

Review

Host Cell Factors in Filovirus Entry: Novel Players, New Insights

Heike Hofmann-Winkler *, Franziska Kaup and Stefan Pöhlmann *

German Primate Center, Infection Biology Unit, Kellnerweg 4, 37077 Göttingen, Germany;

E-Mail: franziska.kaup@dpz.eu

* Authors to whom correspondence should be addressed;

E-Mails: HHofmann-Winkler@dpz.eu (H.H.-W.); s.poehlmann@dpz.eu (S.P.);

Tel.: +49-3851-497 (H.H.-W.); +49-551-3851-150 (S.P.); Fax: +49-551-3851-184 (H.H.-W.); +49-551-3851-184 (S.P.).

Received: 23 October 2012; in revised form: 12 November 2012 / Accepted: 13 November 2012 /

Published: 26 November 2012

Abstract: Filoviruses cause severe hemorrhagic fever in humans with high case-fatality rates. The cellular factors exploited by filoviruses for their spread constitute potential targets for intervention, but are incompletely defined. The viral glycoprotein (GP) mediates filovirus entry into host cells. Recent studies revealed important insights into the host cell molecules engaged by GP for cellular entry. The binding of GP to cellular lectins was found to concentrate virions onto susceptible cells and might contribute to the early and sustained infection of macrophages and dendritic cells, important viral targets. Tyrosine kinase receptors were shown to promote macropinocytic uptake of filoviruses into a subset of susceptible cells without binding to GP, while interactions between GP and human T cell Ig mucin 1 (TIM-1) might contribute to filovirus infection of mucosal epithelial cells. Moreover, GP engagement of the cholesterol transporter Niemann-Pick C1 was demonstrated to be essential for GP-mediated fusion of the viral envelope with a host cell membrane. Finally, mutagenic and structural analyses defined GP domains which interact with these host cell factors. Here, we will review the recent progress in elucidating the molecular interactions underlying filovirus entry and discuss their implications for our understanding of the viral cell tropism.

Keywords: filovirus; Ebola; Marburg; entry; cathepsins; TIM-1; Axl; NPC1

1. Introduction

Filovirus infection causes severe hemorrhagic fever in humans and non-human primates. Outbreaks of filovirus hemorrhagic fever occur in equatorial Africa and are associated with high case-fatality rates [1]. At present, neither vaccines nor antiviral drugs have been approved for combating filovirus infection. The *filoviridae* family comprises two genera, *Ebolavirus* (the ebolaviruses) and *Marburgvirus* (the marburgviruses). The genus *Marburgvirus* includes a single species, *Marburg marburgvirus*, which has two members, Marburg virus (MARV) and Ravn virus (RAVV). The genus *Ebolavirus* includes five species, each of which has a single member: *Zaire ebolavirus* (Ebola virus, EBOV), *Sudan ebolavirus* (Sudan virus, SUDV), *Tai Forest ebolavirus* (Tai Forest virus, TAFV), *Bundibugyo ebolavirus* (Bundibugyo virus, BDBV) and *Reston ebolavirus* (Reston virus, RESTV) [2,3]. Filoviruses exhibit different virulence in humans: EBOV and MARV infection is associated with case-fatality rates of up to 90% [4,5] while RESTV seems to be apathogenic [6–8]. Nevertheless, infection of non-human primates with RESTV can induce hemorrhagic fever [9]. Evidence is emerging that African [10–13], Asian [14] and possibly also European [15] bats are natural reservoirs of filoviruses and these animals could transmit the virus directly to humans or via intermediate hosts, including gorillas [16,17] and swine [6,14,18]. Thus, filoviruses pose a threat to human and animal health in different continents, but virulence factors and pathogenesis are incompletely understood.

Deciphering filovirus pathogenesis requires the elucidation of filovirus interactions with host cells. The entry of filoviruses into target cells is the first essential step in the viral life cycle, and the viral and cellular factors involved in this process are potential targets for antiviral strategies. Infectious filovirus entry is mediated by the viral glycoprotein (GP), which is the only viral envelope protein and thus constitutes the sole target for the neutralizing antibody response. Consequently, defining which domains in GP are essential for cellular entry and can be targeted by the humoral immune response is pivotal to the design of effective vaccines. In addition, insights into the multiple, sequential interactions of GP with host cell factors required for cellular entry can define novel targets for therapeutic inhibition. Key components of the filovirus entry cascade have been identified in the recent years, and some of these discoveries have already been translated into novel antiviral approaches in cell culture and small animal models [19–24]. Here, we will review the current knowledge of the host cell factors involved in the cellular entry of filoviruses and we will discuss the implications of key findings in this area for our understanding of viral pathogenesis.

2. The Filoviral Glycoprotein: Structure and Function

The filovirus particle is predominantly of filamentous nature and consists of seven structural proteins and a non-segmented, single-stranded RNA genome of negative polarity. In the viral envelope, a single type I transmembrane glycoprotein is incorporated, which is a virulence factor [25] and the sole viral determinant of host cell entry. Thus, GP mediates attachment of virions to host cells and fusion of the viral envelope with a host cell membrane, which is essential for delivery of viral proteins and nucleic acid into the host cell cytoplasm [26–30]. To facilitate virus entry, GP interacts with host cell factors, and the expression pattern of these factors is believed to be a central determinant

of filovirus cell tropism. In addition, the various soluble forms of GP produced in the context of ebolavirus infection might modulate viral pathogenicity, as reviewed elsewhere [31].

2.1. Biosynthesis and Posttranslational Modification of the Filovirus Glycoprotein

The filoviral GP is synthesized as a precursor protein, GP₀, in the secretory pathway of infected cells. Upon transport of GP₀ into the Golgi apparatus, subtilisin-like proprotein convertases, in particular furin, process the precursor protein into two subunits, the surface unit GP₁ and the transmembrane unit GP₂ [32]. Both subunits remain covalently linked by an intermolecular disulfide bond and trimers of GP₁-GP₂ heterodimers (GP_{1,2}) are inserted into the cellular and the viral membrane [33]. The filoviral GP_{1,2} is heavily glycosylated and harbors numerous consensus sites for N-linked glycosylation and GP₁ glycosylation has been determined on the molecular level [34,35]. In addition, a mucin-like region (MLR) at the C-terminus of GP₁, which is highly variable among filovirus species [28,33,36–38], is extensively modified by O-linked glycans. The MLR is dispensable for entry into cell lines, as lentiviral vectors (pseudotypes) bearing GP_{1,2} mutants with a deleted MLR show comparable or even enhanced capacity to transduce certain cell lines (for instance Vero cells) compared to particles carrying wild type GP_{1,2} [28,36,39–41]. However, the MLR seems to be important for association with several host cell lectins, as discussed below, and might have an important role in immune evasion and filovirus pathogenesis.

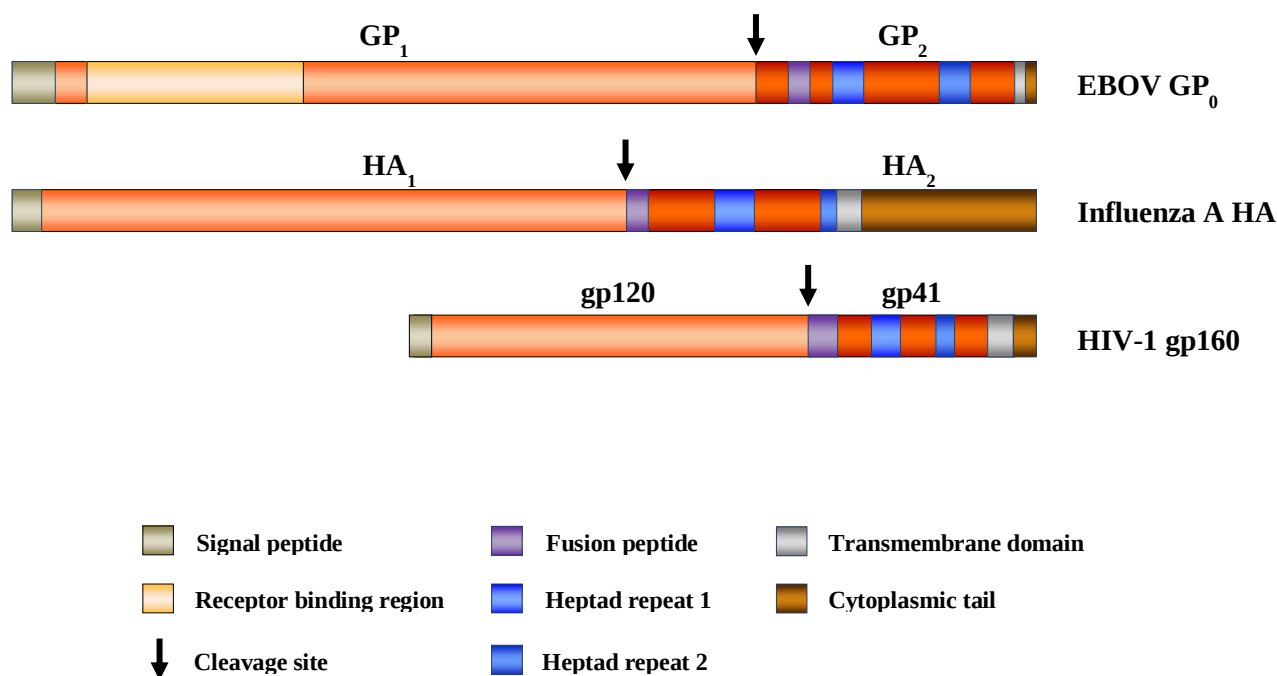
2.2. The Filovirus Glycoprotein Is a Class I Membrane Fusion Protein

Mutagenic analyses and resolution of the structure of GP_{1,2} at the atomic level identified features also present in other viral glycoproteins, termed class I membrane fusion proteins (Figure 1), including the human immunodeficiency viruses (HIV) envelope protein and the influenza viruses hemagglutinin [37,42,43]. Thus, class I membrane fusion proteins form metastable trimers which are oriented perpendicular to the viral membrane. Each monomer consists of an N-terminal surface subunit, which contains a receptor-binding domain, and a C-terminal transmembrane unit inserted in the viral membrane, which harbors domains integral to the membrane fusion reaction, a hydrophobic fusion peptide or fusion loop and two heptad repeats (HR). The surface and transmembrane units are separated by a proteolytic cleavage site, and processing of this site by a host cell protease primes the proteins for membrane fusion. The membrane fusion involves a major conformation rearrangement of the transmembrane unit, resulting in the formation of a characteristic, highly stable trimer-of-hairpins with a central α -helical coiled-coil [44].

As predicted for class I membrane fusion proteins, the surface subunit, GP₁, of the filoviral GP contains a receptor binding region (RBR), which facilitates GP binding to host cells [26–30]. The RBR localizes to the N-terminus of GP₁ and is relatively conserved between EBOV- and MARV-GP (47% amino acid identity) which are believed to share a cellular receptor [36,45]. GP₁ and GP₂ are separated by a protease sensitive site, which is cleaved by subtilisin-like proprotein convertases, in particular furin. However, in contrast to other class I membrane fusion proteins, the furin consensus site is dispensable for cellular entry [46,47] and priming of GP is facilitated by endosomal cathepsins, as discussed below. The transmembrane unit GP₂ contains an N-terminal fusion loop and two HRs, which are conserved between the GP₂ subunits of ebola- and marburgviruses. In addition, a synthetic peptide

corresponding to the C-terminal HR sequence of EBOV-GP₂ inhibited both EBOV and MARV infection [19], indicating that both viruses use similar strategies to facilitate membrane fusion [39,48–53]. The membrane fusion reaction results in the formation of a thermostable trimer-of-hairpins structure [42,43], as expected for a class I membrane fusion protein.

Figure 1. Domain organization of selected viral class I membrane fusion proteins. Viral class I membrane fusion proteins contain an N-terminal surface unit, which binds to cellular receptors, and a C-terminal transmembrane unit, which harbors the membrane fusion machinery. The glycoproteins are synthesized as inactive precursor proteins, which are primed for membrane fusion by proteolytic processing at the border between both subunits (the activating cleavage site is indicated by an arrow). For Ebola virus (EBOV)-viral glycoprotein (GP₀), the receptor binding region (RBR) is shown. The C-terminal membrane fusion subunits of the respective glycoproteins, which anchor the proteins in the viral membranes, contain the following characteristic architectural elements required for membrane fusion: heptad repeats and a hydrophobic fusion peptide. The figure is adapted from [44,54].



