

# Comparative Analysis of Host Cell Entry of Ebola Virus From Sierra Leone, 2014, and Zaire, 1976

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**The ongoing Ebola virus (EBOV) disease (EVD) epidemic in Western Africa is the largest EVD outbreak recorded to date and requires the rapid development and deployment of antiviral measures. The viral glycoprotein (GP) facilitates host cell entry and, jointly with cellular interaction partners, constitutes a potential target for antiviral intervention. However, it is unknown whether the GPs of the currently and previously circulating EBOVs use the same mechanisms for cellular entry and are thus susceptible to inhibition by the same antivirals and cellular defenses. Here, we show that the GPs of the EBOVs circulating in 1976 and 2014 transduce the same spectrum of target cells, use the same cellular factors for host cell entry, and are comparably susceptible to blockade by antiviral interferon-induced transmembrane proteins and neutralizing antibody KZ52. Thus, the viruses responsible for the ongoing EVD epidemic should be fully susceptible to established antiviral strategies targeting GP and cellular entry factors.**

**Keywords.** Ebola virus; West Africa; entry; glycoprotein.

Ebolaviruses, from the family *Filoviridae*, cause a severe and frequently fatal disease in humans [1]. The first documented outbreak of Ebola virus (EBOV, formerly Zaire ebolavirus) disease (EVD) occurred in Zaire and was associated with a case-fatality rate of 88% [2]. Subsequently, 22 EVD outbreaks were recorded in sub-Saharan Africa [2]. These outbreaks generally occurred in relatively remote areas, were contained by quarantine measures, and caused a maximum of 425 cases per outbreak (during the 2000–2001 EVD outbreak in Uganda). The ongoing EVD epidemic, which originated in Guinea [3], differs markedly from previous outbreaks: it affects Western Africa and is associated with a higher death toll than all previously recorded outbreaks jointly [4]. The massive scale of the epidemic is likely due to the

delayed public health response. However, the currently circulating viruses harbor >30 unique amino acid substitutions [5], and it is currently unclear whether these mutations allow for more-efficient spread in and between humans, compared with viruses responsible for past outbreaks.

The EBOV glycoprotein (GP) is inserted into the viral envelope and facilitates viral entry into target cells [6, 7]. Moreover, GP is the sole target for the neutralizing antibody response. GP is synthesized in the constitutive secretory pathway of infected cells and consists of a surface unit, GP1, and a transmembrane unit, GP2 [6, 7]. The GP1 subunit contains an N-terminal receptor binding domain (RBD) [8, 9], which binds to host cell receptors, and a C-terminal mucin-like domain (MLD), while the GP2 subunit contains functional elements required for fusion of the viral envelope with a host cell membrane. Cellular entry of EBOVs commences with uptake of virions into host cell endosomes. This process can be promoted by recognition of phosphatidylserine in the viral envelope by TIM proteins [10] and, via bridging molecules, TAM receptor tyrosine kinases (Tyro3, Axl, and Mer) [11] on the host cell surface or by binding of GP to host cell lectins [12, 13]. Within the endosome, cysteine proteases

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remove the MLD [14], which allows the subsequent interaction of GP with the cholesterol transporter NPC-1 [15, 16], and drug-induced accumulation of endosomal cholesterol blocks infectious entry [17, 18]. Finally, an incompletely characterized stimulus triggers the membrane fusion reaction [19], which allows the release of the ribonucleoprotein complex into the host cell cytoplasm.

All viral and cellular factors involved in the entry of EBOVs into host cells are potential targets for antiviral intervention. However, the GP of the EBOV, which is currently circulating in West Africa harbors 8 unique substitutions in GP [5], and it is unknown whether these changes alter the entry cascade and/or render the virus resistant to inhibitors or neutralizing antibodies. The present study addressed this question by comparing host cell entry mediated by GPs of the EBOVs circulating in 1976 and 2014.

## MATERIAL AND METHODS

### Cell Culture

The adherent human and simian cell lines cells were cultivated in Dulbecco's modified Eagle's medium (PAA Laboratories) and the suspension cells in Roswell Park Memorial Institute 1640 medium (PAA Laboratories), supplemented with 10% fetal bovine serum (Biocrom) and antibiotics. The following cell lines were used: 293T (human embryonic kidney), Huh7 (human hepatoma), U373 (human glioblastoma), HOS (human osteosarcoma), HeLa (human cervix carcinoma), RPE (human retinal pigment epithelial), EA-hy (human endothelial hybrid), Raji (human B cell), Jurkat (human T cell), CEMx174 (human T-cell/B-cell hybrid), sMAGI (rhesus macaque mamma carcinoma), LLC-MK2 (rhesus macaque kidney), Vero (African green monkey kidney), and COS (African green monkey kidney). Differentiation of human THP-1 monocytes into THP-1 macrophages was achieved by incubation with phorbol-12-myristate-13-acetate (PMA; 10 ng/mL) for 48 hours. Bat cell lines were propagated as described elsewhere [20]. All cells were grown in a humidified atmosphere at 37°C and 5% CO<sub>2</sub>.

