25-Hydroxycholesterol Inhibition of Lassa Virus Infection through Aberrant GP1 Glycosylation

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ABSTRACT  Lassa virus (LASV) infection is a major public health concern due to high fatality rates and limited effective treatment. The interferon-stimulated gene cholesterol 25-hydroxylase (CH25H) encodes an enzyme that catalyzes the production of 25-hydroxycholesterol (25HC). 25HC is involved in regulating cholesterol biosynthesis and has recently been identified as a potent antiviral targeting enveloped virus entry. Here, we show a previously unrecognized role of CH25H in inhibiting LASV glycoprotein glycosylation and the production of infectious virus. Overexpression of CH25H or treatment with 25HC decreased LASV G1 glycoprotein N-glycan maturation and reduced the production of infectious LASV. Depletion of endogenous CH25H using small interfering RNA (siRNA) enhanced the levels of fully glycosylated G1 and increased infectious LASV production. Finally, LASV particles produced from 25HC-treated cells were found to be less infectious, to incorporate aberrantly glycosylated GP1 species, and to be defective in binding alpha-dystroglycan, an attachment and entry receptor. Our findings identify a novel role for CH25H in controlling LASV propagation and indicate that manipulation of the expression of CH25H or the administration of 25HC may be a useful anti-LASV therapy.

IMPORTANCE  Lassa fever is an acute viral hemorrhagic fever in humans caused by Lassa virus (LASV). No vaccine for LASV is currently available. Treatment is limited to the administration of ribavirin, which is only effective when given early in the course of illness. Cholesterol 25-hydroxylase (CH25H) is a recently identified interferon-stimulated gene (ISG); it encodes an enzyme that catalyzes the production of 25-hydroxycholesterol (25HC), which inhibits several viruses. Here, we identify a novel antiviral mechanism of 25HC that is dependent on inhibiting the glycosylation of Lassa virus (LASV) glycoprotein and reducing the infectivity of LASV as a means of suppressing viral replication. Since N-linked glycosylation is a critical feature of other enveloped-virus glycoproteins, 25HC may be a broad inhibitor of virus infectivity.
been predominantly attributed to its enzymatic activity through the production of 25HC, which has been shown to block infection by several enveloped viruses (14–17). The antiviral function of 25HC involves multiple mechanisms, including blocking fusion of the viral envelope with host membranes (14), inhibiting viral replication (14, 15), and inhibiting the formation of viral replication complexes on intracellular membranes (17). 25HC was also reported to inhibit a postentry step of viral infection by repressing the activation of SREBP (16), a transcription factor required for lipid and cholesterol biosynthesis. More recently, 25HC has also been shown to block the replication of nonenveloped viruses (18). These findings demonstrate that 25HC has broad-spectrum antiviral effects. Understanding the mechanisms by which 25HC exerts antiviral activity may help to identify strategies and targets for potential intervention.

Here, we report that 25HC restricted LASV infection by inducing the biosynthesis of an aberrantly glycosylated form of GP1, which was efficiently incorporated into LASV particles. Incorporation of the aberrantly glycosylated GP1 was accompanied by reduced LASV infectivity and binding to alpha-dystroglycan (alpha-DG), a cell surface protein involved in LASV attachment and endocytosis (19, 20). Our findings suggest that 25HC can restrict virus infection by a previously unrecognized mechanism linking improper glycosylation and binding of the viral glycoprotein to a cellular receptor.

RESULTS

25HC impairs LASV production. We first tested the ability of 25HC to reduce the production of infectious LASV. Huh7 cells were treated for 1 h with either ethanol (vehicle control) or indicated concentrations of 25-hydroxycholesterol (25HC) and then infected with LASV at a multiplicity of infection (MOI) of 0.1. The LASV titers were reduced by both concentrations of 25HC at 48 and 72 h postinfection (hpi). At 5 μM, 25HC inhibited the production of infectious LASV titers by ~1.1 log, and 10 μM of 25HC inhibited LASV titers by 1.4 log (Fig. 1A). These decreases in viral titers were not due to cellular toxicity, as no significant cytotoxic effects were measured over the same time period (Fig. 1B).