2.3. Filovirus Fusion with Host Cell Membranes

In the native, pre-fusion form of GP_{1,2}, the N-terminal HR is hidden in the GP₁-GP₂ trimeric structure by the RBR [37], which is protected by a glycan cap, whereas the fusion loop curls around the outside of GP₁ [37,45,55]. Priming of GP_{1,2} for membrane fusion occurs in host cell endosomes, where pH-dependent proteases remove the glycan cap [56,57], which in turn leads to the exposure of the fusion loop and allows its insertion into the host cell membrane [37,58]. Thereafter, HR1 and HR2 fold back onto each other, resulting in the formation of a thermostable trimer-of-hairpin structure [42,43,59], which brings viral and target cell membranes into close vicinity and ultimately

facilitates membrane fusion. Several host cell factors are involved in priming and triggering of filovirus GP_{1,2} for membrane fusion and will be discussed in detail below.

3. Cell and Organ Tropism of Filoviruses

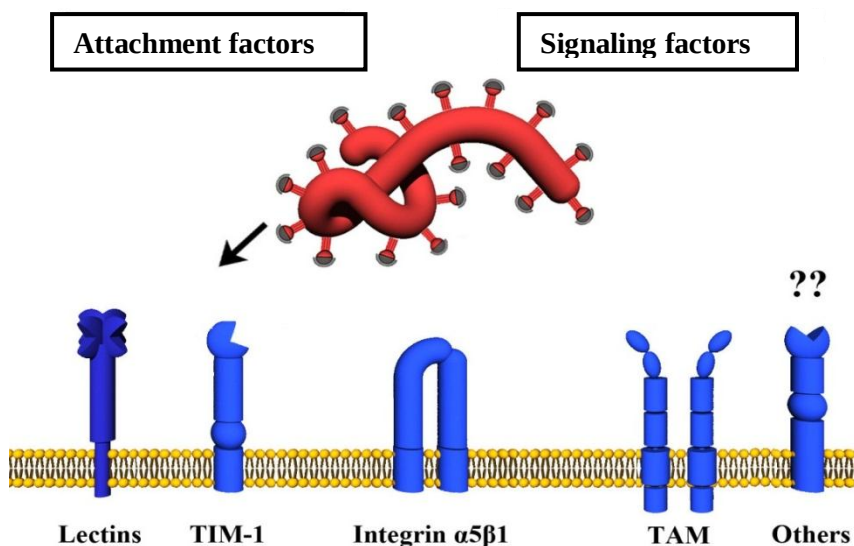
The cellular molecules involved in filovirus entry govern the spectrum of cells susceptible to filovirus infection. Before we discuss GP_{1,2} interactions with these factors, we will therefore provide a brief overview of the filovirus cell tropism. Filoviruses exhibit a broad cell and organ tropism in infected humans and non-human primates. However, at the early stages of the infection, cells of the mononuclear phagocyte system are mainly targeted, in particular macrophages and dendritic cells in the spleen, lymph nodes and liver [60–70]. Infection of these cells not only amplifies the virus [71] and ensures its rapid dissemination [72], but also triggers the uncontrolled release of pro-inflammatory cytokines [60,66,70], a hallmark of filovirus pathogenesis.

Secondary targets of filovirus infection are mainly fibroblasts and endothelial cells located in many different organs, including liver, kidney and testis, and these cells are permissive to robust, lytic viral replication [64,73]. It has been proposed that ebolavirus infection of the vascular endothelium and the ensuing GP_{1,2}-induced cell rounding might result in loss of vascular integrity and hemorrhage [28]. However, the vascular endothelium is a late target in EBOV infected non-human primates, and no obvious cytotoxic effects are associated with its infection [64], suggesting that hemorrhage is not a direct consequence of ebolavirus infection of vascular endothelial cells. Apart from fibroblasts and endothelial cells, virtually all cell types are susceptible to filovirus infection, and infectious EBOV and MARV could be isolated from all tissues tested [64,67,74–78]. The only cells refractory to the otherwise pantropic filoviruses are lymphocytes [65,67], and experiments with pseudotypes showed that these cells are not susceptible to GP_{1,2}-driven host cell entry, potentially because of lack of host cell factors required for appropriate uptake and intracellular trafficking [26,27,27,79–84].

4. Host Cell Factors Promoting Infectious Filovirus Entry

The entry of enveloped viruses into host cells commences with the attachment of the virus to the cell surface, which is frequently promoted by relatively nonspecific interactions between the viral GP and cellular attachment-promoting factors. Subsequently, highly specific engagement of cellular molecules by the viral GP is essential to trigger uptake of virions into target cells and/or fusion of the viral with a host cell membrane. A virus entry receptor is usually defined as a cellular binding partner of a viral GP which is essential for infectious viral entry into host cells. Given the complexity of filovirus entry, which involves both cell surface molecules and intracellular proteins, only some of which physically interact with GP, we will not employ the classical receptor definition in our description of filovirus entry. Instead, discriminate between attachment factors, which interact with GP_{1,2} at the cellular membrane and promote viral attachment to cells, signaling factors, which induce filovirus uptake through activating signaling cascades (Figure 2), and endo-/lysosomal host factors, which prime and activate GP_{1,2} for membrane fusion (Figure 3).

Figure 2. Host cell surface proteins involved in filovirus uptake. Cellular lectins bind glycans present on the filoviral GP_{1,2} and thereby promote attachment to target cells. The surface molecule TIM-1 interacts with GP_{1,2} inserted in the viral membrane. TAM proteins do not bind to GP_{1,2} but elicit signals which promote viral uptake by macropinocytosis. Integrins might interact with GP_{1,2} and are important for intracellular processing of GP_{1,2}.



4.1. Attachment Factors

4.1.1. Carbohydrate-Binding Host Cell Factors (Lectins)

The cellular lectins DC-SIGN, DC-SIGNR, LSECtin, ASPGR-1 and hMGL [41,85–89] can promote filovirus entry into transfected cells (Table 1). An initial study suggested that expression of some of these lectins might be sufficient to render target cells susceptible to filovirus infection [85]. However, subsequent work showed that lectins merely facilitate usage of low levels of so far unidentified molecules important for filovirus entry [81,90,91]. Augmentation of filovirus infection by lectins depends on binding of these proteins to glycans on GP_{1,2}, and the respective lectins exhibit differences in their carbohydrate specificities, which are reflected by differential requirements for GP_{1,2} binding. Thus, the extensively O-glycosylated MLR is dispensable for GP_{1,2} binding to DC-SIGN and DC-SIGNR [92], which mainly recognize high-mannose glycans, while deletion of the MLR impedes interactions with hMGL, which recognizes terminal galactose-/N-acetylgalactosamine [41,50,93]. In addition, differences in lectin engagement among the ebolavirus species and MARV have been noted [41,92]. The expression of the GP-binding lectins on relevant filovirus target cells, including tissue macrophages and dendritic cells (hMGL, DC-SIGN) [94,95], hepatocytes (ASGPR-1) [96] and liver and lymph node sinusoidal endothelial cells (LSECtin, DC-SIGNR) [97,98] suggests that they could modulate filovirus spread in the infected host. However, there is currently little evidence that endogenous lectin expression appreciably augments filovirus entry into primary target cells [90], and the role of lectins in filovirus spread in animal models has not been examined. In the context of HIV-1 infection, it has been demonstrated that viral binding to DC-SIGN on dendritic cells triggers signal transduction and thereby commandeers the cell to undergo aberrant maturation and to produce

immunosuppressive cytokines [99], which is believed to promote viral spread. Whether a similar mechanism is operative during filovirus infection is unknown. Finally, it is noteworthy that secreted lectins can modulate filovirus infection. Thus, recombinant mannose-binding C-type lectin (MBL) was shown to protect mice from a lethal EBOV infection potentially by targeting the virus for phagocytosis and complement-directed lysis [100].

Table 1. Overview and expression of lectins involved in filovirus attachment.

GP-binding lectins	Tissue distribution	Reference
ASGPR-1	• hepatocytes	[86]
hMGL	• monocyte-derived immature dendritic cells • intermediate precursors of macrophages	[41]
DC-SIGN	• dendritic cells • fetal macrophages • decidual macrophages • alveolar macrophages • platelets • activated primary B-cells	[85,85,88,90,101–103]
DC-SIGNR	• endothelial cells in the placenta • endothelial cells in the liver and lymph node sinuses	[85]
LSEctin	• sinusoidal endothelial cells in liver, lymph nodes and bone marrow • thymic dendritic cells • monocyte-derived macrophages • peripheral blood cells	[34,87,102]

4.1.2. TIM-1

The human T cell Ig mucin 1 (TIM-1) surface molecule was initially found to be a cellular receptor for hepatitis A virus [104]. By a bioinformatics-based correlation analysis between gene expression profiles and susceptibility of cell lines to EBOV-GP_{1,2}-driven infection, TIM-1 was recently also identified as an entry factor for filoviruses [105]. TIM-1 is a type I membrane glycoprotein with an extracellular IgV domain and a mucin-like domain predicted to be heavily O-glycosylated [106]. The IgV domain allows highly specific recognition of phosphatidylserine exposed on the surface of apoptotic cells, and TIM-1 was shown to be involved in the clearance of apoptotic cells [107,108]. TIM-1 is expressed on activated T-cells, epithelial cells, conjunctiva and renal tissue [105,109,110] as well as certain cell lines including the liver cell line Huh7 [105] (Table 2), which is permissive for filovirus replication [111].

A physical interaction between the receptor binding domain of EBOV-GP₁ and soluble TIM-1 could be demonstrated [105], and soluble TIM-1 was shown to block EBOV-GP_{1,2}-driven infection, indicating that TIM-1-dependent filovirus entry requires GP_{1,2} interactions with this protein. RNA knock-down of endogenous TIM-1 inhibited pseudovirus entry into otherwise susceptible cell lines, and ectopic expression of TIM-1 on TIM-1-negative cell lines enhanced infection [105], indicating that TIM-1 indeed promotes filovirus infection of certain cell lines. However, macrophages and dendritic

cells, which are important targets of filovirus infection, lack TIM-1 expression [110], suggesting that so far unidentified cellular factors facilitate filovirus entry into these cells. Finally, it would be interesting to determine whether TIM-1 intracellular signaling [112,113] might contribute to filovirus GP-mediated cellular uptake.

Table 2. Overview of host cell factors involved in filovirus entry.

Host factor	Expression	Analysis in cell lines	Reference
TIM-1	• Epithelia of conjunctiva and cornea • Injured kidney tubuli epithelia • Activated Th2 cells • Human airway epithelia • Mast cells	• 786-O • A498 • ACHN • CAKI-1 • TK-10 • UO-31 • RXF393 • Huh7 • Vero E6 • 293T-H3	[105,109,110]
Axl	• broad expression in cells of the mature immune, nervous, reproductive and vascular systems	• Vero E6 • HT1080 • 293 • HeLa • Cos7 • A549 • SNB19 • HuVECs • SN12C • HFF	[114–120]
Integrin $\alpha V \beta 1$	• Ubiquitous	• Hela • HepG2 • Hep3B	[121–124]
Cathepsin B and L	• Ubiquitous	• Vero E6 • MEF • 293T	[24,56,57,125–128]
NPC1	• Ubiquitous	• HUVEC • Vero E6 • Cloned HAP-1 • others	[20,129,130]

4.2. Signaling Factors

4.2.1. TAM Family of Tyrosine Kinase Receptors

Another interesting group of molecules shown to be involved in ebolavirus infectious entry are members of the Tyro3/Axl/Mer (TAM) family of receptor tyrosine kinases [119,131]. These proteins contain a kinase domain and an adhesion-molecule-like extracellular domain and play important roles

in diverse biological processes including cell proliferation and survival, cell adhesion and cytokine release [131]. Members of the TAM family are evolutionarily conserved between vertebrate species and the respective proteins are widely expressed in adult tissues, most prominently in the brain, lung, kidney, lymphatic tissue and the vascular system [118,119] (Table 2). The natural ligands for TAM receptors are the structurally homologous proteins Gas6 and protein S [132,133]. These secreted proteins bind to phosphatidylserine residues exposed by apoptotic cells [134,135], and ligand-activated TAM receptors have been found to inhibit inflammation pathways in macrophages and dendritic cells [120].