### Plasmids and Antibodies

The nucleotide sequence encoding the GP of the currently circulating EBOV (Makona variant; GenBank accession number KM233105; Ebola virus/H.sapiens-wt/SLE/2014/Makona-G3838) was synthesized (GeneArt, Life Technologies) and inserted into pcDNA3.1zeo via *Hind*III and *Xba*I sites. Expression plasmids for the GPs of EBOV strain Mayinga from 1976 (EBOV-GP 1976), Lassa fever virus, influenza A virus (strain A/WSN/33), and vesicular stomatitis virus (VSV), as well as 3-component human immunodeficiency virus type 1 (HIV-1)- and MLV-based vector systems for analysis of GP-driven transduction have been described elsewhere [18, 18, 21–23]. Plasmids encoding the lectins DC-SIGN, DC-SIGNR, LSECtin, ASGPR-1, Clec5A, and folate receptor  $\alpha$  were also described previously

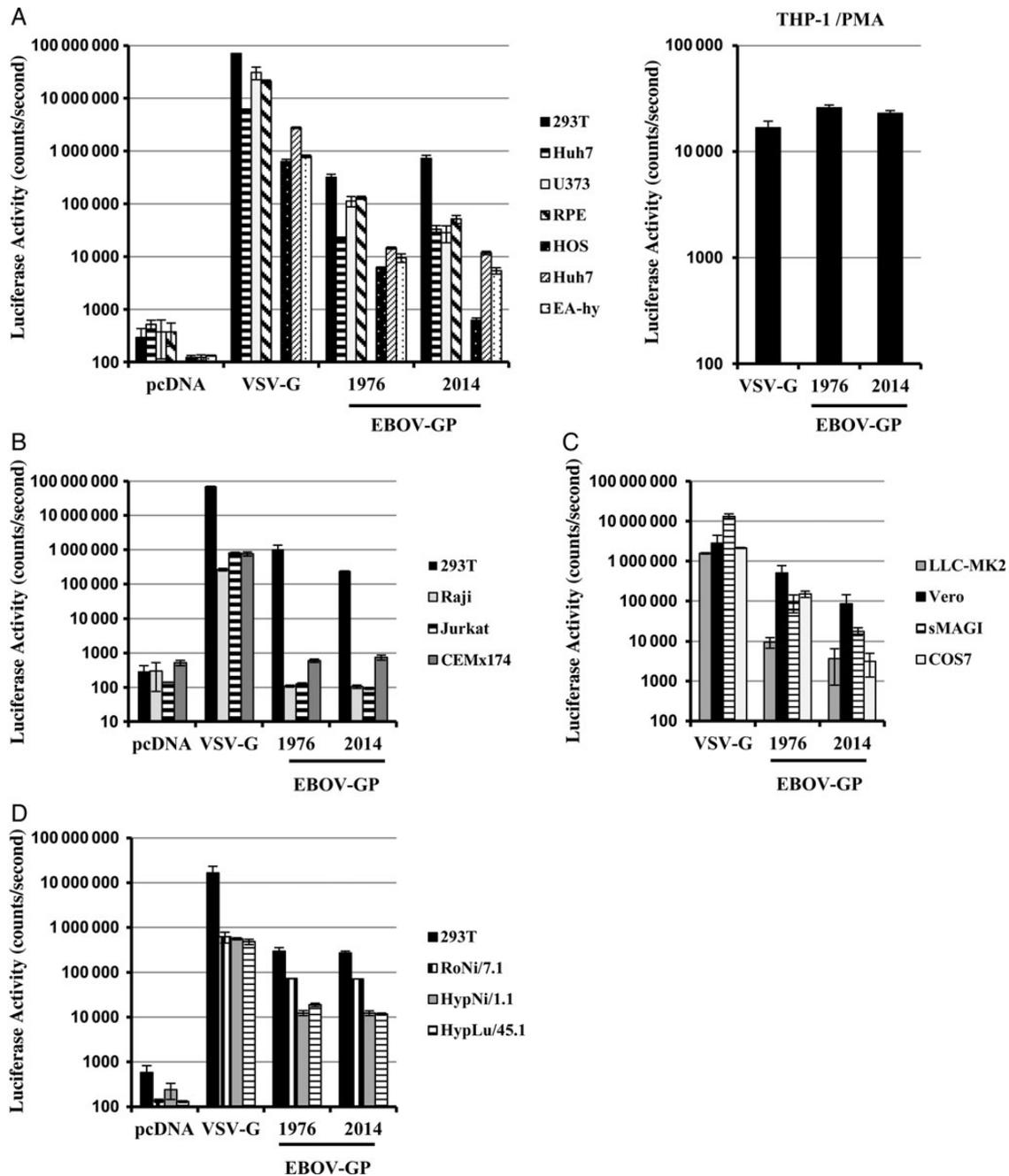
[21, 24–26]. Monoclonal antibodies KZ52 and 3B11 directed against EBOV-GP [27, 28] were kindly provided by Prof S. Becker, Marburg. Monoclonal antibody 183-H12-5C recognizing HIV-1 Gag was obtained from the National Institutes of Health (NIH) AIDS Reagent Program.