LASV infectivity is reduced by 25HC treatment. Next, we used quantitative reverse transcription-PCR (qRT-PCR) to determine LASV RNA copy numbers in supernatants of LASV-infected cells. In contrast to LASV infectious titers (Fig. 1A), the extracellular LASV RNA levels from control and 25HC-treated samples increased to 1 × 10⁹ copies per ml at 72 hpi, and 25HC treatment did not affect viral RNA release (Fig. 1C). To highlight the differences between LASV titers and viral RNA copy numbers due to 25HC treatment, we calculated the ratio of infectious LASV (50% tissue culture infective dose [TCID₅₀]/ml) to viral RNA copy number (Fig. 1D). In 25HC-treated cells, the ratio was reduced to ~15% of the values for controls at 48 and 72 hpi, suggesting that the loss in LASV infectivity was most notable after 24 hpi.
Previous reports suggest that 25HC blocks cell entry of enveloped viruses (14). To assess whether the impaired LASV infectivity was due to inhibited LASV entry, we measured the levels of cell-associated LASV S genome at an early time point after infection. Huh7 cells pretreated with 25HC were infected with LASV at an MOI of 1. S segment RNA levels were measured by qRT-PCR and normalized to the level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. As shown by the results in Fig. S1 in the supplemental material, the S genome levels did not differ significantly among the samples, indicating that 25HC did not affect the levels of internalized LASV genome. On the other hand, treatment with the positive-control BIBX 1382, previously shown to inhibit LASV glycoprotein-dependent entry (21), significantly reduced the S genome levels. These results are consistent with 25HC not affecting the initial stages of LASV entry.

**25HC alters the electrophoretic mobility of glycosylated GP1.** To understand the mechanism by which 25HC reduces LASV infectivity, we examined the viral proteins in the lysates of LASV-infected cells. Western blot analysis using a monoclonal antibody (Mab) recognizing the GP1 region detected GP1 migrating at ~75 kDa and the cleavage product GP1 as a diffuse 40- to 42-kDa band in lysates of untreated cells (Fig. 2A). At 48 h, treatment with 5 and 10 μM 25HC decreased the levels of the 42-kDa species but not the 75-kDa species. At 72 hpi, the intensity of the 42-kDa band shifted below ~40 kDa. 25HC treatment also changed the GP1 cleavage efficiency [%GP1/(GP1 + GP)] was unaffected. 25HC treatment caused decreased LASV NP levels at 24 and 48 hpi, but the NP levels were similar to those in the vehicle control at 72 hpi. Thus, 25HC increased GP1 mobility on SDS-PAGE gels and reduced infectious LASV production (Fig. 2A and 1A).

**25HC inhibits LASV GP1 glycosylation in LASV GPC-transfected cells.** Cleavage of LASV GPC by SKI-1/S1P is essential for the production of infectious virus. The reduced LASV titers in 25HC-treated cells (Fig. 1A) could be due to reduced cleavage or reduced glycosylation of LASV GPC. Thus, we next looked at the expression of GPC and GP1 in Huh7 cells transfected with LASV GPC and treated with 25HC or ethanol vehicle control. Cells were treated with 25HC, and cell lysates were analyzed by Western blotting with antibodies for GP1 or actin. GPC-transfected and ethanol-treated cells showed GPC and GP1 electrophoretic mobilities similar to those of the LASV-infected cells (compare Fig. 2B and A), with the precursor protein migrating at ~75 kDa and the majority of GP1 detected at ~40 to 42 kDa. After 24 h of treatment, 25HC did not have a significant effect on GP1 glycosylation in GPC-transfected cells (see Fig. S2 in the supplemental material). However, after 48 h, similar to the results for LASV-infected cells, 5 μM or 10 μM of 25HC resulted in a distinct shift in GP1 mobility on SDS-PAGE gels, from ~40 to 42 kDa to below ~40 kDa. The unprocessed GP1 band shifted also, from ~75 kDa to ~73 kDa, after 25HC treatment. Although the overall levels of GPC and GP1 appeared slightly reduced upon 25HC treatment, 25HC did not affect GP1 cleavage efficiency, as demonstrated by quantifying GP1 and GP1 levels in cell lysates (Fig. 2B). No effect on the level or the migration of the endogenous protein mannose 6-phosphate receptor (M6PR), which contains 5 potential N-linked glycosylation sites (22), was detected by 25HC treatment. Thus, 25HC specifically increased GP1 mobility on SDS-PAGE gels but did not affect GP1 cleavage efficiency in the absence of other viral proteins.