By introduction of a cDNA library derived from permissive VeroE6 cells into non-susceptible Jurkat lymphocytes, Axl was identified as an EBOV entry factor expressed at the cell surface [117]. The ectopic expression of Axl and the TAM family members DTK and MER on lymphoid cells allowed for transduction of GP_{1,2}-harboring pseudotypes, which could be blocked by Axl-specific antibodies, confirming that TAM-proteins could promote filovirus entry [117], although the mechanism underlying the antibody-mediated blockade of viral entry is currently unclear. Mutational analysis revealed that both the extracellular ligand binding domain and the cytoplasmic tail of Axl were required for efficient GP_{1,2}-mediated entry [116,117]. A role of TAM family proteins in filovirus entry was confirmed by an independent study, which showed that expression of an mRNA encoding for Axl correlates with susceptibility of cell lines to EBOV infection [114]. The use of TAM protein-specific antibodies and siRNA knockdown indeed identified several cell lines, in which EBOV-GP_{1,2}-facilitated entry was dependent on Axl expression. In contrast, down-regulation of Axl-expression in other cell lines did not compromise GP_{1,2}-driven entry [114,115,117]. So far, all studies failed to detect a direct interaction between EBOV-GP₁ and Axl. However, Axl expression was shown to augment internalization of EBOV-GP_{1,2}-bearing pseudovirions and virus-cell fusion [114], and this activity correlated with enhanced macropinocytosis in Axl-expressing cells [115]. Thus, Axl might promote filovirus uptake by macropinocytosis, which was previously shown to be a pathway exploited by filoviruses for cellular uptake [136,137]. Indeed, inhibition of Axl and blockade of PI3K, which is important for macropinocytosis and Axl-dependent signaling, both inhibit filovirus entry in a cell type-dependent fashion [21,125]. Finally, it is noteworthy that Gas6 can promote Sindbis virus entry by bridging phosphatidylserine present in the viral envelope to Axl localized on target cells [138]. Whether a similar mechanism operates in the context of filovirus entry remains to be investigated. The observation that recombinant Gas6 inhibits Axl- and DTK-dependent GP_{1,2}-mediated entry [117] might argue against this hypothesis.

4.2.2. $\alpha 5\beta 1$ -Integrin

Integrins are cell surface expressed heterodimeric type I transmembrane glycoproteins, which are composed of two non-covalently linked subunits (α and β) [122]. The integrin family in mammals comprises eighteen α and eight β subunits which can assemble into 24 different heterodimers [139]. These heterodimers, in turn, convey specificity to cell-cell and cell-extracellular matrix adhesion, immune cell recruitment, extravasation, and signaling events [121,140]. Members of the integrin family have been discovered as attachment factors or receptors for a large number of enveloped and non-enveloped viruses, including herpesviruses [141–143], adenoviruses [144,145], hantaviruses

[146–149], picornaviruses [150–152], and reoviruses [153,154]. The association with viral surface proteins and microbial pathogens are followed by multiple signaling events, some of which promote cytoskeletal reorganization and thus facilitate receptor-mediated endocytosis (as reviewed in [155,156] and references therein). It is not surprising that numerous viruses hijack integrins for infectious entry, as they are widely expressed in various tissues throughout the body (Table 2).

Expression of EBOV-GP_{1,2} was initially shown to interfere with surface expression of various cellular membrane proteins, including $\alpha 3$ and $\beta 1$ integrins [40,124]. However, a more recent study suggested that GP_{1,2} expression does not reduce cell surface levels of integrins but rather sterically occludes epitopes in these proteins otherwise recognized by antibodies [157]. Experiments with EBOV-GP_{1,2}-bearing pseudotypes demonstrated that soluble recombinant $\beta 1$ integrin or $\beta 1$ -reactive antibodies diminish GP_{1,2} driven entry, suggesting that GP_{1,2} might need to engage $\beta 1$ integrins for infectious entry [124]. However, a direct interaction between EBOV-GP_{1,2} and integrins remains to be demonstrated. Work by Schornberg and colleagues provided evidence that $\alpha 5\beta 1$ -integrin is required for expression of the double chain forms of cathepsin B and L and for full cathepsin L activity [123], an endosomal protease involved in priming of GP_{1,2} for membrane fusion, as discussed below. In contrast, $\alpha 5\beta 1$ -integrin was dispensable for GP_{1,2}-mediated binding and uptake into target cells [123]. These observations suggest that $\alpha 5\beta 1$ -integrin indirectly promotes GP_{1,2}-driven entry by ensuring activity of GP_{1,2}-priming cysteine proteases or by stimulating the protease maturation pathway, which might be required for viral entry. Whether physical interactions of GP_{1,2} with $\alpha 5\beta 1$ -integrin also contribute to filovirus entry remains to be determined.

4.3. Endo-/Lysosomal Host Cell Factors

The membrane fusion reaction driven by the GPs of enveloped viruses can be triggered by several stimuli. For some viruses, engagement of a receptor at the cell surface activates fusion with the plasma membrane at neutral pH [158]. Alternatively, receptor binding resulting in virus internalization, and membrane fusion is stimulated by protonation of the viral GP in the acidic environment of the endosome [158]. The mild pH (6.5–6) of early endosomes is sufficient to trigger membrane fusion facilitated by the Nipah and Hendra virus GPs [159,160], while the low pH (5.5–4) environment of late endosomes and/or lysosomes activates the membrane fusion proteins of influenza, bunya and dengue viruses [161–163]. In addition, some viruses require both low pH and receptor engagement as triggers for membrane fusion [164], while others are triggered upon receptor engagement at the cell surface, but ultimately fuse with the endosomal membrane [165], indicating that complex determinants govern the nature of the trigger and the subcellular location of membrane fusion reaction.

Many viral GPs are synthesized as inactive precursor proteins which transit into a membrane fusion-competent state only upon proteolytic cleavage by host cell proteases, a process termed priming. A prominent example is the influenza virus, which depends on cleavage of its hemagglutinin by host cell proteases for acquisition of infectivity, and the nature of the cleavage sequences in HA determines the virulence of avian influenza viruses [166]. The subtilisin-like proprotein convertase furin is responsible for priming of several viral GPs in the Golgi apparatus of infected cells, and furin consensus sites are present in GP₀ of all filoviruses excluding RESTV, which harbors an incomplete furin recognition site [32]. Despite of its conservation, several studies indicate that this motive is

dispensable for filoviral spread in cell culture and infected animals [46,47], and the reason for its presence is unknown.

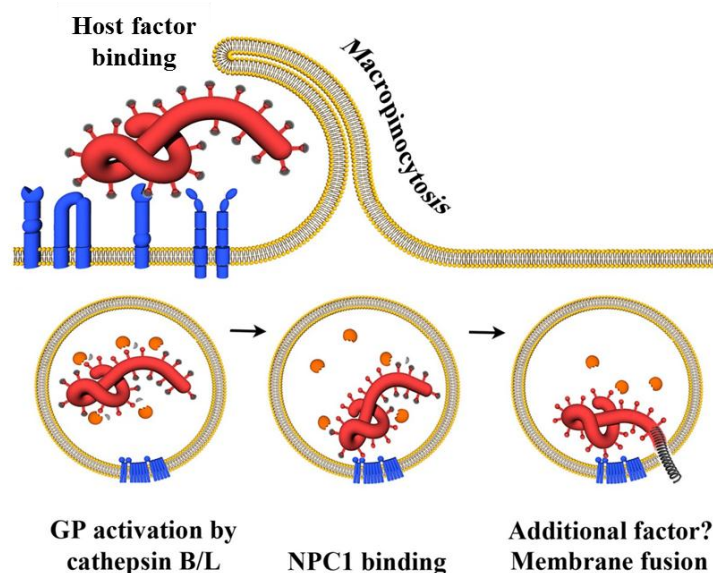
It has long been noticed that filoviruses depend on low pH for infectious cellular entry [26]. However, it has also been demonstrated that low pH does not trigger the fusion activity of GP_{1,2} [167]. This conundrum has been resolved by a study demonstrating that filoviruses are activated by endo-/lysosomal cysteine proteases, which require a low pH environment for their enzymatic activity [57].

4.3.1. Cathepsins B and L

Cathepsins comprise serine, aspartic and cysteine proteases and carry out diverse biological functions, including antigen processing for MHCII presentation [168–170]. Cysteine proteases of the papain family, some of which are localized in endosomes, are expressed as preproenzymes and are activated by proteolysis in the endoplasmatic reticulum and the late endosome/lysosome [168–170]. The low pH environment present in the latter compartment is essential for cathepsin enzymatic activity.

Chandran and colleagues demonstrated that two lysosomal cathepsins, cathepsin B and L, cleave filovirus GP_{1,2} and that cathepsin activity is essential for GP_{1,2}-driven host cell entry [57]. This report showed that cathepsins B and L prime the filovirus GP_{1,2} for membrane fusion and proposed that cathepsin cleavage of virion-associated GP_{1,2} occurs in a sequential fashion: First, cathepsin L and/or B cleave GP_{1,2} into an 18 kDa form, which is fully infectious but still requires cathepsin B activity for infectious entry. Subsequently, the 18 kDa form is processed by cathepsin B and cleavage might be sufficient to trigger membrane fusion [57]. An alternative model for GP_{1,2} activation has been proposed by subsequent studies. Thus, Schornberg and colleagues demonstrated that processing of virion-inserted GP_{1,2} by recombinant cathepsin B and L or the bacterial protease thermolysin yielded a 19 kDa form of GP₁ and was associated with a notable increase in infectivity [56], a finding confirmed by others [126]. Processing of GP₁ into the 19 kDa form proceeded via 50 kDa and 20 kDa intermediates and it was speculated that the 20 kDa form might differ from the 18 kDa form observed by Chandran and colleagues only in the presence of a N-linked glycan [56]. Virions bearing the 19 kDa form were largely resistant to cathepsin B but not L inhibitors but remained sensitive to a lysosomotropic agent and a cysteine protease inhibitor. On the basis of these findings, a two-step model was proposed, suggesting that GP_{1,2} must first be processed by cathepsins B and L before the activity of a third lysosomal factor, potentially a thiol reductase, triggers GP_{1,2}-dependent membrane fusion [56,171]. Indeed, subsequent studies provided evidence that the 19 kDa form represents a metastable conformation in which the fusion machinery is not yet exposed [37,172–174] and which can be triggered for membrane fusion by low pH and reduction [173]. In addition, it was demonstrated that cleavage of GP_{1,2} removes a glycan cap and the MLR, while the N-terminal RBR and GP₂ remain in the molecule [172,175,176]. In sum, proteolytic processing by cathepsins B and L primes GP_{1,2} for membrane fusion and exposes the RBR. Subsequently, an incompletely understood stimulus triggers membrane fusion and these final steps of the lysosomal escape of filoviruses critically depend on GP_{1,2} binding to NPC1 (Figure 3), as discussed below.

Figure 3. Infectious entry of filoviruses into target cells. After interaction between GP_{1,2} and cellular surface molecules, virions are internalized via macropinocytosis into the endosomal compartment. Subsequently, the endosomal cysteine proteases cathepsin B and L proteolytically process GP_{1,2}, thereby removing the glycan cap (indicated by grey caps) and allowing primed GP₁ binding to Niemann-Pick C1 (NPC1), which is essential for the following virus-host membrane fusion process. Finally, a so far incompletely understood stimulus triggers the membrane fusion activity in GP₂.



Despite the importance of cathepsin B and L in priming EBOV-GP_{1,2} for membrane fusion in several cell lines, the dependence on these particular proteases for viral entry is not universal among filoviruses. A requirement for cathepsin B activity during entry of EBOV-, TAFV- and BDBV-but not SUDV-, RESTV and MARV-GP-bearing pseudotypes has been described, and the same group showed that particles harboring the GP_{1,2} of EBOV, SUDV and MARV exhibited enhanced transduction efficiency when cathepsin L was active in concert with cathepsin B. In contrast, entry of RESTV was dependent on a cysteine protease distinct from cathepsins B and L [125]. Furthermore, it has been reported that cathepsin L activity is dispensable for ebolavirus GP_{1,2}-driven entry into Vero cells and mouse embryonic fibroblasts [57,125] as well as human monocyte-derived dendritic cells [128]. Moreover, the observation of the failure of EBOV-GP_{1,2}-bearing pseudotypes to transduce CatB^{-/-}/CatL^{-/-} mouse embryonic fibroblasts can be overcome by ectopic expression of CatB, suggests that a protease other than CatL is required for a post-CatB cleavage step necessary for membrane fusion [125]. In addition, transduction of primary human macrophages by EBOV-GP_{1,2}-carrying pseudotypes was shown to be dependent on both cathepsin B and L, whereas MARV-GP_{1,2}-facilitated entry was not blocked efficiently by cathepsin B/L inhibitors, suggesting that MARV-GP_{1,2} might employ a so far unknown protease for priming in macrophages [177]. It is also noteworthy that many studies investigating the role of cathepsins in filovirus host cell entry were performed with GP_{1,2}-bearing vectors and not with authentic filoviruses. It would thus be interesting to examine the effect of cathepsin B and L knock-out on filoviral spread and pathogenicity, particularly in the light of efforts to develop cathepsin inhibitors as treatment for SARS-coronavirus [178] and filovirus infection.