### Production of Retroviral and Rhabdoviral Pseudotypes and Virus-Like Particles (VLPs)

The generation of retroviral vectors pseudotyped with heterologous viral GPs (pseudotypes) was carried out as described before [18]. In brief, 293T cells underwent calcium phosphate transfection with an expression plasmid encoding the GP of choice in combination with plasmids encoding HIV-1 *gag-pol* and plasmid pCSWF-*luc* [29] or plasmids encoding MLV *gag-pol* and packaging plasmid MLV-*luc* [23]. The culture supernatants were harvested 48 hours after transfection, passed through filters (pore size, 0.45  $\mu$ m), aliquoted, and stored at –80°C. For production of rhabdoviral particles, a protocol described elsewhere [18] was followed. VLPs were produced by expression of HIV-1 Gag in combination with the GP of interest in 293T cells, followed by centrifugation of the VLP-containing supernatant through a 20% sucrose cushion [30]. The presence of Gag and GP in VLP preparations was analyzed by Western blot, using antibodies against HIV-p24 (NIH) and monoclonal antibody 3B11 directed against EBOV-GP [31].

### Transduction Experiments

For all transduction experiments, target cells were seeded in 96-well plates at a density of  $3 \times 10^4$  cells/well. Cells were then incubated for 8 hours with 50  $\mu$ L of medium containing pseudoparticles bearing the GP of interest. Transduction efficiency was determined by quantification of luciferase activities in cell lysates at 30 hours (rhabdoviral pseudotypes) or 72 hours after transduction (retroviral pseudotypes), using commercially available kits (Promega, PJK). In some experiments, target cells were transduced to express interferon-induced transmembrane (IFITM) proteins or were transfected to express cellular lectins, as described previously [18], before transduction with GP-bearing pseudotypes. Alternatively, expression of EBOV entry factors was inhibited by small interfering RNAs (siRNAs). For this, siRNA knock down in target cells was performed 24 hours prior to transduction by transfection of 5 pmol of siRNA (all Santa Cruz), using Lipofectamine 2000 (Life Technologies). To determine whether cationic amphiphiles (U18666A, Merck; clomiphene or terconazole, Sigma-Aldrich) or protease inhibitors (CatL inhibitor III, Merck; CA074Me, Calbiochem; CA074; Sigma-Aldrich; AEBSF, Roth) influence transduction efficiency, the inhibitors were diluted in appropriate solvent as recommended by the manufacturer. Target cells were preincubated with inhibitor for 60 minutes at 37°C before addition of pseudotypes, and the culture medium was replaced by fresh culture medium without inhibitor after 8 hours.



**Figure 1.** Pseudotypes bearing glycoprotein (GP) from ebolavirus (EBOV) strain Mayinga from 1976 (EBOV-GP 1976) and EBOV variant Makona from 2014 (EBOV-GP 2014) mediate entry into the same spectrum of cell lines. The indicated cell lines of human (A and B), monkey (C), and bat (D) origin were transduced with equal volumes of pseudotypes carrying the indicated GPs or no GP (pcDNA). Transduction efficiency was quantified by a luciferase assay 72 hours after transduction. Each experiment was performed with triplicate samples and repeated at least 3 times, using at least 2 independent pseudotype preparations. Error bars indicate standard deviations. HypLu/45.1, cell line derived from lung from *H. monstrosus*; HypNi/1.1, cell line derived from kidney from *Hypsignathus monstrosus*; RoNi/7.1, kidney cell line from *Rousettus aegyptiacus*.

### Stability Test

To assess virion stability, pseudotypes bearing EBOV-GP or the GP of VSV (VSV-G) were normalized for comparable infectivity. Subsequently, the pseudotypes were incubated at defined temperatures for increasing periods, frozen at  $-80^{\circ}\text{C}$  at a given time point, and used for transduction of 293T target

cells. Seventy-two hours after transduction, transduction efficiency was quantified by a luciferase assay.

### Antibody-Mediated Neutralization

Pseudotypes carrying the GPs of the respective EBOV isolate or VSV-G as a control were normalized for comparable infectivity

and incubated with monoclonal antibody KZ52 at indicated dilutions for 1 hour at 37°C. Thereafter, the antibody/pseudotype mixtures were added to 293T cells, the cells were incubated for 72 hours, and luciferase activities in cell lysates were determined.

