudotype virus with a much higher titer is needed for research and diagnosis. We found that the use of CCHFV GP with a truncated carboxyl terminus resulted in a higher titer of pseudotyped VSV (30 times higher than background) (Fig. 1). Although the precise reason(s) why the truncation of the carboxyl terminus of CCHFV GP produced high viral titers is unclear, the altered localization of Gn and Gc and/or proper assembly with the VSV proteins may have been involved.
Nonetheless, because of its high titer and characteristics that were recognized in authentic CCHFV in terms of the pH- and cholesterol dependence of infection (Table 1, Figs. 2 and 3), the pseudotyped VSV with truncated CCHFV GP (e.g., del10 mutant in Table 1) is potentially useful for studies on the entry mechanisms of CCHFV and serosurveillance experiments, such as the measurement of neutralizing activities. As CCHFV requires high biosafety
containment, this pseudotype virus will be a powerful tool for studying CCHFV.

Because the CCHFV envelope protein is a glycoprotein [10, 35] and some phleboviruses, such as Rift Valley fever virus, Uukuniemi virus [33], and SFTSV [34], used a C-type lectin, DC-SIGN, as a receptor, we expected C-type lectins to be receptors for CCHFV. In this study, we found that CCHFVpv efficiently infected DC-SIGN-expressing cells, although we examined only two C-type lectins (Fig. 5), indicating that DC-SIGN is a possible novel entry factor for CCHFV. The result suggests that the role of DC-SIGN in cell entry is conserved among members of the family Bunyaviridae. CCHFV initially replicates in dendritic cells and tissue resident macrophages, and the virus then migrates to a broad range of tissues and organs, including regional lymph nodes, spleen and liver [6, 41, 42]. As DC-SIGN is present on the surface of dendritic cells [28, 43] and macrophages [44], the results of this study agree with the tropism of this virus and suggest that DC-SIGN has an important role in the infection of dendritic cells and macrophages. However, as the inhibitory effect by mannan or anti-DC-SIGN antibody was only approximately 50% (Fig. 5c and d), the mechanism of enhancement of CCHFVpv infection by DC-SIGN might be nonspecific or could involve interactions other than those with the carbohydrate-recognition domain of DC-SIGN.

The effects of two C-type lectins on CCHFVpv infection differed from the effects on EBOVpv and Lassapv infection (Fig. 5b). CCHFVpv infection was enhanced by DC-SIGN but not by LSECtin. In contrast, Lassapv infection was enhanced by both lectins, and EBOVpv infection was enhanced to a much greater extent by LSECtin than by DC-SIGN. Such different features suggest that the glycosylation status of the CCHFV envelope GP is different from that of the Ebola and Lassa virus GPs. However, there are several possible reasons why LSECtin expression does not enhance CCHFVpv infection. For example, it is possible that the molecule does not bind the CCHFVpv particle, resulting in no involvement (no enhancement) in the viral infection. A second possibility is that the molecule binds the CCHFVpv particle but that the binding does not result in enhancement of infection due to inadequate interaction with a putative factor or cofactor necessary for infection. A third possibility is that binding of the CCHFVpv particle with LSECtin enhances transport to a degradation pathway.

There is a possibility that other molecules that have not yet been examined may enhance CCHFV infection more than DC-SIGN. To investigate the entry mechanism of CCHFV more precisely, further experiments will be needed including examination of other C-type lectins (such as mannose receptor, L-SIGN, or langerin), identification of still unknown receptors, and binding and internalization assays. In addition, candidate molecules/pathways that are eventually found to be involved in CCHFVpv entry into cells should be studied using wild-type CCHFV, because it is unclear whether the entry mechanisms of CCHFVpv and CCHFV are completely identical. Nevertheless, this pseudotype virus can provide novel insights into the mechanism of CCHFV infection and can be developed as a surrogate model for CCHFV research.

Acknowledgements This work was supported in part by grants for Research on Emerging and Re-emerging Infectious Diseases from the Ministry of Health, Labor, and Welfare of Japan (H25-Shinko-Ippan-004) and by Grants-in-Aid for Exploratory Research (26600227, 25670222) from the Japan Society for the Promotion of Science (JSPS).