To test whether the change in GP1 mobility on SDS-PAGE gels was due to changes in glycosylation, the lysates of cells transfected with LASV GPC were treated with endoglycosidase H (Endo-H) or peptide-N-glycosidase F (PNGase F). Treatment with PNGase F, which removes all N-linked glycans, caused the majority of GP1 to migrate at between ~20 and ~24 kDa (instead of ~40 to 42); this pattern was observed regardless of whether 25HC was used (Fig. 2C, lanes 3, 6, and 9). Without 25HC, Endo-H digestion, which removes high-mannose species while leaving the most complex and hybrid glycans intact, resulted in a shift of intensity of the ~40- to 42-kDa GP1 band to just below ~40 kDa in the lysates of control cells (Fig. 2C, lane 1 versus lane 2). In contrast, when 25HC-treated lysates were digested with Endo-H, the intensity of the GP1 species migrating below ~40 kDa was decreased, and multiple additional GP1 bands migrating from ~40 kDa to ~22 kDa became apparent (Fig. 2C, lanes 2, 5, and 8, top). Together, this indicated that the GP1 species below ~40 kDa are more sensitive to Endo-H digestion in 25HC-treated lysates than in lysates of control cells; this increase in sensitivity suggests that the maturation of GP1 N-glycans was impaired by 25HC. In contrast, GP2 sensitivity to Endo-H digestion was minimally affected in the presence and absence of 25HC (Fig. 2C lanes 2, 5, and 8, bottom). Taken together, these results indicate that 25HC treatment leads to the production of aberrantly glycosylated forms of GP1 that contain more immature N-glycans.

**Overexpression of CH25H alters GP1 electrophoretic mobility and LASV production.** 25HC is generated by the enzyme CH25H. To confirm a role for CH25H in GP glycosylation, we overexpressed this enzyme in Huh7 cells. Huh7 cells transfected with Myc-tagged CH25H or with empty plasmid were transfected with LASV GPC, and GP1 expression was analyzed by Western blotting. As a negative control, we used Myc-tagged IFITM3, another ISG that can block virus entry by disrupting intracellular cholesterol homeostasis (23, 24). The expression of CH25H and IFITM3 was confirmed by Western blotting (Fig. 3A). As seen with 25HC treatment, CH25H transfection altered the migration of GP1 to below 40 kDa (Fig. 3A). In contrast, no shift in GP1 band intensity was observed in cells transfected with either empty plasmid or IFITM3.

To investigate whether the expression of CH25H also affected GP1 glycosylation during LASV infection, as it does in GPC-transfected cells, we overexpressed CH25H in Huh7 cells and infected these cells with LASV. The expression of CH25H, but not of IFITM3, shifted the GP1 band at 72 hpi (Fig. 3B), similar to 25HC treatment (Fig. 2A). Furthermore, cells overexpressing CH25H produced 5 times less LASV than infected cells that received the empty vector (Fig. 3C). In comparison, IFITM3 overexpression did not affect LASV growth (Fig. 3C). Thus, CH25H overexpression altered LASV GP1 glycosylation and decreased LASV production. Taken together, the similar effects of CH25H expression and 25HC treatment suggest that 25HC synthesized by CH25H is responsible for the effects of CH25H transfection.

**Knocking down CH25H expression increases LASV production.** To evaluate the role of endogenous CH25H in LASV GP1 glycosylation and the reduction of LASV titers, we silenced CH25H mRNA expression under sterol-depleted conditions. Sterol depletion was used to increase CH25H expression to help detect changes in GP1 glycosylation and LASV titers. In LASV-infected cells, small interfering RNA (siRNA) targeting CH25H reduced its expression by more than 85% (Fig. 3D), and no change
in CH25H levels was observed in control cells treated with siRNA targeting IFITM3 or scrambled siRNA. Cells treated with CH25H siRNA showed increased intensity in the GP1 band at 42 kDa, consistent with more complex N-glycan content in GP1 (Fig. 3E). Increased intensity of the 42-kDa GP1 band was also accompanied by an ~fourfold increase in LASV titers compared to the titers in cells transfected with control or IFITM3 siRNA (Fig. 3F). No change in NP levels was detected in cells transfected with CH25H siRNA. These results suggested that knocking down CH25H increased the yield of the 42-kDa GP1 species and enhanced LASV replication.