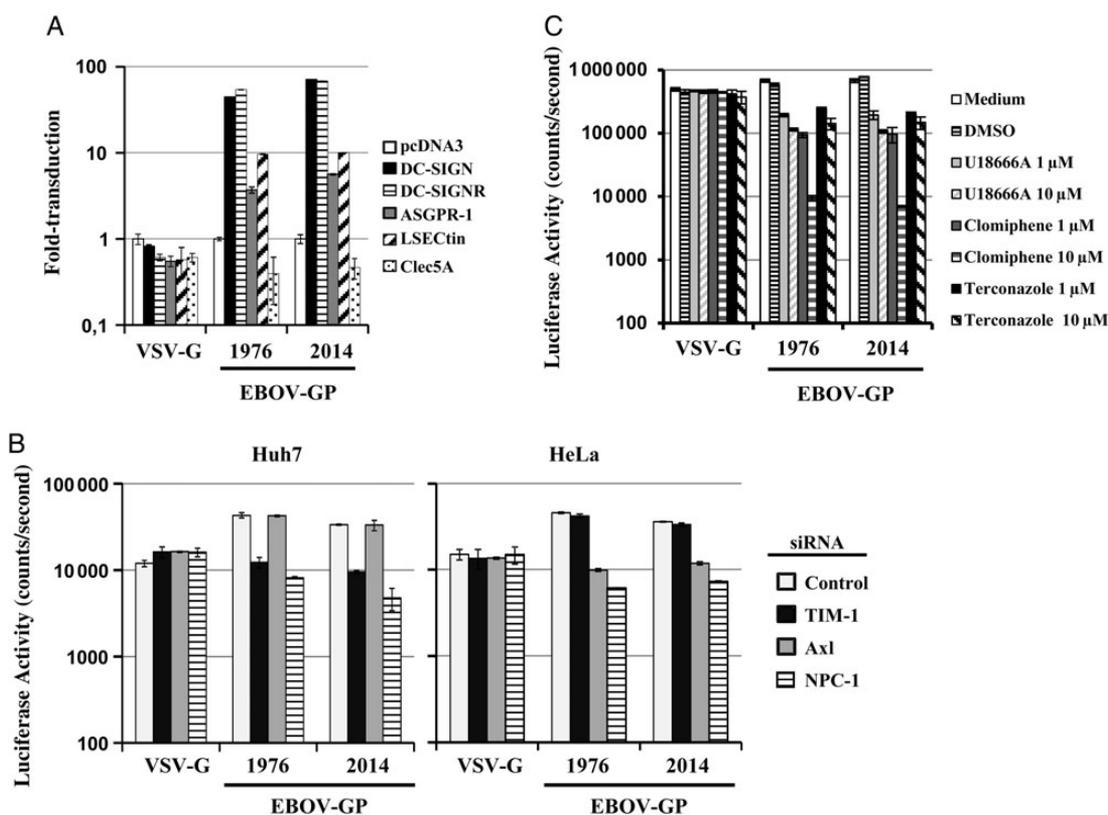
## RESULTS

### GPs of EBOVs Responsible for the 1976 Outbreak in Zaire and the 2014 Epidemic in West Africa Facilitate Entry Into an Identical Spectrum of Cells

Ebolaviruses infect a broad spectrum of cell types in cell culture [32], but macrophages and dendritic cells constitute early and sustained targets in the infected host [33]. In contrast, lymphocytes are refractory to infection, both in vitro and in vivo [32,33]. To analyze whether previously and currently circulating EBOVs show differences in host cell tropism and entry, we comparatively analyzed pseudotypes bearing the GPs of EBOV, strain Mayinga,

1976 (EBOV-GP 1976) and EBOV variant Makona (EBOV-GP 2014) [34], which differ in 1 amino acid residue in the RBD and 16 amino acid residues in the MLD (Supplementary Figure 1). Pseudotypes bearing VSV-G served as positive control, while pseudotypes bearing no GP were used as negative control. Both EBOV GPs were efficiently expressed in transfected cells and incorporated into retroviral particles (Supplementary Figure 2), and both mediated entry into the human cell lines 293T, Huh7, U373, RPE, HOS, and EA-hy with comparable efficiencies (Figure 1A). Similarly, transduction of macrophages obtained upon PMA treatment of THP-1 cells was comparable (Figure 1A). In contrast, both GPs were unable to facilitate transduction of a human B-cell line (Raji), a human T-cell line (Jurkat) and a human T/B hybrid cell line (CEMx174; Figure 1B).

EBOV is highly pathogenic for nonhuman primates (NHPs), which are used as animal models for EVD in humans [35]. Therefore, we also analyzed whether EBOV-GP 1976 and



**Figure 2.** Comparable entry factor use by pseudotypes bearing glycoprotein (GP) from ebolavirus (EBOV) strain Mayinga from 1976 (EBOV-GP 1976) and EBOV variant Makona from 2014 (EBOV-GP 2014). *A*, 293T cells transfected to express the indicated lectins or transfected with empty plasmid (pcDNA3) were transduced with infectivity-normalized pseudotypes bearing the indicated viral GPs. Seventy-two hours after transduction, luciferase activities were determined in cell lysates. The results of a single, representative experiment performed with triplicate samples are shown. Transduction of control transfected cells (pcDNA) was set as 1. Similar results were obtained in 5 independent experiments, using different pseudotype preparations. *B*, Huh7 cells (left panel) and HeLa cells (right panel) underwent small interfering RNA (siRNA) transfection and were subsequently transduced with infectivity-normalized pseudotypes. Luciferase activities in cell lysates were measured 72 hours after transduction. The experiment was performed with triplicate samples and is representative of 3 independent experiments. *C*, 293T cells were preincubated with solvent (dimethyl sulfoxide [DMSO]) or the respective cationic amphiphiles in the indicated concentrations and subsequently transduced with pseudotypes bearing the indicated GPs. Luciferase activities were measured 72 hours after transduction. Two additional experiments yielded comparable results. Error bars indicate standard deviations.