The respective knock-out mice required for such studies have been described [179–181]. A study by Wong and colleagues indicates that blockade of cathepsin activity might result in the development of resistant viruses. Resistance was associated with mutations at the border between GP₁ and GP₂, which increased susceptibility to proteolytic cleavage and allowed GP_{1,2} priming by cysteine cathepsins other than cathepsin B and L [182]. Finally, it is noteworthy that expression of integrins previously suggested to promote filovirus entry is required for activity of cathepsin B and L [123], indicating an intricate interplay between some of the host cell molecules exploited by filoviruses for cellular entry.

4.3.2. NPC1

An endosomal factor required for filovirus entry after GP_{1,2} priming by cathepsins has recently been discovered by two independent studies as the Niemann-Pick C1 (NPC1) protein. Cote and colleagues discovered that filoviruses depend on NPC1 for cellular entry by screening a library of chemical compounds for entry inhibitors [20], while Carette and coworkers found in a screen of haploid human cells that mutations in NPC1 are not compatible with filovirus GP_{1,2}-driven entry [129]. Finally, a recent study found that CHO cells selected for resistance to EBOV-GP_{1,2}-dependent entry harbored a defect in the *NPC1* gene [183]. The NPC1 protein is highly conserved among species and is ubiquitously expressed in human tissues, with the highest expression in the liver [184,185] (Table 2). The protein is an integral membrane protein of late endosomes and lysosomes and exhibits a polytopic orientation, forming several luminal and cytoplasmatic loops. NPC1 is a cholesterol transporter and mutations in the *NPC1* gene result in fatal, progressive neurodegenerative disorder, Niemann-Pick C1 disease, due to a defect in the export of cholesterol from lysosomes [186]. The abnormal accumulation of cholesterol in turn leads to altered protein and lipid trafficking [187,188]. Which lines of evidence suggest that NPC1 facilitates filovirus entry? NPC1 interacts with primed GP₁ [20,130] and the contribution of NPC1 to infectious entry of filoviruses can be separated from its cholesterol transport activity [20,129], indicating that the protein directly facilitates entry. NPC1-deficient cells, including primary fibroblasts derived from NPC1 patients, were resistant to filovirus infection, but still allowed for efficient cellular entry of several other viruses [20,129]. Furthermore, transduction of the wild type *NPC1* gene into NPC1-defective, patient-derived cells or NPC1-negative CHO cells fully restored infection [20,129] and directed expression of NPC1 in non-susceptible reptilian cells or haploid hamster CHO-K1 cell clones was sufficient to render these cells susceptible to GP_{1,2}-mediated infection [130]. Additionally, siRNA knockdown of NPC-1 in HeLa cells resulted in reduced virus uptake [20]. Finally, heterozygous *NPC1*^{-/+} mice were protected against filovirus infection in sharp contrast to wild type mice [129]. In sum, these results show that NPC1 plays a key role in cellular entry of ebolaviruses.

As the NPC1 protein is localized on the endosomal and lysosomal membranes, it was proposed to act downstream of filovirus GP_{1,2} engagement of attachment and signaling factors at the cell surface. Indeed, GP_{1,2}-mediated viral uptake was readily detectable in NPC1-deficient cells, where the virions accumulated in early endosomes, indicating that membrane fusion was not triggered [129]. Proteolytic processing of GP_{1,2} and thus exposure of the RBR was a prerequisite of NPC1 binding, as only the cleaved 19 kDa form was able to physically interact with NPC1 [20,130]. Mapping studies revealed that the 19 kDa form binds to the second luminal domain of NPC1 [130] and cell surface presentation

of this domain in the context of an artificial receptor molecule was sufficient to allow entry of pseudotypes carrying thermolysin-primed filovirus GP_{1,2} [130]. It can be speculated that the interaction between the 19 kDa form of GP_{1,2} and NPC1 might expose the GP₂ residues involved in membrane fusion. However, fusion of pseudotypes bearing the primed 19 kDa GP_{1,2} with the plasma membrane of target cells expressing the second loop of NPC1 at their surface could not be induced by low pH treatment. Thus, binding of primed GP_{1,2} to NPC1 is not sufficient to trigger membrane fusion [130].

Besides filoviruses, flaviviruses have also been found to depend on molecules involved in cholesterol transport for infectious entry into host cells. The cholesterol-transport inhibitor U18666A interfered with hepatitis C and Dengue virus (strain TSV01) infection [189,190], and a factor with 40% amino acid identity to NPC1 (named NPC1-like 1, which is only expressed in human hepatocytes and the intestine) was shown to be involved in uptake of hepatitis C virus [191]. In contrast to filovirus infection, however, hepatitis C virus entry mediated by the NPC1-like 1 protein was dependent on cholesterol uptake and no evidence for an interaction between hepatitis C virus and NPC1-like 1 protein has been reported so far [191].

Taken together, the NPC1 protein is a filovirus receptor with unexpected characteristics: In comparison to cell surface receptors used by other viruses, NPC1 is unique in recognizing the filoviral glycoprotein only after uptake into the cell and after proteolytic processing. The identification of small molecule inhibitors directly or indirectly inhibiting NPC1-usage by GP_{1,2} for host cell entry [20,129] makes it an attractive candidate for antiviral therapy.

5. Conclusions

Host cell entry is the first essential step in filovirus infection. Cellular lectins can concentrate vectors bearing GP_{1,2} at the cellular surface and can thereby promote infectious entry. However, lectin expression usually does not render cells susceptible to GP_{1,2}-driven entry and a role of lectins in the cell tropism of filoviruses in the infected host remains to be demonstrated. TAM tyrosine kinase receptors and TIM-1 can augment entry of filoviruses into a subset of susceptible cell lines and into some primary cells but are not universally required for filovirus infection. In fact, neither TIM-1 nor the TAM family member Axl are expressed to appreciable amounts in human macrophages, key viral targets, and the factors regulating viral uptake into these cells remain to be elucidated. The respective studies might reveal that filoviruses can use diverse cell surface factors for uptake into TIM-1-, Axl-negative cells, which might account for the broad cell tropism of filoviruses. In contrast to Axl and TIM-1, the broadly expressed endosomal/lysosomal protein NPC1 is required for filovirus entry into all cellular systems tested so far and seems to play a key role in filovirus entry. Further experiments will clarify whether NPC1 alone is sufficient for triggering membrane fusion or whether another host cell factor is involved.

Acknowledgments

The authors would like to thank Dominik Dahlmann for technical support with figures. This work was funded by the Leibniz Foundation.

Conflict of Interest

The authors declare no conflict of interest.

References and Notes

1. Mahanty, S.; Bray, M. Pathogenesis of filoviral haemorrhagic fevers. *Lancet Infect. Dis.* **2004**, *4*, 487–498.
2. Kuhn, J.H.; Becker, S.; Ebihara, H.; Geisbert, T.W.; Jahrling, P.B.; Kawaoka, Y.; Netesov, S.V.; Nichol, S.T.; Peters, C.J.; Volchkov, V.E.; *et al.* Family Filoviridae. In *Virus Taxonomy — Ninth Report of the International Committee on Taxonomy of Viruses*; King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J., Eds.; Elsevier/Academic Press: London, UK, 2001; pp. 665–671.
3. Adams, M.J.; Carstens, E.B. Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses (2012). *Arch. Virol.* **2012**, *157*, 1411–1422.
4. Hutchinson, K.L.; Rollin, P.E. Cytokine and chemokine expression in humans infected with Sudan Ebola virus. *J. Infect. Dis.* **2007**, *196*, S357–S363.
5. Kuhn, J.H.; Dodd, L.E.; Wahl-Jensen, V.; Radoshitzky, S.R.; Bavari, S.; Jahrling, P.B. Evaluation of perceived threat differences posed by filovirus variants. *Biosecur. Bioterror.* **2011**, *9*, 361–371.
6. Barrette, R.W.; Metwally, S.A.; Rowland, J.M.; Xu, L.; Zaki, S.R.; Nichol, S.T.; Rollin, P.E.; Towner, J.S.; Shieh, W.J.; Batten, B.; *et al.* Discovery of swine as a host for the Reston ebolavirus. *Science* **2009**, *325*, 204–206.
7. Miranda, M.E.; Ksiazek, T.G.; Retuya, T.J.; Khan, A.S.; Sanchez, A.; Fulhorst, C.F.; Rollin, P.E.; Calaor, A.B.; Manalo, D.L.; Roces, M.C.; *et al.* Epidemiology of Ebola (subtype Reston) virus in the Philippines, 1996. *J. Infect. Dis.* **1999**, *179*, S115–S119.
8. Rollin, P.E.; Williams, R.J.; Bressler, D.S.; Pearson, S.; Cottingham, M.; Pucak, G.; Sanchez, A.; Trappier, S.G.; Peters, R.L.; Greer, P.W.; *et al.* Ebola (subtype Reston) virus among quarantined nonhuman primates recently imported from the Philippines to the United States. *J. Infect. Dis.* **1999**, *179*, S108–S114.
9. Jahrling, P.B.; Geisbert, T.W.; Jaax, N.K.; Hanes, M.A.; Ksiazek, T.G.; Peters, C.J. Experimental infection of cynomolgus macaques with Ebola-Reston filoviruses from the 1989–1990 U.S. epizootic. *Arch. Virol. Suppl.* **1996**, *11*, 115–134.
10. Leroy, E.M.; Kumulungui, B.; Pourrut, X.; Rouquet, P.; Hassanin, A.; Yaba, P.; Delicat, A.; Paweska, J.T.; Gonzalez, J.P.; Swanepoel, R. Fruit bats as reservoirs of Ebola virus. *Nature* **2005**, *438*, 575–576.
11. Pourrut, X.; Souris, M.; Towner, J.S.; Rollin, P.E.; Nichol, S.T.; Gonzalez, J.P.; Leroy, E. Large serological survey showing cocirculation of Ebola and Marburg viruses in Gabonese bat populations, and a high seroprevalence of both viruses in *Rousettus aegyptiacus*. *BMC Infect. Dis.* **2009**, *9*, 159.
12. Towner, J.S.; Amman, B.R.; Sealy, T.K.; Carroll, S.A.; Comer, J.A.; Kemp, A.; Swanepoel, R.; Paddock, C.D.; Balinandi, S.; Khristova, M.L.; *et al.* Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. *PLoS Pathog.* **2009**, *5*, e1000536.