Aberrantly glycosylated GP1 incorporates into virions. A possible cause for reduced LASV infectivity in 25HC-treated cells may be the incorporation of aberrantly glycosylated GP1 into LASV particles. To determine whether virus particles released from 25HC-treated cells contain aberrantly glycosylated GP1, supernatants of control or 25HC-treated cells were sedimented through a 20% sucrose cushion. No uncleaved GPC (~75 kDa)
was detected in the pelleted virions (Fig. 4A, left versus right), as previously reported (6). The NP levels were comparable in all samples. Virus released from control cells contained only the typically glycosylated form of GP1, migrating between 40 and 42 kDa. In contrast, in cells treated with 25HC, the majority of GP1 present in the pelleted LASV migrated below 40 kDa, indicating that 25HC treatment resulted in efficient incorporation of aberrantly glycosylated GP1 into LASV particles.
LASSV virions containing aberrantly glycosylated GP1 are defective in alpha-DG binding. The initial attachment of LASSV to the cell surface is dependent on the host glycoprotein alpha-DG. To test whether the aberrantly glycosylated LASSV particles produced after 25HC treatment were defective in alpha-DG binding, we performed a binding assay (20). In this assay, alpha-DG–Fc fusion proteins were immobilized on 96-well plates and incubated with semipurified LASSV preparations obtained from control and 25HC-treated cells. Equal volumes of purified LASSV were incubated on alpha-DG, and bound fractions were measured by determining the optical density (OD) at 450 nm. *, $P < 0.05$; **, $P < 0.005$.

DISCUSSION

Here, we demonstrate that 25HC has antiviral activity against LASSV that is linked to the production of virus particles with reduced infectivity. LASSV particles produced in the presence of 25HC contained aberrantly glycosylated GP1, the subunit of the viral glycoprotein responsible for receptor binding. These particles bound poorly to alpha-DG, the cell surface attachment and entry factor for LASSV. Thus, 25HC inhibits LASSV infection by a novel mechanism that alters the maturation of N-linked glycans, leading to impaired receptor binding.

The correct folding and cleavage of arenavirus GP are critical for the incorporation of GP1 and GP2 into virions, and inhibition of cleavage results in the release of noninfectious virus particles devoid of surface glycoproteins (6). 25HC reduced LASSV infectivity, but not through defective GPC cleavage. Instead, 25HC blocked the maturation of N-linked glycans on the viral glycoprotein subunits, rendering them more sensitive to digestion with Endo-H than the untreated forms; the majority of cell-associated GPC, GP1, and GP2 glycoproteins were sensitive to Endo-H in our experiments, but the sensitivity of GP2 to Endo-H was not affected by 25HC treatment. Although we did not extensively investigate these apparent differences, the more extensive glycosylation of GP1 (GP1 has 7 N-glycan sites, versus 4 in GP2) (10) might facilitate the detection of changes in GP1 glycosylation after Endo-H treatment, while changes in GP2 are harder to detect. Since Endo-H only removes immature N-glycans that are rich in mannose residues, the addition and/or trimming of N-glycans might be crucial for 25HC-induced reduction of LASSV infectivity.

Indeed, abnormal N-glycan maturation due to changing the SKI-/S1P cleavage motif (RSLK) to a furin motif (RRKR) in Junin virus, a New World arenavirus, resulted in reduced viral spread, suggesting that glycan maturation might affect arenavirus infectivity in general (25). The aberrantly glycosylated GP1 was readily incorporated into viral particles, though the ability of these particles to bind alpha-DG was significantly compromised. Although inhibitors of trimming oligomannosyl-rich chains had no effect on the production of infectious LCMV, another arenavirus that uses alpha-DG as a receptor (26), the loss of N-glycans in GP1 of LCMV has been associated with reduction in infectivity (11). Mutating 3 of the 4 N-glycans of LCMV GP1 produces noninfectious virulike particles without altering GPC processing, suggesting that GP1’s glycan composition can be critical for the infectivity of other arenaviruses (11). Therefore, we propose that the inhibition of LASSV infectivity by 25HC is at least partly due to inefficient binding of the aberrantly glycosylated GP1 to alpha-DG.