EBOV-GP 2014 transduce NHP-derived cell lines. Transduction of the LLC-MK2 and sMAGI cell lines was comparable to transduction of the Vero cell line (Figure 1C). In contrast, EBOV-GP 1976 was more efficient than EBOV-GP 2014 at transducing COS7 cells. Finally, the ability of EBOV-GP 1976 and EBOV-GP 2014 to transduce bat cell lines (derived from *Rousettus aegyptiacus* and *Hypsignathus monstrosus*) was examined, since *H. monstrosus*, *Epomops franqueti*, and *Myonycteris torquata* can serve as natural reservoir for EBOVs [36]. However, no appreciable differences were observed (Figure 1D). In sum, these results suggest that EBOV variants circulating in 1976 and 2014 exhibit a comparable cell tropism.

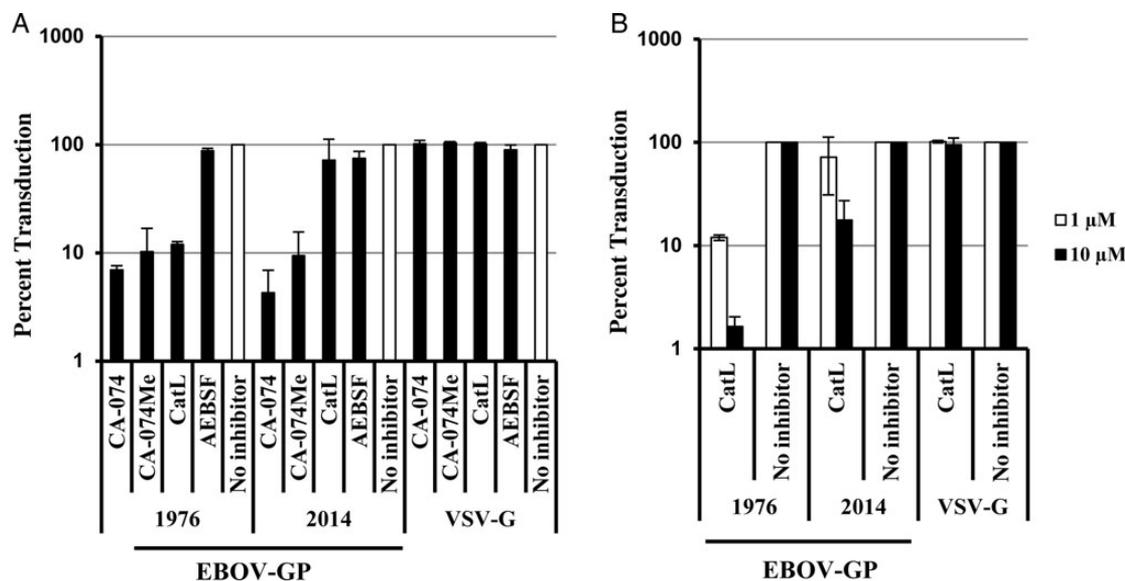
### GPs of EBOVs Circulating in 1976 and 2014 Exhibit Comparable Use of Entry Factors

Lectins, TIM-1, and TAM kinase Axl can augment entry of EBOVs into certain target cells [6]. In contrast, NPC-1 is universally required for entry (Dahlmann et al, submitted). We addressed whether EBOV-GP 1976 and EBOV-GP 2014 show differential dependence on these factors for host cell entry. The lectins DC-SIGN, DC-SIGNR, ASGPR1, and LSECtin are known to augment GP-driven entry [12, 13, 37] and promoted transduction driven by EBOV-GP 1974 and EBOV-GP 2014 to similar extents while expression of CLEC5A, which binds Dengue virus [38], had no effect (Figure 2A). Moreover, both EBOV-GP 1976 and EBOV-GP 2014 used TIM-1 and Axl for entry

into certain target cells (Figure 2B), while expression of NPC-1 was universally required for entry (Figure 2B and 2C). Finally, entry driven by both GPs was comparably inhibited by U18666A, which induces an NPC-1 knockout phenotype in cells [39], and related compounds (Figure 2C), in the absence of unwanted cytotoxic effects (not shown). Thus, our studies revealed no obvious difference in entry factor use by EBOV-GP 1976 and EBOV-GP 2014.