13. Kuzmin, I.V.; Niezgoda, M.; Franka, R.; Agwanda, B.; Markotter, W.; Breiman, R.F.; Shieh, W.J.; Zaki, S.R.; Rupprecht, C.E. Marburg virus in fruit bat, Kenya. *Emerg. Infect. Dis.* **2010**, *16*, 352–354.
14. Taniguchi, S.; Watanabe, S.; Masangkay, J.S.; Omatsu, T.; Ikegami, T.; Alviola, P.; Ueda, N.; Iha, K.; Fujii, H.; Ishii, Y.; *et al.* Reston Ebolavirus antibodies in bats, the Philippines. *Emerg. Infect. Dis.* **2011**, *17*, 1559–1560.
15. Negrodo, A.; Palacios, G.; Vazquez-Moron, S.; Gonzalez, F.; Dopazo, H.; Molero, F.; Juste, J.; Quetglas, J.; Savji, N.; de la Cruz, M.M.; *et al.* Discovery of an ebolavirus-like filovirus in europe. *PLoS Pathog.* **2011**, *7*, e1002304.
16. Leroy, E.M.; Rouquet, P.; Formenty, P.; Souquiere, S.; Kilbourne, A.; Froment, J.M.; Bermejo, M.; Smit, S.; Karesh, W.; Swanepoel, R.; *et al.* Multiple Ebola virus transmission events and rapid decline of central African wildlife. *Science* **2004**, *303*, 387–390.
17. Bermejo, M.; Rodriguez-Teijeiro, J.D.; Illera, G.; Barroso, A.; Vila, C.; Walsh, P.D. Ebola outbreak killed 5000 gorillas. *Science* **2006**, *314*, 1564.
18. Sayama, Y.; Demetria, C.; Saito, M.; Azul, R.R.; Taniguchi, S.; Fukushi, S.; Yoshikawa, T.; Iizuka, I.; Mizutani, T.; Kurane, I.; *et al.* A seroepidemiologic study of Reston ebolavirus in swine in the Philippines. *BMC Vet. Res.* **2012**, *8*, 82.
19. Miller, E.H.; Harrison, J.S.; Radoshitzky, S.R.; Higgins, C.D.; Chi, X.; Dong, L.; Kuhn, J.H.; Bavari, S.; Lai, J.R.; Chandran, K. Inhibition of Ebola virus entry by a C-peptide targeted to endosomes. *J. Biol. Chem.* **2011**, *286*, 15854–15861.
20. Cote, M.; Misasi, J.; Ren, T.; Bruchez, A.; Lee, K.; Filone, C.M.; Hensley, L.; Li, Q.; Ory, D.; Chandran, K.; *et al.* Small molecule inhibitors reveal Niemann-Pick C1 is essential for Ebola virus infection. *Nature* **2011**, *477*, 344–348.
21. Saeed, M.F.; Kolokoltsov, A.A.; Freiberg, A.N.; Holbrook, M.R.; Davey, R.A. Phosphoinositide-3 kinase-Akt pathway controls cellular entry of Ebola virus. *PLoS Pathog.* **2008**, *4*, e1000141.
22. Barrientos, L.G.; O'Keefe, B.R.; Bray, M.; Sanchez, A.; Gronenborn, A.M.; Boyd, M.R. Cyanovirin-N binds to the viral surface glycoprotein, GP1,2 and inhibits infectivity of Ebola virus. *Antivir. Res.* **2003**, *58*, 47–56.
23. Basu, A.; Li, B.; Mills, D.M.; Panchal, R.G.; Cardinale, S.C.; Butler, M.M.; Peet, N.P.; Majgier-Baranowska, H.; Williams, J.D.; Patel, I.; *et al.* Identification of a small-molecule entry inhibitor for filoviruses. *J. Virol.* **2011**, *85*, 3106–3119.
24. Shah, P.P.; Wang, T.; Kaletsky, R.L.; Myers, M.C.; Purvis, J.E.; Jing, H.; Huryn, D.M.; Greenbaum, D.C.; Smith, A.B., III; Bates, P.; *et al.* A small-molecule oxocarbazate inhibitor of human cathepsin L blocks severe acute respiratory syndrome and ebola pseudotype virus infection into human embryonic kidney 293T cells. *Mol. Pharmacol.* **2010**, *78*, 319–324.
25. Groseth, A.; Marzi, A.; Hoenen, T.; Herwig, A.; Gardner, D.; Becker, S.; Ebihara, H.; Feldmann, H. The Ebola virus glycoprotein contributes to but is not sufficient for virulence *in vivo*. *PLoS Pathog.* **2012**, *8*, e1002847.
26. Takada, A.; Robison, C.; Goto, H.; Sanchez, A.; Murti, K.G.; Whitt, M.A.; Kawaoka, Y. A system for functional analysis of Ebola virus glycoprotein. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 14764–14769.

27. Wool-Lewis, R.J.; Bates, P. Characterization of Ebola virus entry by using pseudotyped viruses: Identification of receptor-deficient cell lines. *J. Virol.* **1998**, *72*, 3155–3160.
28. Yang, Z.Y.; Duckers, H.J.; Sullivan, N.J.; Sanchez, A.; Nabel, E.G.; Nabel, G.J. Identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury. *Nat. Med.* **2000**, *6*, 886–889.
29. Feldmann, H.; Volchkov, V.E.; Volchkova, V.A.; Klenk, H.D. The glycoproteins of Marburg and Ebola virus and their potential roles in pathogenesis. *Arch. Virol. Suppl.* **1999**, *15*, 159–169.
30. Hoenen, T.; Groseth, A.; Falzarano, D.; Feldmann, H. Ebola virus: Unravelling pathogenesis to combat a deadly disease. *Trends Mol. Med.* **2006**, *12*, 206–215.
31. Takada, A.; Kawaoka, Y. The pathogenesis of Ebola hemorrhagic fever. *Trends Microbiol.* **2001**, *9*, 506–511.
32. Volchkov, V.E.; Feldmann, H.; Volchkova, V.A.; Klenk, H.D. Processing of the Ebola virus glycoprotein by the proprotein convertase furin. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 5762–5767.
33. Sanchez, A.; Yang, Z.Y.; Xu, L.; Nabel, G.J.; Crews, T.; Peters, C.J. Biochemical analysis of the secreted and virion glycoproteins of Ebola virus. *J. Virol.* **1998**, *72*, 6442–6447.
34. Powlesland, A.S.; Fisch, T.; Taylor, M.E.; Smith, D.F.; Tissot, B.; Dell, A.; Pöhlmann, S.; Drickamer, K. A novel mechanism for LSECtin binding to Ebola virus surface glycoprotein through truncated glycans. *J. Biol. Chem.* **2008**, *283*, 593–602.
35. Ritchie, G.; Harvey, D.J.; Stroehrer, U.; Feldmann, F.; Feldmann, H.; Wahl-Jensen, V.; Royle, L.; Dwek, R.A.; Rudd, P.M. Identification of N-glycans from Ebola virus glycoproteins by matrix-assisted laser desorption/ionisation time-of-flight and negative ion electrospray tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 571–585.
36. Manicassamy, B.; Wang, J.; Rumschlag, E.; Tymen, S.; Volchkova, V.; Volchkov, V.; Rong, L. Characterization of Marburg virus glycoprotein in viral entry. *Virology* **2007**, *358*, 79–88.
37. Lee, J.E.; Fusco, M.L.; Hessel, A.J.; Oswald, W.B.; Burton, D.R.; Saphire, E.O. Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor. *Nature* **2008**, *454*, 177–182.
38. Sanchez, A.; Trappier, S.G.; Mahy, B.W.; Peters, C.J.; Nichol, S.T. The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 3602–3607.
39. Jeffers, S.A.; Sanders, D.A.; Sanchez, A. Covalent modifications of the ebola virus glycoprotein. *J. Virol.* **2002**, *76*, 12463–12472.
40. Simmons, G.; Wool-Lewis, R.J.; Baribaud, F.; Netter, R.C.; Bates, P. Ebola virus glycoproteins induce global surface protein down-modulation and loss of cell adherence. *J. Virol.* **2002**, *76*, 2518–2528.
41. Takada, A.; Fujioka, K.; Tsuiji, M.; Morikawa, A.; Higashi, N.; Ebihara, H.; Kobasa, D.; Feldmann, H.; Irimura, T.; Kawaoka, Y. Human macrophage C-type lectin specific for galactose and N-acetylgalactosamine promotes filovirus entry. *J. Virol.* **2004**, *78*, 2943–2947.
42. Malashkevich, V.N.; Schneider, B.J.; McNally, M.L.; Milhollen, M.A.; Pang, J.X.; Kim, P.S. Core structure of the envelope glycoprotein GP2 from Ebola virus at 1.9-Å resolution. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 2662–2667.

43. Weissenhorn, W.; Carfi, A.; Lee, K.H.; Skehel, J.J.; Wiley, D.C. Crystal structure of the Ebola virus membrane fusion subunit, GP2, from the envelope glycoprotein ectodomain. *Mol. Cell* **1998**, *2*, 605–616.
44. Kielian, M.; Rey, F.A. Virus membrane-fusion proteins: More than one way to make a hairpin. *Nat. Rev. Microbiol.* **2006**, *4*, 67–76.
45. Kuhn, J.H.; Radoshitzky, S.R.; Guth, A.C.; Warfield, K.L.; Li, W.; Vincent, M.J.; Towner, J.S.; Nichol, S.T.; Bavari, S.; Choe, H.; *et al.* Conserved receptor-binding domains of Lake Victoria marburgvirus and Zaire ebolavirus bind a common receptor. *J. Biol. Chem.* **2006**, *281*, 15951–15958.
46. Neumann, G.; Geisbert, T.W.; Ebihara, H.; Geisbert, J.B.; Daddario-DiCaprio, K.M.; Feldmann, H.; Kawaoka, Y. Proteolytic processing of the Ebola virus glycoprotein is not critical for Ebola virus replication in nonhuman primates. *J. Virol.* **2007**, *81*, 2995–2998.
47. Neumann, G.; Feldmann, H.; Watanabe, S.; Lukashevich, I.; Kawaoka, Y. Reverse genetics demonstrates that proteolytic processing of the Ebola virus glycoprotein is not essential for replication in cell culture. *J. Virol.* **2002**, *76*, 406–410.
48. Jaskierny, A.J.; Panahi, A.; Feig, M. Effect of flanking residues on the conformational sampling of the internal fusion peptide from Ebola virus. *Proteins* **2011**, *79*, 1109–1117.
49. Watanabe, S.; Takada, A.; Watanabe, T.; Ito, H.; Kida, H.; Kawaoka, Y. Functional importance of the coiled-coil of the Ebola virus glycoprotein. *J. Virol.* **2000**, *74*, 10194–10201.
50. Usami, K.; Matsuno, K.; Igarashi, M.; Denda-Nagai, K.; Takada, A.; Irimura, T. Involvement of viral envelope GP2 in Ebola virus entry into cells expressing the macrophage galactose-type C-type lectin. *Biochem. Biophys. Res. Commun.* **2011**, *407*, 74–78.
51. Adam, B.; Lins, L.; Stroobant, V.; Thomas, A.; Brasseur, R. Distribution of hydrophobic residues is crucial for the fusogenic properties of the Ebola virus GP2 fusion peptide. *J. Virol.* **2004**, *78*, 2131–2136.
52. Ito, H.; Watanabe, S.; Sanchez, A.; Whitt, M.A.; Kawaoka, Y. Mutational analysis of the putative fusion domain of Ebola virus glycoprotein. *J. Virol.* **1999**, *73*, 8907–8912.
53. Ruiz-Arguello, M.B.; Goni, F.M.; Pereira, F.B.; Nieva, J.L. Phosphatidylinositol-dependent membrane fusion induced by a putative fusogenic sequence of Ebola virus. *J. Virol.* **1998**, *72*, 1775–1781.
54. Hofmann, H.; Pöhlmann, S. Cellular entry of the SARS coronavirus. *Trends Microbiol.* **2004**, *12*, 466–472.
55. Manicassamy, B.; Wang, J.; Jiang, H.; Rong, L. Comprehensive analysis of ebola virus GP1 in viral entry. *J. Virol.* **2005**, *79*, 4793–4805.
56. Schornberg, K.; Matsuyama, S.; Kabsch, K.; Delos, S.; Bouton, A.; White, J. Role of endosomal cathepsins in entry mediated by the Ebola virus glycoprotein. *J. Virol.* **2006**, *80*, 4174–4178.
57. Chandran, K.; Sullivan, N.J.; Felbor, U.; Whelan, S.P.; Cunningham, J.M. Endosomal proteolysis of the Ebola virus glycoprotein is necessary for infection. *Science* **2005**, *308*, 1643–1645.
58. Dias, J.M.; Kuehne, A.I.; Abelson, D.M.; Bale, S.; Wong, A.C.; Halfmann, P.; Muhammad, M.A.; Fusco, M.L.; Zak, S.E.; Kang, E.; *et al.* A shared structural solution for neutralizing ebolaviruses. *Nat. Struct. Mol. Biol.* **2011**, *18*, 1424–1427.