The mechanism by which 25HC induces changes in the GP1 glycans is currently unknown, but we speculate that Golgi-complex and trans-Golgi network-associated glycosidases and/or mannosidases involved in GP1 maturation might be affected by 25HC. Since the conversion of high-mannose oligosaccharide to a complex type occurs in the medial to trans-Golgi network (27), our data suggest that LASSV GP1 is transported to the cis-Golgi compartment. This is consistent with our findings that 25HC did not affect LASSV GPC cleavage, a process that occurs in the endoplasmic reticulum (ER) and/or cis-Golgi compartment. Accordingly, blocking the complex-N-glycan maturation of HIV glycoprotein GP120 by inhibiting mannosidase I has been linked to
reduced virion infectivity without changes in GP160 cleavage (28). Alternatively, a 25HC-induced alteration of normal transport of GPC or its cleaved products might affect the extent of GP1 glycan maturation, although the release of LASV particles following 25HC treatment appeared unimpaired.

CH25H is an interferon-stimulated gene, and its expression is upregulated during infection with hepatitis C and influenza viruses (16, 29). How LASV infection upregulates CH25H is unknown, especially since LASV-infected human cells produce undetectable or very low levels of type-I IFN (30, 31). Further investigations are required to understand the mechanism of CH25H transcriptional activation by LASV. CH25H knockdown enhanced LASV titers and increased the levels of the ~42-kDa GP1 normally found in the virus. LASV containing the ~42-kDa GP1 had better receptor binding activity than LASV bearing the aberrantly glycosylated GP1 induced by 25HC. This suggests that the enhanced LASV titers seen after CH25H knockdown are probably due to increased LASV infectivity, as CH25H overexpression and 25HC inversely affected both virus titers and glycosylation. However, further experiments are necessary to rule out the possibility that CH25H depletion increases the release of viral particles.

While the exact mechanism by which CH25H inhibits GP1 glycosylation remains to be elucidated, our data demonstrate the importance of CH25H and the unexpected role of GP1 glycosylation in LASV infectivity. Future experiments are warranted to more precisely address the biochemical changes of glycans associated with 25HC treatment and the effect of these changes on LASV’s interactions with its cell surface receptor.

MATERIALS AND METHODS

Biosafety. All work with infectious virus was conducted in a BSL-4 laboratory at the Centers for Disease Control and Prevention (CDC, Atlanta, GA), following the guidelines of CDC standard operating procedures.

Cells, virus, plasmids, reagents, and antibodies. Vero-E6 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Life Technologies, Inc., Grand Island, NY, USA) supplemented with 10% (vol/vol) fetal calf serum (FCS, HyClone; Thermo Fisher Scientific, Waltham, MA, USA) and penicillin-streptomycin (Life Technologies, Inc.). In some samples, sterol-depleted medium (lipoprotein-deficient serum; Sigma-Aldrich, St. Louis, MO, USA) was used instead of medium with FCS. Huh7 cells were propagated in DMEM with 10% (vol/vol) FCS and 1 X nonessential amino acids (Life Technologies, Inc.). 25HC was from Sigma-Aldrich. LASV (Josiah strain) was from the CDC Viral Special Pathogens Branch reference collection; all work with LASV was done under BSL-4 conditions. The plasmid encoding LASV-GPC has been previously described (21, 32). Plasmids encoding Myc-tagged CH25H and Myc-tagged IFITM3 were purchased from Origene (Rockville, MD, USA). The following antibodies were used in this study: mouse monoclonal antibody against LASV NP (703079-Lassa Josiah HMAF), mouse monoclonal antibody against LASV GPC (52-74-7), monoclonal antibody against LASV GP2 (81001-52-2085-0006-BG12-alpha-LASV2G), and a mix of 5 monoclonal LASV antibodies for immunofluorescence (SPR628 anti-LASV 5-MAb mixture). The anti-Myc antibody was from Origene, and the mouse monoclonal antiactin antibody was from Sigma-Aldrich (St. Louis, MO, USA).

Transfection and infection. To determine the effects of 25HC on GP1 glycosylation in LASV GPC-transfected cells, Huh7 cells were plated at 2.5 X 10⁴ per well in 12-well plates. The following day, cells were transiently transfected with 2 μg of empty expression vector (pCMV6) or plasmid expressing Myc-tagged CH25H or Myc-tagged IFITM3 using TransIT-LT1 (Mirus). At 24 h posttransfection, cells were washed twice with DMEM and then retransfected with plasmid expressing LASV GPC as described above.