### Subtle Differences in Dependence on Cellular Cysteine Proteases

The enzymatic activity of the pH-dependent endosomal/lysosomal cysteine proteases cathepsin B (catB) and cathepsin L (catL) is required for cellular entry of pseudotypes bearing EBOV-GP 1976 and authentic EBOV 1976 [14]. We used a panel of inhibitors previously employed to study EBOV-GP protease use [40], to determine whether EBOV-GP 1976 and EBOV-GP 2014 exhibit differences in their protease requirements. Entry driven by both proteins was comparably inhibited by the cysteine protease inhibitors CA074Me and CA074, which efficiently inhibit catB but have little or no effect on catL and catS activity [40]. In contrast, the serine protease inhibitor AEBSF had no effect on GP-driven entry, and none of the compounds tested modulated entry driven by VSV-G (Figure 3A), as expected [40]. These observations indicate that EBOV-GP 1976 and EBOV-GP 2014 both depend on catB activity for



**Figure 3.** Subtle differences in the protease requirements of pseudotypes bearing glycoprotein (GP) from Ebola virus (EBOV) strain Mayinga from 1976 (EBOV-GP 1976) and EBOV variant Makona from 2014 (EBOV-GP 2014). *A*, 293T cells were preincubated with solvent or protease inhibitor (CA-074, 5 μM; CA-074Me, 0.4 μM; CatL, 1 μM; and AEBSF, 5 μg/mL) and thereafter transduced with infectivity-normalized pseudotypes and incubated for 72 hours in the presence of pseudotype and inhibitor. Then, luciferase activities were determined in cell extracts. Transduction in the absence of inhibitor was set as 100%. The average of 3 independent experiments is shown. Error bars indicate standard error of the mean (SEM). *B*, 293T cells were preincubated with solvent or protease inhibitor CatL III (CatL) at either 1 μM (white bars) or 10 μM (black bars), followed by transduction with infectivity-normalized pseudotypes. Luciferase activities were determined 72 hours after transduction. Transduction in the absence of inhibitor was set as 100%. The average of 3 independent experiments is shown. Error bars indicate SEM.

efficient transduction of 293T cells. However, EBOV-GP 2014 was less susceptible than EBOV-GP 1976 to blockade by a third inhibitor, CatL (Figure 3B), which inhibits catB and catL activity to similar extents [40], suggesting subtle differences in the protease dependence of these GPs.

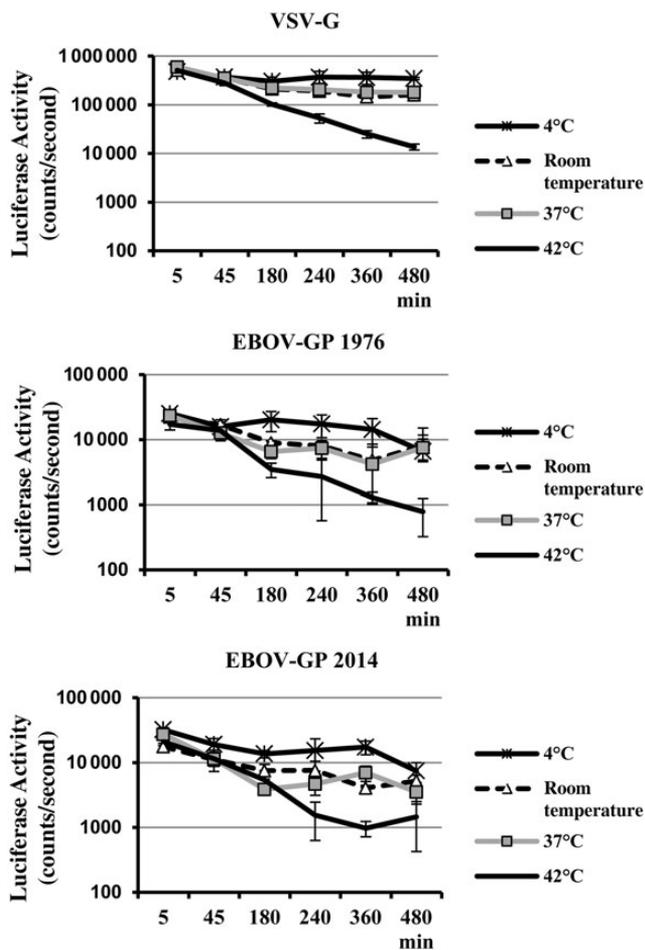
### Particles Bearing GPs of EBOVs From 1976 and 2014 Show Comparable Stability

HIV-derived particles rapidly lose infectivity when stored at room temperature [41], and this loss might be due to inactivation of the viral envelope protein. Therefore, we asked whether retroviral particles bearing EBOV-GP 1976 and EBOV-GP 2014 exhibit different stabilities. Incubation of viral particles bearing