59. Gregory, S.M.; Harada, E.; Liang, B.; Delos, S.E.; White, J.M.; Tamm, L.K. Structure and function of the complete internal fusion loop from Ebolavirus glycoprotein 2. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 11211–11216.
60. Stroher, U.; West, E.; Bugany, H.; Klenk, H.D.; Schnittler, H.J.; Feldmann, H. Infection and activation of monocytes by Marburg and Ebola viruses. *J. Virol.* **2001**, *75*, 11025–11033.
61. Gupta, M.; Mahanty, S.; Ahmed, R.; Rollin, P.E. Monocyte-derived human macrophages and peripheral blood mononuclear cells infected with ebola virus secrete MIP-1alpha and TNF-alpha and inhibit poly-IC-induced IFN-alpha *in vitro*. *Virology* **2001**, *284*, 20–25.
62. Bosio, C.M.; Aman, M.J.; Grogan, C.; Hogan, R.; Ruthel, G.; Negley, D.; Mohamadzaeh, M.; Bavari, S.; Schmaljohn, A. Ebola and Marburg viruses replicate in monocyte-derived dendritic cells without inducing the production of cytokines and full maturation. *J. Infect. Dis.* **2003**, *188*, 1630–1638.
63. Geisbert, T.W.; Jahrling, P.B.; Hanes, M.A.; Zack, P.M. Association of Ebola-related Reston virus particles and antigen with tissue lesions of monkeys imported to the United States. *J. Comp. Pathol.* **1992**, *106*, 137–152.
64. Geisbert, T.W.; Hensley, L.E.; Larsen, T.; Young, H.A.; Reed, D.S.; Geisbert, J.B.; Scott, D.P.; Kagan, E.; Jahrling, P.B.; Davis, K.J. Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: Evidence that dendritic cells are early and sustained targets of infection. *Am. J. Pathol.* **2003**, *163*, 2347–2370.
65. Geisbert, T.W.; Hensley, L.E.; Gibb, T.R.; Steele, K.E.; Jaax, N.K.; Jahrling, P.B. Apoptosis induced *in vitro* and *in vivo* during infection by Ebola and Marburg viruses. *Lab. Invest.* **2000**, *80*, 171–186.
66. Feldmann, H.; Geisbert, T.W. Ebola haemorrhagic fever. *Lancet* **2011**, *377*, 849–862.
67. Ryabchikova, E.I.; Kolesnikova, L.V.; Luchko, S.V. An analysis of features of pathogenesis in two animal models of Ebola virus infection. *J. Infect. Dis.* **1999**, *179*, S199–S202.
68. Zaki, S.R.; Goldsmith, C.S. Pathologic features of filovirus infections in humans. *Curr. Top. Microbiol. Immunol.* **1999**, *235*, 97–116.
69. Mahanty, S.; Hutchinson, K.; Agarwal, S.; McRae, M.; Rollin, P.E.; Pulendran, B. Cutting edge: Impairment of dendritic cells and adaptive immunity by Ebola and Lassa viruses. *J. Immunol.* **2003**, *170*, 2797–2801.
70. Hartman, A.L.; Towner, J.S.; Nichol, S.T. Ebola and marburg hemorrhagic fever. *Clin. Lab. Med.* **2010**, *30*, 161–177.
71. Feldmann, H.; Bugany, H.; Mahner, F.; Klenk, H.D.; Drenckhahn, D.; Schnittler, H.J. Filovirus-induced endothelial leakage triggered by infected monocytes/macrophages. *J. Virol.* **1996**, *70*, 2208–2214.
72. Bray, M.; Geisbert, T.W. Ebola virus: The role of macrophages and dendritic cells in the pathogenesis of Ebola hemorrhagic fever. *Int. J. Biochem. Cell Biol.* **2005**, *37*, 1560–1566.
73. Schnittler, H.J.; Mahner, F.; Drenckhahn, D.; Klenk, H.D.; Feldmann, H. Replication of Marburg virus in human endothelial cells. A possible mechanism for the development of viral hemorrhagic disease. *J. Clin. Invest* **1993**, *91*, 1301–1309.
74. Johnson, E.; Jaax, N.; White, J.; Jahrling, P. Lethal experimental infections of rhesus monkeys by aerosolized Ebola virus. *Int. J. Exp. Pathol.* **1995**, *76*, 227–236.

75. Ryabchikova, E.; Strelets, L.; Kolesnikova, L.; Pyankov, O.; Sergeev, A. Respiratory Marburg virus infection in guinea pigs. *Arch. Virol.* **1996**, *141*, 2177–2190.
76. Ryabchikova, E.; Kolesnikova, L.; Smolina, M.; Tkachev, V.; Pereboeva, L.; Baranova, S.; Grazhdantseva, A.; Rassadkin, Y. Ebola virus infection in guinea pigs: Presumable role of granulomatous inflammation in pathogenesis. *Arch. Virol.* **1996**, *141*, 909–921.
77. Jaax, N.K.; Davis, K.J.; Geisbert, T.J.; Vogel, P.; Jaax, G.P.; Topper, M.; Jahrling, P.B. Lethal experimental infection of rhesus monkeys with Ebola-Zaire (Mayinga) virus by the oral and conjunctival route of exposure. *Arch. Pathol. Lab. Med.* **1996**, *120*, 140–155.
78. Davis, K.J.; Anderson, A.O.; Geisbert, T.W.; Steele, K.E.; Geisbert, J.B.; Vogel, P.; Connolly, B.M.; Huggins, J.W.; Jahrling, P.B.; Jaax, N.K. Pathology of experimental Ebola virus infection in African green monkeys. Involvement of fibroblastic reticular cells. *Arch. Pathol. Lab. Med.* **1997**, *121*, 805–819.
79. Chan, S.Y.; Speck, R.F.; Ma, M.C.; Goldsmith, M.A. Distinct mechanisms of entry by envelope glycoproteins of Marburg and Ebola (Zaire) viruses. *J. Virol.* **2000**, *74*, 4933–4937.
80. Ito, H.; Watanabe, S.; Takada, A.; Kawaoka, Y. Ebola virus glycoprotein: Proteolytic processing, acylation, cell tropism, and detection of neutralizing antibodies. *J. Virol.* **2001**, *75*, 1576–1580.
81. Simmons, G.; Rennekamp, A.J.; Chai, N.; Vandenberghe, L.H.; Riley, J.L.; Bates, P. Folate receptor alpha and caveolae are not required for Ebola virus glycoprotein-mediated viral infection. *J. Virol.* **2003**, *77*, 13433–13438.
82. Peters, C.J.; Jahrling, P.B.; Ksiazek, T.G.; Johnson, E.D.; Lupton, H.W. Filovirus contamination of cell cultures. *Dev. Biol. Stand.* **1992**, *76*, 267–274.
83. Van der Groen, G. *Ebola Virus Hemorrhagic Fever*; Amsterdam Elsevier/North Holland Biomedical Press: Amsterdam, The Netherlands, 1978.
84. Yang, Z.; Delgado, R.; Xu, L.; Todd, R.F.; Nabel, E.G.; Sanchez, A.; Nabel, G.J. Distinct cellular interactions of secreted and transmembrane Ebola virus glycoproteins. *Science* **1998**, *279*, 1034–1037.
85. Alvarez, C.P.; Lasala, F.; Carrillo, J.; Muniz, O.; Corbi, A.L.; Delgado, R. C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in cis and in trans. *J. Virol.* **2002**, *76*, 6841–6844.
86. Becker, S.; Spiess, M.; Klenk, H.D. The asialoglycoprotein receptor is a potential liver-specific receptor for Marburg virus. *J. Gen. Virol.* **1995**, *76*, 393–399.
87. Gramberg, T.; Hofmann, H.; Moller, P.; Lalor, P.F.; Marzi, A.; Geier, M.; Krumbiegel, M.; Winkler, T.; Kirchhoff, F.; Adams, D.H.; *et al.* LSECTin interacts with filovirus glycoproteins and the spike protein of SARS coronavirus. *Virology* **2005**, *340*, 224–236.
88. Marzi, A.; Gramberg, T.; Simmons, G.; Moller, P.; Rennekamp, A.J.; Krumbiegel, M.; Geier, M.; Eisemann, J.; Turza, N.; Saunier, B.; *et al.* DC-SIGN and DC-SIGNR interact with the glycoprotein of Marburg virus and the S protein of severe acute respiratory syndrome coronavirus. *J. Virol.* **2004**, *78*, 12090–12095.
89. Simmons, G.; Reeves, J.D.; Grogan, C.C.; Vandenberghe, L.H.; Baribaud, F.; Whitbeck, J.C.; Burke, E.; Buchmeier, M.J.; Soilleux, E.J.; Riley, J.L.; *et al.* DC-SIGN and DC-SIGNR bind ebola glycoproteins and enhance infection of macrophages and endothelial cells. *Virology* **2003**, *305*, 115–123.

90. Marzi, A.; Moller, P.; Hanna, S.L.; Harrer, T.; Eisemann, J.; Steinkasserer, A.; Becker, S.; Baribaud, F.; Pöhlmann, S. Analysis of the interaction of Ebola virus glycoprotein with DC-SIGN (dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin) and its homologue DC-SIGNR. *J. Infect. Dis.* **2007**, *196*, S237–S246.
91. Matsuno, K.; Nakayama, E.; Noyori, O.; Marzi, A.; Ebihara, H.; Irimura, T.; Feldmann, H.; Takada, A. C-type lectins do not act as functional receptors for filovirus entry into cells. *Biochem. Biophys. Res. Commun.* **2010**, *403*, 144–148.
92. Marzi, A.; Wegele, A.; Pöhlmann, S. Modulation of virion incorporation of Ebolavirus glycoprotein: Effects on attachment, cellular entry and neutralization. *Virology* **2006**, *352*, 345–356.
93. Matsuno, K.; Kishida, N.; Usami, K.; Igarashi, M.; Yoshida, R.; Nakayama, E.; Shimojima, M.; Feldmann, H.; Irimura, T.; Kawaoka, Y.; *et al.* Different potential of C-type lectin-mediated entry between Marburg virus strains. *J. Virol.* **2010**, *84*, 5140–5147.
94. Geijtenbeek, T.B.; Torensma, R.; van Vliet, S.J.; van Duijnhoven, G.C.; Adema, G.J.; van, K.Y.; Figdor, C.G. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell* **2000**, *100*, 575–585.
95. Higashi, N.; Fujioka, K.; Denda-Nagai, K.; Hashimoto, S.; Nagai, S.; Sato, T.; Fujita, Y.; Morikawa, A.; Tsuiji, M.; Miyata-Takeuchi, M.; *et al.* The macrophage C-type lectin specific for galactose/N-acetylgalactosamine is an endocytic receptor expressed on monocyte-derived immature dendritic cells. *J. Biol. Chem.* **2002**, *277*, 20686–20693.
96. Spiess, M. The asialoglycoprotein receptor: A model for endocytic transport receptors. *Biochemistry* **1990**, *29*, 10009–10018.
97. Bashirova, A.A.; Geijtenbeek, T.B.; van Duijnhoven, G.C.; van Vliet, S.J.; Eilering, J.B.; Martin, M.P.; Wu, L.; Martin, T.D.; Viebig, N.; Knolle, P.A.; *et al.* A dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN)-related protein is highly expressed on human liver sinusoidal endothelial cells and promotes HIV-1 infection. *J. Exp. Med.* **2001**, *193*, 671–678.
98. Pöhlmann, S.; Soilleux, E.J.; Baribaud, F.; Leslie, G.J.; Morris, L.S.; Trowsdale, J.; Lee, B.; Coleman, N.; Doms, R.W. DC-SIGNR, a DC-SIGN homologue expressed in endothelial cells, binds to human and simian immunodeficiency viruses and activates infection in trans. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 2670–2675.
99. Gringhuis, S.I.; van der Vlist, M.; van den Berg, L.M.; den, D.J.; Litjens, M.; Geijtenbeek, T.B. HIV-1 exploits innate signaling by TLR8 and DC-SIGN for productive infection of dendritic cells. *Nat. Immunol.* **2010**, *11*, 419–426.
100. Michelow, I.C.; Lear, C.; Scully, C.; Prugar, L.I.; Longley, C.B.; Yantosca, L.M.; Ji, X.; Karpel, M.; Brudner, M.; Takahashi, K.; *et al.* High-dose mannose-binding lectin therapy for Ebola virus infection. *J. Infect. Dis.* **2011**, *203*, 175–179.
101. Baribaud, F.; Doms, R.W.; Pöhlmann, S. The role of DC-SIGN and DC-SIGNR in HIV and Ebola virus infection: Can potential therapeutics block virus transmission and dissemination? *Expert. Opin. Ther. Targets.* **2002**, *6*, 423–431.