References

1. Bente D, Forrester N, Watts D et al (2013) Crimean-Congo hemorrhagic fever: history, epidemiology, pathogenesis, clinical syndrome and genetic diversity. Antivir Res 100:159–189. doi:10.1016/j.antiviral.2013.07.006
2. Mertens M, Schmidt K, Oezkal A, Groschup MH (2013) The impact of Crimean-Congo hemorrhagic fever virus on public health. Antiviral Res 96:248–260. doi:10.1016/j.antiviral.2013.02.007
3. Whitehouse CA (2004) Crimean-Congo hemorrhagic fever. Antiviral Res 64:145–160. doi:10.1016/j.antiviral.2004.08.001
4. Papa A, Weber F, Hewson R et al (2015) Meeting report: First International Conference on Crimean-Congo hemorrhagic fever. Antiviral Res 120:57–65. doi:10.1016/j.antiviral.2015.05.005
5. Messina JP, Pigott DM, Golding N et al (2015) The global distribution of Crimean-Congo hemorrhagic fever. Trans R Soc Trop Med Hyg 109:503–513. doi:10.1093/trstmh/trv050
6. Akinci E, Bodur H, Leblebicioğlu H (2013) Pathogenesis of crimean-congo hemorrhagic Fever. Vector Borne Zoonotic Dis 13:429–437. doi:10.1089/vbz.2012.1061
7. Papa A, Ma B, Kouidou S et al (2002) Genetic characterization of the M RNA segment of Crimean Congo hemorrhagic fever virus strains, China. Emerg Infect Dis 8:50–53
8. Nursmarkov T, Samsyzhan Y, Atshabar B et al (2015) Crimean-Congo haemorrhagic fever virus in Kazakhstan (1948–2013). Int J Infect Dis 38:19–23. doi:10.1016/j.ijid.2015.07.007
9. Virus Taxonomy (2014) EC 46, Montreal, Canada, July 2014, Email ratification 2015 (MSL #29)
10. Sanchez AJ, Vincent MJ, Nichol ST (2002) Characterization of the glycoproteins of Crimean-Congo hemorrhagic fever virus. J Virol 76:7263–7275
11. Vincent MJ, Sanchez AJ, Erickson BR et al (2003) Crimean-Congo hemorrhagic fever virus glycoprotein proteolytic processing by subtilase SKI-1. J Virol 77:8640–8649
12. Bergeron E, Vincent MJ, Nichol ST (2007) Crimean-Congo hemorrhagic fever virus glycoprotein processing by the endoprotease SKI-1/S1P is critical for virus infectivity. J Virol 81:13271–13276. doi:10.1128/JVI.01647-07
13. Bertolotti-Ciarlet A, Smith J, Strecker K et al (2005) Cellular localization and antigenic characterization of crimean-congo hemorrhagic fever virus glycoproteins. J Virol 79:6152–6161. doi:10.1128/JVI.79.10.6152-6161.2005
14. Simon M, Johansson C, Mirazimi A (2009) Crimean-Congo hemorrhagic fever virus entry and replication is clathrin-, pH- and cholesterol-dependent. J Gen Virol 90:210–215. doi:10.1099/vir.0.006387-0
15. Garrison AR, Radosztsky SR, Kota KP et al (2013) Crimean-Congo hemorrhagic fever virus utilizes a clathrin- and early endosome-dependent entry pathway. Virology 444:45–54. doi:10.1016/j.virol.2013.05.030

16. Shi X, Kohl A, Li P, Elliott RM (2007) Role of the cytoplasmic tail domains of Bunyamwera orthobunyavirus glycoproteins Gn and Gc in virus assembly and morphogenesis. J Virol 81:10151–10160. doi:10.1128/JVI.00753-07

17. Overby AK, Popov VL, Pettersson RF, Neve EPA (2007) The cytoplasmic tails of Uukuniemi Virus (Bunyaviridae) G(N) and G(C) glycoproteins are important for intracellular targeting and the budding of virus-like particles. J Virol 81:11381–11391. doi:10.1128/JVI.00767-07

18. Carnece X, Ermonval M, Kreher F et al (2014) Role of the cytosolic tails of Rift Valley fever virus envelope glycoproteins in viral morphogenesis. Virology 448:1–14. doi:10.1016/j.virol.2013.09.023

19. Tamin A, Harcourt BH, Lo MK et al (2009) Development of a neutralization assay for Nipah virus using pseudotype particles. J Virol Methods 160:1–6. doi:10.1016/j.jviromet.2009.02.025

20. Fukushima S, Mizutani T, Saijo M et al (2005) Vesicular stomatitis virus pseudotyped with severe acute respiratory syndrome coronavirus spike protein. J Gen Virol 86:2269–2274. doi:10.1099/vir.0.80955-0

21. Ogino M, Ebihara H, Lee B-H et al (2003) Use of Vesicular Stomatitis Virus Pseudotypes Bearing Hantaan or Seoul Virus Envelope Proteins in a Rapid and Safe Neutralization Test. Clin Vaccine Immunol 10:154–160. doi: 10.1128/CDLI.10.1.154-160.2003

22. Tani H, Iha K, Shimozima M et al (2014) Analysis of Lujo virus cell entry using pseudotyped vesicular stomatitis virus. J Virol 88:7317–7330. doi:10.1128/JVI.00512-14

23. Taniguchi S, Sayama Y, Nagata N et al (2012) Analysis of the humoral immune responses among cynomolgus macaque naturally infected with Reston virus during the 1996 outbreak in the Philippines. BMC Vet Res 8:189. doi:10.1186/1746-6148-8-189