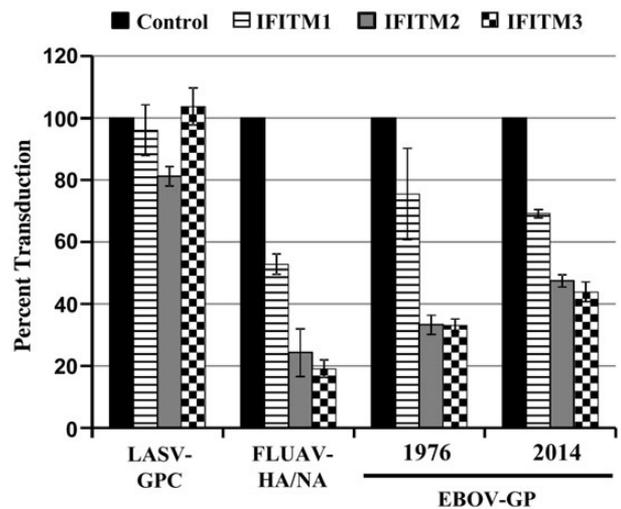
VSV-G, EBOV-GP 1976, and EBOV-GP 2014 for up to 8 hours at 4°C, room temperature, and 37°C modestly and comparably reduced particle infectivity, and this loss occurred slightly more rapidly at room temperature and 37°C as compared to 4°C (Figure 4). A more profound loss was observed upon incubation at 42°C, but again no differences were observed between EBOV-GP 1976 and EBOV-GP 2014 (Figure 4), suggesting that the stability of these GPs is comparable.

### Entry Driven by GPs of EBOVs Circulating in 1976 and 2014 Is Comparably Inhibited by IFITM Proteins and Neutralizing Antibody KZ52

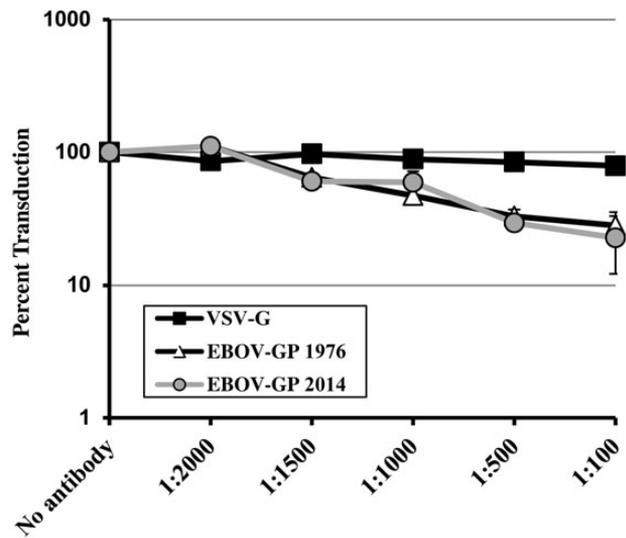
IFITM proteins 1, 2, and 3 inhibit cellular entry of EBOV [42] and several other viral pathogens [42, 43] and might reduce EBOV amplification in the infected host, raising the question of whether viruses circulating in 2014 are less susceptible to IFITM protein inhibition than those responsible for the 1976 outbreak in Zaire. Engineered expression of IFITM proteins in 293T cells had no appreciable impact on transduction mediated by Lassa fever virus GPC but reduced influenza virus HA-driven transduction, with inhibition by IFITM protein 3 being most efficient (Figure 5), in keeping with published data [42]. Transduction by the 2 EBOV-GPs tested was also inhibited by IFITM protein expression, and no appreciable differences in inhibition efficiency were observed (Figure 5). Thus, the virus



**Figure 4.** Pseudoparticles bearing glycoprotein (GP) from Ebola virus (EBOV) strain Mayinga from 1976 (EBOV-GP 1976) and EBOV variant Makona from 2014 (EBOV-GP 2014) exhibit comparable stability. Pseudotypes normalized for comparable infectivity were incubated at the indicated temperatures for different periods, frozen at  $-80^{\circ}\text{C}$ , and used for transduction of 293T target cells. Seventy-two hours after transduction, transduction efficiency was quantified by luciferase assay. The results are representative of 3 independent experiments performed with triplicate samples. Error bars indicate standard deviations. Abbreviation: VSV-G, glycoprotein of vesicular stomatitis virus.



**Figure 5.** Pseudoparticles bearing glycoprotein (GP) of Ebola virus (EBOV) strain Mayinga from 1976 (EBOV-GP 1976) and EBOV variant Makona from 2014 (EBOV-GP 2014) are susceptible to inhibition by interferon-induced transmembrane (IFITM) proteins. 293T cells engineered to express IFITM proteins 1, 2, or 3 or no IFITM protein (control) were transduced with pseudoparticles bearing the indicated GPs. Luciferase activities were assessed 72 hours after transduction; transduction efficiency in cells expressing no IFITM protein (control) was set as 100%. The average of 2 separate experiments performed with triplicate samples is shown. Error bars indicate standard deviations.



**Figure 6.** Host cell entry driven by pseudotypes bearing glycoprotein (GP) of ebolavirus (EBOV) strain Mayinga from 1976 (EBOV-GP 1976) and EBOV variant Makona from 2014 (EBOV-GP 2014) is inhibited by a neutralizing antibody. Pseudotypes carrying the indicated GPs were normalized for comparable infectivity and incubated with the indicated dilutions of monoclonal antibody KZ52 for 1 hour at 37°C. Thereafter, the complexes were used for transduction of 293T cells, and luciferase activities in cell lysates were measured 72 hours after transduction. Transduction in the absence of antibody was set as 100%. The results of a single experiment performed with triplicate samples are shown and are representative of 3 independent experiments. Error bars indicate standard deviations.

circulating in 2014 is likely to be fully susceptible to blockade by IFITM proteins.