102. Gramberg, T.; Soilleux, E.; Fisch, T.; Lalor, P.F.; Hofmann, H.; Wheeldon, S.; Cotterill, A.; Wegele, A.; Winkler, T.; Adams, D.H.; *et al.* Interactions of LSECtin and DC-SIGN/DC-SIGNR with viral ligands: Differential pH dependence, internalization and virion binding. *Virology* **2008**, *373*, 189–201.
103. Lin, G.; Simmons, G.; Pöhlmann, S.; Baribaud, F.; Ni, H.; Leslie, G.J.; Haggarty, B.S.; Bates, P.; Weissman, D.; Hoxie, J.A.; *et al.* Differential N-linked glycosylation of human immunodeficiency virus and Ebola virus envelope glycoproteins modulates interactions with DC-SIGN and DC-SIGNR. *J. Virol.* **2003**, *77*, 1337–1346.
104. Kaplan, G.; Totsuka, A.; Thompson, P.; Akatsuka, T.; Moritsugu, Y.; Feinstone, S.M. Identification of a surface glycoprotein on African green monkey kidney cells as a receptor for hepatitis A virus. *EMBO J.* **1996**, *15*, 4282–4296.
105. Kondratowicz, A.S.; Lennemann, N.J.; Sinn, P.L.; Davey, R.A.; Hunt, C.L.; Moller-Tank, S.; Meyerholz, D.K.; Rennert, P.; Mullins, R.F.; Brindley, M.; *et al.* T-cell immunoglobulin and mucin domain 1 (TIM-1) is a receptor for Zaire Ebolavirus and Lake Victoria Marburgvirus. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 8426–8431.
106. McIntire, J.J.; Umetsu, S.E.; Akbari, O.; Potter, M.; Kuchroo, V.K.; Barsh, G.S.; Freeman, G.J.; Umetsu, D.T.; DeKruyff, R.H. Identification of Tapr (an airway hyperreactivity regulatory locus) and the linked Tim gene family. *Nat. Immunol.* **2001**, *2*, 1109–1116.
107. Kobayashi, N.; Karisola, P.; Pena-Cruz, V.; Dorfman, D.M.; Jinushi, M.; Umetsu, S.E.; Butte, M.J.; Nagumo, H.; Chernova, I.; Zhu, B.; *et al.* TIM-1 and TIM-4 glycoproteins bind phosphatidylserine and mediate uptake of apoptotic cells. *Immunity* **2007**, *27*, 927–940.
108. Miyanishi, M.; Tada, K.; Koike, M.; Uchiyama, Y.; Kitamura, T.; Nagata, S. Identification of Tim4 as a phosphatidylserine receptor. *Nature* **2007**, *450*, 435–439.
109. Umetsu, S.E.; Lee, W.L.; McIntire, J.J.; Downey, L.; Sanjanwala, B.; Akbari, O.; Berry, G.J.; Nagumo, H.; Freeman, G.J.; Umetsu, D.T.; *et al.* TIM-1 induces T cell activation and inhibits the development of peripheral tolerance. *Nat. Immunol.* **2005**, *6*, 447–454.
110. Ichimura, T.; Asseldonk, E.J.; Humphreys, B.D.; Gunaratnam, L.; Duffield, J.S.; Bonventre, J.V. Kidney injury molecule-1 is a phosphatidylserine receptor that confers a phagocytic phenotype on epithelial cells. *J. Clin. Invest.* **2008**, *118*, 1657–1668.
111. Kuhn, J.H. Filoviruses. A compendium of 40 years of epidemiological, clinical, and laboratory studies. *Arch. Virol. Suppl.* **2008**, *20*, 13–360.
112. de Souza, A.J.; Oak, J.S.; Jordanhazy, R.; DeKruyff, R.H.; Fruman, D.A.; Kane, L.P. T cell Ig and mucin domain-1-mediated T cell activation requires recruitment and activation of phosphoinositide 3-kinase. *J. Immunol.* **2008**, *180*, 6518–6526.
113. de Souza, A.J.; Oriss, T.B.; O'malley, K.J.; Ray, A.; Kane, L.P. T cell Ig and mucin 1 (TIM-1) is expressed on *in vivo*-activated T cells and provides a costimulatory signal for T cell activation. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 17113–17118.
114. Brindley, M.A.; Hunt, C.L.; Kondratowicz, A.S.; Bowman, J.; Sinn, P.L.; McCray, P.B., Jr.; Quinn, K.; Weller, M.L.; Chiorini, J.A.; Maury, W. Tyrosine kinase receptor Axl enhances entry of Zaire ebolavirus without direct interactions with the viral glycoprotein. *Virology* **2011**, *415*, 83–94.

115. Hunt, C.L.; Kolokoltsov, A.A.; Davey, R.A.; Maury, W. The Tyro3 receptor kinase Axl enhances macropinocytosis of Zaire ebolavirus. *J. Virol.* **2011**, *85*, 334–347.
116. Shimojima, M.; Ikeda, Y.; Kawaoka, Y. The mechanism of Axl-mediated Ebola virus infection. *J. Infect. Dis.* **2007**, *196*, S259–S263.
117. Shimojima, M.; Takada, A.; Ebihara, H.; Neumann, G.; Fujioka, K.; Irimura, T.; Jones, S.; Feldmann, H.; Kawaoka, Y. Tyro3 family-mediated cell entry of Ebola and Marburg viruses. *J. Virol.* **2006**, *80*, 10109–10116.
118. Crosier, K.E.; Crosier, P.S. New insights into the control of cell growth; the role of the Axl family. *Pathology* **1997**, *29*, 131–135.
119. Linger, R.M.; Keating, A.K.; Earp, H.S.; Graham, D.K. TAM receptor tyrosine kinases: Biologic functions, signaling, and potential therapeutic targeting in human cancer. *Adv. Cancer Res.* **2008**, *100*, 35–83.
120. Rothlin, C.V.; Ghosh, S.; Zuniga, E.I.; Oldstone, M.B.; Lemke, G. TAM receptors are pleiotropic inhibitors of the innate immune response. *Cell* **2007**, *131*, 1124–1136.
121. Berman, A.E.; Kozlova, N.I. Integrins: Structure and functions. *Membr. Cell Biol.* **2000**, *13*, 207–244.
122. Hynes, R.O. Integrins: Versatility, modulation, and signaling in cell adhesion. *Cell* **1992**, *69*, 11–25.
123. Schornberg, K.L.; Shoemaker, C.J.; Dube, D.; Abshire, M.Y.; Delos, S.E.; Bouton, A.H.; White, J.M. Alpha5beta1-integrin controls ebolavirus entry by regulating endosomal cathepsins. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 8003–8008.
124. Takada, A.; Watanabe, S.; Ito, H.; Okazaki, K.; Kida, H.; Kawaoka, Y. Downregulation of beta1 integrins by Ebola virus glycoprotein: Implication for virus entry. *Virology* **2000**, *278*, 20–26.
125. Misasi, J.; Chandran, K.; Yang, J.Y.; Considine, B.; Filone, C.M.; Cote, M.; Sullivan, N.; Fabozzi, G.; Hensley, L.; Cunningham, J. Filoviruses require endosomal cysteine proteases for entry but exhibit distinct protease preferences. *J. Virol.* **2012**, *86*, 3284–3292.
126. Kaletsky, R.L.; Simmons, G.; Bates, P. Proteolysis of the Ebola virus glycoproteins enhances virus binding and infectivity. *J. Virol.* **2007**, *81*, 13378–13384.
127. Sanchez, A. Analysis of filovirus entry into vero e6 cells, using inhibitors of endocytosis, endosomal acidification, structural integrity, and cathepsin (B and L) activity. *J. Infect. Dis.* **2007**, *196*, S251–S258.
128. Martinez, O.; Johnson, J.; Manicassamy, B.; Rong, L.; Olinger, G.G.; Hensley, L.E.; Basler, C.F. Zaire Ebola virus entry into human dendritic cells is insensitive to cathepsin L inhibition. *Cell Microbiol.* **2010**, *12*, 148–157.
129. Carette, J.E.; Raaben, M.; Wong, A.C.; Herbert, A.S.; Obernosterer, G.; Mulherkar, N.; Kuehne, A.I.; Kranzusch, P.J.; Griffin, A.M.; Ruthel, G.; *et al.* Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. *Nature* **2011**, *477*, 340–343.
130. Miller, E.H.; Obernosterer, G.; Raaben, M.; Herbert, A.S.; Deffieu, M.S.; Krishnan, A.; Ndungo, E.; Sandesara, R.G.; Carette, J.E.; Kuehne, A.I.; *et al.* Ebola virus entry requires the host-programmed recognition of an intracellular receptor. *EMBO J.* **2012**, *31*, 1947–1960.
131. Lemke, G.; Rothlin, C.V. Immunobiology of the TAM receptors. *Nat. Rev. Immunol.* **2008**, *8*, 327–336.

132. Stitt, T.N.; Conn, G.; Gore, M.; Lai, C.; Bruno, J.; Radziejewski, C.; Mattsson, K.; Fisher, J.; Gies, D.R.; Jones, P.F.; *et al.* The anticoagulation factor protein S and its relative, Gas6, are ligands for the Tyro 3/Axl family of receptor tyrosine kinases. *Cell* **1995**, *80*, 661–670.
133. Godowski, P.J.; Mark, M.R.; Chen, J.; Sadick, M.D.; Raab, H.; Hammonds, R.G. Reevaluation of the roles of protein S and Gas6 as ligands for the receptor tyrosine kinase Rse/Tyro 3. *Cell* **1995**, *82*, 355–358.
134. Scott, R.S.; McMahon, E.J.; Pop, S.M.; Reap, E.A.; Caricchio, R.; Cohen, P.L.; Earp, H.S.; Matsushima, G.K. Phagocytosis and clearance of apoptotic cells is mediated by MER. *Nature* **2001**, *411*, 207–211.
135. Hafizi, S.; Dahlback, B. Gas6 and protein S. Vitamin K-dependent ligands for the Axl receptor tyrosine kinase subfamily. *FEBS J.* **2006**, *273*, 5231–5244.
136. Nanbo, A.; Imai, M.; Watanabe, S.; Noda, T.; Takahashi, K.; Neumann, G.; Halfmann, P.; Kawaoka, Y. Ebolavirus is internalized into host cells via macropinocytosis in a viral glycoprotein-dependent manner. *PLoS Pathog.* **2010**, *6*, e1001121.
137. Saeed, M.F.; Kolokoltsov, A.A.; Albrecht, T.; Davey, R.A. Cellular entry of ebola virus involves uptake by a macropinocytosis-like mechanism and subsequent trafficking through early and late endosomes. *PLoS Pathog.* **2010**, *6*, e1001110.
138. Morizono, K.; Xie, Y.; Olafsen, T.; Lee, B.; Dasgupta, A.; Wu, A.M.; Chen, I.S. The soluble serum protein Gas6 bridges virion envelope phosphatidylserine to the TAM receptor tyrosine kinase Axl to mediate viral entry. *Cell Host Microbe* **2011**, *9*, 286–298.
139. Hynes, R.O. Cell adhesion: Old and new questions. *Trends Cell Biol.* **1999**, *9*, M33–M37.
140. Cary, L.A.; Han, D.C.; Guan, J.L. Integrin-mediated signal transduction pathways. *Histol. Histopathol.* **1999**, *14*, 1001–1009.
141. Akula, S.M.; Pramod, N.P.; Wang, F.Z.; Chandran, B. Integrin alpha3beta1 (CD 49c/29) is a cellular receptor for Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) entry into the target cells. *Cell* **2002**, *108*, 407–419.
142. Tugizov, S.M.; Berline, J.W.; Palefsky, J.M. Epstein-Barr virus infection of polarized tongue and nasopharyngeal epithelial cells. *Nat. Med.* **2003**, *9*, 307–314.
143. Feire, A.L.; Koss, H.; Compton, T. Cellular integrins function as entry receptors for human cytomegalovirus via a highly conserved disintegrin-like domain. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 15470–15475.
144. Mathias, P.; Wickham, T.; Moore, M.; Nemerow, G. Multiple adenovirus serotypes use alpha v integrins for infection. *J. Virol.* **1994**, *68*, 6811–6814.
145. Wickham, T.J.; Mathias, P.; Cheresch, D.A.; Nemerow, G.R. Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalization but not virus attachment. *Cell* **1993**, *73*, 309–319.
146. Gavrilovskaya, I.N.; Brown, E.J.; Ginsberg, M.H.; Mackow, E.R. Cellular entry of hantaviruses which cause hemorrhagic fever with renal syndrome is mediated by beta3 integrins. *J. Virol.* **1999**, *73*, 3951–3959.
147. Gavrilovskaya, I.N.; Shepley, M.; Shaw, R.; Ginsberg, M.H.; Mackow, E.R. beta3 Integrins mediate the cellular entry of hantaviruses that cause respiratory failure. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 7074–7079.