24. Sayama Y, Demetria C, Saito M et al (2012) A seroepidemiologic study of Reston ebolavirus in swine in the Philippines. BMC Vet Res 8:82. doi:10.1186/1746-6148-8-82

25. Tani H, Komoda Y, Matsuo E et al (2007) Replication-competent recombinant vesicular stomatitis virus encoding hepatitis C virus envelope proteins. J Virol 81:8601–8612. doi:10.1128/JVI.00608-07

26. Takada A, Robison C, Goto H et al (1997) A system for functional analysis of Ebola virus glycoprotein. Proc Natl Acad Sci USA 94:14764–14769

27. Xiao X, Feng Y, Zhu Z, Dimitrov DS (2011) Identification of a putative Crimean-Congo hemorrhagic fever virus entry factor. Biochem Biophys Res Commun 411:253–258. doi:10.1016/j.bbrc.2011.06.109

28. Geijtenbeek TB, Kwon DS, Tornesma R et al (2000) DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell 100:587–597

29. de Witte L, de Vries RD, van der Vlist M et al (2008) DC-SIGN and CD150 have distinct roles in transmission of measles virus from dendritic cells to T-lymphocytes. PLoS Pathog 4:e1000049. doi:10.1371/journal.ppat.1000049

30. Tassaneetrithep B, Burgess TH, Granelli-Piperno A et al (2003) DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. J Exp Med 197:823–829. doi:10.1084/jem.20021840

31. Marzi A, Gramberg T, Simmons G et al (2004) DC-SIGN and DC-SIGNR interact with the glycoprotein of Marburg virus and the S protein of severe acute respiratory syndrome coronavirus. J Virol 78:12090–12095. doi:10.1128/JVI.78.21.12090-12095.2004

32. Shimojima M, Ströher U, Ebihara H et al (2012) Identification of cell surface molecules involved in dystroglycan-independent Lassa virus cell entry. J Virol 86:2067–2078. doi:10.1128/JVI.06451-11

33. Hofmann H, Li X, Zhang X et al (2013) Severe fever with thrombocytopenia virus glycoproteins are targeted by neutralizing antibodies and can use DC-SIGN as a receptor for pH-dependent entry into human and animal cell lines. J Virol 87:4384–4394. doi:10.1128/JVI.02628-12

34. Erickson BR, Deyde V, Sanchez AJ et al (2007) N-linked glycosylation of Gn (but not Gc) is important for Crimean Congo hemorrhagic fever virus glycoprotein localization and transport. Virology 361:348–355. doi:10.1016/j.virol.2006.11.023

35. Shimojima M, Takada A, Ebihara H et al (2006) Tyro3 family-mediated cell entry of Ebola and Marburg viruses. J Virol 80:10109–10116. doi:10.1128/JVI.01157-06

36. Shimojima M, Nishimura Y, Miyazawa T et al (2002) A feline CD2 homologue interacts with human red blood cells. Immunology 105:360–366

37. Carneiro FA, Ferradosa AS, Da Poian AT (2001) Low pH-induced conformational changes in vesicular stomatitis virus glycoprotein involve dramatic structure reorganization. J Biol Chem 276:62–67. doi:10.1074/jbc.M008753200

38. Powlesland AS, Fisch T, Taylor ME et al (2008) A novel mechanism for LSECtin binding to Ebola virus surface glycoprotein through truncated glycans. J Biol Chem 283:593–602. doi:10.1074/jbc.M076292200

39. Shtanko O, Nikitina RA, Altuntas CZ et al (2014) Crimean-Congo hemorrhagic fever virus entry into host cells occurs through the multivesicular body and requires ESCRT regulators. PLoS Pathog 10:e1004390. doi:10.1371/journal.ppat.1004390

40. Connolly-Andersen A-M, Douagi I, Kraus AA, Mirazimi A (2009) Crimean Congo hemorrhagic fever virus infects human monocyte-derived dendritic cells. Virology 390:157–162. doi:10.1016/j.virol.2009.06.010

41. Burt FJ, Swanepoel R, Shieh WJ et al (1997) Immunohistochemical and in situ localization of Crimean-Congo hemorrhagic fever (CCHF) virus in human tissues and implications for CCHF pathogenesis. Arch Pathol Lab Med 121:839–846

42. Geijtenbeek TBH, Engering A, Van Kooyk Y (2002) DC-SIGN, a C-type lectin on dendritic cells that unveils many aspects of dendritic cell biology. J Leukoc Biol 71:921–931

43. Tassaneetrithep B, Burgess TH, Granelli-Piperno A et al (2003) DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. J Exp Med 197:823–829. doi:10.1084/jem.20021840

44. Zhang F, Ren S, Zuo Y (2014) DC-SIGN, DC-SIGNR and LSECtin: C-type lectins for infection. Int Rev Immunol 30:186–202. doi:10.1080/02619238.2013.859287