Host cell entry of EBOVs can be inhibited by GP-specific antibodies, and some neutralizing antibodies were shown to exert antiviral activity in the host. We assessed inhibition of EBOV-GP 1976-driven and EBOV-GP 2014-driven entry by antibody KZ52 (obtained from an EVD survivor), which targets GP2 [44] and displays antiviral activity in guinea pigs but not monkeys [27]. Entry driven by both GPs was comparably inhibited by preincubation of particles with KZ52, while this antibody had no effect on entry driven by VSV-G (Figure 6), indicating that the epitope recognized by KZ52 is conserved between EBOV 1976 and EBOV 2014.

## DISCUSSION

The ongoing EVD epidemic in Western Africa is of unprecedented proportions and calls for the large-scale assessment of existing options for antiviral intervention and the development of new antiviral strategies. However, amino acid substitutions unique to the GP of the currently circulating EBOV [5] might render the viruses nonsusceptible to antivirals and cellular defenses targeting GP or cellular factors promoting GP-driven entry. The present study indicates that this scenario does not

apply: no major differences in host cell entry driven by EBOV-GP 1976 and EBOV-GP 2014 were observed, and both GPs were comparably susceptible to blockade by inhibitors and antiviral host cell proteins.

EBOV-GP 2014 contains a unique amino acid substitution in the RBD [5, 8], which may alter the requirement for host cell receptors and could modulate viral cell tropism. Our results suggest that this is not the case: entry mediated by EBOV-GP 1976 and EBOV-GP 2014 was dependent on expression of the cholesterol transporter NPC-1 and was inhibited by compounds that induce accumulation of endosomal cholesterol. Moreover, expression of endogenous TIM-1 and Axl, which can augment GP-driven entry by binding directly (TIM-1) or via a bridging molecule (Axl) to PtdSer in the viral envelope [45, 46], promoted entry driven by both EBOV-GP 1976 and EBOV-GP 2014. The MLD of GP is heavily modified with O-linked glycans and is required for binding to certain host cell lectins [47]. The observation that the MLD of EBOV-GP 2014 contains 16 amino acid substitutions relative to the MLD of EBOV-GP 1976 suggested that engagement of cellular lectins might differ between these GPs. However, again no appreciable differences were observed. In keeping with the comparable entry factor use, both GPs facilitated entry into an identical spectrum of cell lines derived from bats, NHPs, and humans, including THP-1 cell-derived macrophages. The only consistent difference observed was a reduction of EBOV-GP 2014-mediated transduction of COS7 cells, relative to that mediated by EBOV-GP 1976. This effect might point toward subtle differences in entry factor use, which may not be detectable in the assay system used in the present study.

The removal of the MLD by endosomal cysteine proteases, catB and L, is required for subsequent binding of GP to NPC-1 [14, 19]. Several studies indicate that vectors bearing EBOV-GP, as well as authentic EBOV, depend on the activity of catB/L for infectious entry into cell lines [14, 48, 49], although catB/L dependence seems to vary between *Ebolavirus* species [48, 50], and the activity of these particular proteases is dispensable for EBOV spread in a murine model [48]. The present study suggests that catB/L dependence extends to viruses currently circulating in West Africa. However, reduced susceptibility of EBOV-GP 2014 to the inhibitor CatL, relative to that of EBOV-GP 1976, points toward minor differences in the protease requirements of the respective GPs.

The GPs of EBOVs are cleaved by proprotein convertases in the Golgi apparatus of infected cells, and evidence for cleavage of EBOV-GP 2014 was obtained (data not shown). The cleavage products, the surface unit GP1 and the transmembrane unit GP2, remain covalently associated via a disulphide bond. One could speculate that the stability of this association might be different between EBOV-GP 1976 and EBOV-GP 2014, which could translate into different stability of retroviral particles bearing these GPs. However, a comparable time- and temperature-

dependent loss of infectivity of EBOV-GP 1976-bearing and EBOV-GP 2014-bearing particles was observed, arguing against substantial differences in GP stability, although it cannot be disregarded that GP stability might differ in the context of retroviral and filoviral particles.

Interferon-induced antiviral host factors and neutralizing antibodies can contribute to viral control in the infected host. The present study suggests that EBOV variants from 1976 and 2014 are susceptible to both defense mechanisms. Expression of IFITM proteins comparably inhibited host cell entry driven by EBOV-GP 1976 and EBOV-GP 2014, potentially by increasing endosomal cholesterol levels [17, 18]. Moreover, entry driven by both GPs was blocked by neutralizing antibody KZ52, which was obtained from a human EVD survivor and targets GP2 [44]. Collectively, these results and the observations discussed above suggest that previously and presently circulating EBOV variants might use similar mechanisms for host cell entry, infect a similar spectrum of target cells, and might be comparably susceptible to inhibition by innate and adaptive immune responses.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

## Notes

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**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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