148. Raymond, T.; Gorbunova, E.; Gavrilovskaya, I.N.; Mackow, E.R. Pathogenic hantaviruses bind plexin-semaphorin-integrin domains present at the apex of inactive, bent alphavbeta3 integrin conformers. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 1163–1168.
149. Berinstein, A.; Roivainen, M.; Hovi, T.; Mason, P.W.; Baxt, B. Antibodies to the vitronectin receptor (integrin alpha V beta 3) inhibit binding and infection of foot-and-mouth disease virus to cultured cells. *J. Virol.* **1995**, *69*, 2664–2666.
150. Bergelson, J.M.; Shepley, M.P.; Chan, B.M.; Hemler, M.E.; Finberg, R.W. Identification of the integrin VLA-2 as a receptor for echovirus 1. *Science* **1992**, *255*, 1718–1720.
151. Xing, L.; Huhtala, M.; Pietiainen, V.; Kapyla, J.; Vuorinen, K.; Marjomaki, V.; Heino, J.; Johnson, M.S.; Hyypia, T.; Cheng, R.H. Structural and functional analysis of integrin alpha2I domain interaction with echovirus 1. *J. Biol. Chem.* **2004**, *279*, 11632–11638.
152. Whitton, J.L.; Cornell, C.T.; Feuer, R. Host and virus determinants of picornavirus pathogenesis and tropism. *Nat. Rev. Microbiol.* **2005**, *3*, 765–776.
153. Graham, K.L.; Fleming, F.E.; Halasz, P.; Hewish, M.J.; Nagesha, H.S.; Holmes, I.H.; Takada, Y.; Coulson, B.S. Rotaviruses interact with alpha4beta7 and alpha4beta1 integrins by binding the same integrin domains as natural ligands. *J. Gen. Virol.* **2005**, *86*, 3397–3408.
154. Maginnis, M.S.; Forrest, J.C.; Kopecky-Bromberg, S.A.; Dickeson, S.K.; Santoro, S.A.; Zutter, M.M.; Nemerow, G.R.; Bergelson, J.M.; Dermody, T.S. Beta1 integrin mediates internalization of mammalian reovirus. *J. Virol.* **2006**, *80*, 2760–2770.
155. Stewart, P.L.; Nemerow, G.R. Cell integrins: Commonly used receptors for diverse viral pathogens. *Trends Microbiol.* **2007**, *15*, 500–507.
156. Nemerow, G.R.; Cheresch, D.A. Herpesvirus hijacks an integrin. *Nat. Cell Biol.* **2002**, *4*, E69–E71.
157. Francica, J.R.; Varela-Rohena, A.; Medvec, A.; Plesa, G.; Riley, J.L.; Bates, P. Steric shielding of surface epitopes and impaired immune recognition induced by the ebola virus glycoprotein. *PLoS Pathog.* **2010**, *6*, e1001098.
158. Harrison, S.C. Viral membrane fusion. *Nat. Struct. Mol. Biol.* **2008**, *15*, 690–698.
159. Diederich, S.; Sauerhering, L.; Weis, M.; Altmeyen, H.; Schaschke, N.; Reinheckel, T.; Erbar, S.; Maisner, A. Activation of the Nipah virus fusion protein in MDCK cells is mediated by cathepsin B within the endosome-recycling compartment. *J. Virol.* **2012**, *86*, 3736–3745.
160. Popa, A.; Carter, J.R.; Smith, S.E.; Hellman, L.; Fried, M.G.; Dutch, R.E. Residues in the hendra virus fusion protein transmembrane domain are critical for endocytic recycling. *J. Virol.* **2012**, *86*, 3014–3026.
161. White, J.; Kartenbeck, J.; Helenius, A. Membrane fusion activity of influenza virus. *EMBO J.* **1982**, *1*, 217–222.
162. van der Schaar, H.M.; Rust, M.J.; Chen, C.; Ende-Metselaar, H.; Wilschut, J.; Zhuang, X.; Smit, J.M. Dissecting the cell entry pathway of dengue virus by single-particle tracking in living cells. *PLoS Pathog.* **2008**, *4*, e1000244.
163. Lozach, P.Y.; Huotari, J.; Helenius, A. Late-penetrating viruses. *Curr. Opin. Virol.* **2011**, *1*, 35–43.
164. Mothes, W.; Boerger, A.L.; Narayan, S.; Cunningham, J.M.; Young, J.A. Retroviral entry mediated by receptor priming and low pH triggering of an envelope glycoprotein. *Cell* **2000**, *103*, 679–689.

165. Miyauchi, K.; Kim, Y.; Latinovic, O.; Morozov, V.; Melikyan, G.B. HIV enters cells via endocytosis and dynamin-dependent fusion with endosomes. *Cell* **2009**, *137*, 433–444.
166. Bertram, S.; Glowacka, I.; Steffen, I.; Köhl, A.; Pöhlmann, S. Novel insights into proteolytic cleavage of influenza virus hemagglutinin. *Rev. Med. Virol.* **2010**, *20*, 298–310.
167. Bar, S.; Takada, A.; Kawaoka, Y.; Alizon, M. Detection of cell-cell fusion mediated by Ebola virus glycoproteins. *J. Virol.* **2006**, *80*, 2815–2822.
168. Turk, B.; Turk, D.; Turk, V. Lysosomal cysteine proteases: More than scavengers. *Biochim. Biophys. Acta* **2000**, *1477*, 98–111.
169. Lutgens, S.P.; Cleutjens, K.B.; Daemen, M.J.; Heeneman, S. Cathepsin cysteine proteases in cardiovascular disease. *FASEB J.* **2007**, *21*, 3029–3041.
170. Reiser, J.; Adair, B.; Reinheckel, T. Specialized roles for cysteine cathepsins in health and disease. *J. Clin. Invest.* **2010**, *120*, 3421–3431.
171. White, J.M.; Schornberg, K.L. A new player in the puzzle of filovirus entry. *Nat. Rev. Microbiol.* **2012**, *10*, 317–322.
172. Dube, D.; Brecher, M.B.; Delos, S.E.; Rose, S.C.; Park, E.W.; Schornberg, K.L.; Kuhn, J.H.; White, J.M. The primed ebolavirus glycoprotein (19-kilodalton GP1,2): Sequence and residues critical for host cell binding. *J. Virol.* **2009**, *83*, 2883–2891.
173. Brecher, M.; Schornberg, K.L.; Delos, S.E.; Fusco, M.L.; Saphire, E.O.; White, J.M. Cathepsin cleavage potentiates the Ebola virus glycoprotein to undergo a subsequent fusion-relevant conformational change. *J. Virol.* **2012**, *86*, 364–372.
174. Bale, S.; Liu, T.; Li, S.; Wang, Y.; Abelson, D.; Fusco, M.; Woods, V.L., Jr.; Saphire, E.O. Ebola virus glycoprotein needs an additional trigger, beyond proteolytic priming for membrane fusion. *PLoS Negl. Trop. Dis.* **2011**, *5*, e1395.
175. Hood, C.L.; Abraham, J.; Boyington, J.C.; Leung, K.; Kwong, P.D.; Nabel, G.J. Biochemical and structural characterization of cathepsin L-processed Ebola virus glycoprotein: Implications for viral entry and immunogenicity. *J. Virol.* **2010**, *84*, 2972–2982.
176. Lee, J.E.; Saphire, E.O. Ebolavirus glycoprotein structure and mechanism of entry. *Future Virol.* **2009**, *4*, 621–635.
177. Gnirss, K.; Köhl, A.; Karsten, C.; Glowacka, I.; Bertram, S.; Kaup, F.; Hofmann, H.; Pöhlmann, S. Cathepsins B and L activate Ebola but not Marburg virus glycoproteins for efficient entry into cell lines and macrophages independent of TMPRSS2 expression. *Virology* **2012**, *424*, 3–10.
178. Simmons, G.; Gosalia, D.N.; Rennekamp, A.J.; Reeves, J.D.; Diamond, S.L.; Bates, P. Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 11876–11881.
179. Roth, W.; Deussing, J.; Botchkarev, V.A.; Pauly-Evers, M.; Saftig, P.; Hafner, A.; Schmidt, P.; Schmahl, W.; Scherer, J.; Anton-Lamprecht, I.; *et al.* Cathepsin L deficiency as molecular defect of furless: Hyperproliferation of keratinocytes and perturbation of hair follicle cycling. *FASEB J.* **2000**, *14*, 2075–2086.
180. Benavides, F.; Starost, M.F.; Flores, M.; Gimenez-Conti, I.B.; Guenet, J.L.; Conti, C.J. Impaired hair follicle morphogenesis and cycling with abnormal epidermal differentiation in nackt mice, a cathepsin L-deficient mutation. *Am. J. Pathol.* **2002**, *161*, 693–703.

181. Guicciardi, M.E.; Deussing, J.; Miyoshi, H.; Bronk, S.F.; Svingen, P.A.; Peters, C.; Kaufmann, S.H.; Gores, G.J. Cathepsin B contributes to TNF-alpha-mediated hepatocyte apoptosis by promoting mitochondrial release of cytochrome c. *J. Clin. Invest.* **2000**, *106*, 1127–1137.
182. Wong, A.C.; Sandesara, R.G.; Mulherkar, N.; Whelan, S.P.; Chandran, K. A forward genetic strategy reveals destabilizing mutations in the Ebolavirus glycoprotein that alter its protease dependence during cell entry. *J. Virol.* **2010**, *84*, 163–175.
183. Haines, K.M.; Vande Burgt, N.H.; Francica, J.R.; Kaletsky, R.L.; Bates, P. Chinese hamster ovary cell lines selected for resistance to ebolavirus glycoprotein mediated infection are defective for NPC1 expression. *Virology* **2012**, *432*, 20–28.
184. Loftus, S.K.; Morris, J.A.; Carstea, E.D.; Gu, J.Z.; Cummings, C.; Brown, A.; Ellison, J.; Ohno, K.; Rosenfeld, M.A.; Tagle, D.A.; *et al.* Murine model of Niemann-Pick C disease: Mutation in a cholesterol homeostasis gene. *Science* **1997**, *277*, 232–235.
185. Garver, W.S.; Xie, C.; Repa, J.J.; Turley, S.D.; Dietschy, J.M. Niemann-Pick C1 expression is not regulated by the amount of cholesterol flowing through cells in the mouse. *J. Lipid. Res.* **2005**, *46*, 1745–1754.
186. Carstea, E.D.; Morris, J.A.; Coleman, K.G.; Loftus, S.K.; Zhang, D.; Cummings, C.; Gu, J.; Rosenfeld, M.A.; Pavan, W.J.; Krizman, D.B.; *et al.* Niemann-Pick C1 disease gene: Homology to mediators of cholesterol homeostasis. *Science* **1997**, *277*, 228–231.
187. Karten, B.; Peake, K.B.; Vance, J.E. Mechanisms and consequences of impaired lipid trafficking in Niemann-Pick type C1-deficient mammalian cells. *Biochim. Biophys. Acta* **2009**, *1791*, 659–670.
188. Rosenbaum, A.I.; Maxfield, F.R. Niemann-Pick type C disease: Molecular mechanisms and potential therapeutic approaches. *J. Neurochem.* **2011**, *116*, 789–795.
189. Lai, C.K.; Jeng, K.S.; Machida, K.; Lai, M.M. Hepatitis C virus egress and release depend on endosomal trafficking of core protein. *J. Virol.* **2010**, *84*, 11590–11598.
190. Poh, M.K.; Shui, G.; Xie, X.; Shi, P.Y.; Wenk, M.R.; Gu, F. U18666A, an intra-cellular cholesterol transport inhibitor, inhibits dengue virus entry and replication. *Antivir. Res.* **2012**, *93*, 191–198.
191. Sainz, B., Jr.; Barretto, N.; Martin, D.N.; Hiraga, N.; Imamura, M.; Hussain, S.; Marsh, K.A.; Yu, X.; Chayama, K.; Alrefai, W.A.; *et al.* Identification of the Niemann-Pick C1-like 1 cholesterol absorption receptor as a new hepatitis C virus entry factor. *Nat. Med.* **2012**, *18*, 281–285.