For LASV infection, Huh7 cells were plated at 5 X 10⁴ cells per well in 12-well plates. The next day, cells were treated with ethanol or 25HC in serum-free medium for 1 h. 25HC treatment was done in serum-free medium to enhance the effect of the drug by avoiding potential binding of 25HC to serum lipids and proteins. Cells were then infected with LASV at the indicated MOIs for 1 h. The virus inoculum was removed, and cells were washed with serum-free medium. Fresh medium containing 2% FCS and with or without 25HC was then added. To determine the effects of CH25H or IFITM3 on LASV infection, cells were transfected with either empty vector (pCMV6) or plasmid expressing Myc-tagged CH25H or Myc-tagged IFITM3 using TransIT-LT1 (Mirus) as described above. After 24 h, transfected cells were washed twice with DMEM and then infected with LASV at an MOI of 0.3.

Huh7 cells (2.5 X 10⁵ per well) were reverse transfected and then retransfected 48 h later using DharmaFect 1 (Thermo Fisher Scientific) and 100 nmol siRNA against CH25H or IFITM3 or control siRNA (ON-Target plus SMARTpool siRNA; Thermo Fisher Scientific). Seventy-two hours after the initial transfection, cells were infected with LASV at an MOI of 1 under serum-free conditions. After 1 h of virus adsorption, cells were washed 4 times with serum-free medium and then replated with DMEM containing 5% lipoprotein-deficient serum.

TCID₅₀ determination. Supernatants from LASV-infected cells were harvested at the indicated time points, and virus titrations were performed in Vero-E6 cells. Three days postinfection, the cells were fixed, permeabilized, and stained to visualize viral proteins. Endpoint viral titers were determined, and TCID₅₀ were calculated as described previously (21). The results represent the mean titers and standard deviations calculated from the results of 3 independent experiments.

Quantitative RT-PCR. Huh7 cells were infected with LASV for the indicated times, and then RNA was isolated from cells or from supernatants of infected cells using the MagMAX-96 total RNA isolation kit (Thermo Fisher Scientific). One-step qRT-PCR was performed using an Applied Biosystems 7500 real-time PCR system and primers specific for CH25H or IFITM3 from single-tube TaqMan assays (Thermo Fisher Scientific). The levels of target genes were normalized to the GAPDH levels and with or without 25HC was then added. To determine the effects of CH25H or IFITM3 on LASV infection, cells were transfected with either empty vector (pCMV6) or plasmid expressing Myc-tagged CH25H or Myc-tagged IFITM3 using TransIT-LT1 (Mirus) as described above. After 24 h, transfected cells were washed twice with DMEM and then infected with LASV at an MOI of 0.3.

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Western blotting. At the indicated times after transfection or infection, cell lysates were harvested by adding lysis buffer containing 50 mM NaCl, 5 mM EDTA, 1% NP-40, 0.1% SDS, and 0.5% sodium deoxycholate supplemented with a protease inhibitor cocktail (Sigma-Aldrich). Lysates from infected cells were gamma irradiated at 2 X 10⁶ rad using a high-energy ⁶⁰Co source. Proteins were electrophoretically separated on 4-to-12% NuPAGE gels (Invitrogen) and transferred to nitrocellulose membranes. The membranes were blocked for 1 h with buffer containing Tris-buffered saline, 0.1% Tween 20, and 5% nonfat dry milk and then probed overnight at 4°C with primary antibodies. The membranes were developed using horseradish peroxidase-conjugated secondary antibod-
ies and enhanced chemiluminescence. After the detection of primary antibodies, the membranes were stripped and reprobed with anti-influenza antibody as a loading control. The results shown are representative of 3 independent experiments.

Virion purification. Supernatants from ethanol- or 25HC-treated and LASF-infected cells were clarified by centrifuging at 15,000 × g for 30 min. Clarified supernatants were subjected to ultracentrifugation (100,000 × g for 90 min at 4°C) through a 20% sucrose cushion to collect LASF virions. Virions were suspended in 2× Western lysis buffer, gamma irradiated at 5 × 10⁷ rad using a high-energy 60Co source, and analyzed by Western blotting to detect GP1 and NP. Alternatively, virions were suspended in phosphate-buffered saline (PBS) and used for alpha-DG fusion protein binding enzyme-linked immunosorbent assay (ELISA) under BSL-4 conditions.

Lassa virions and alpha-DG–Fc fusion protein binding ELISA. A plasmid expressing a fusion protein consisting of the signal sequence from CD5, the ectodomain of alpha-DG (amino acids 30 to 430), and the Fc domain of human IgG1 was generated by cloning the alpha-DG sequence amplified from RNA extracted from HEK-293 cells into plasmid pSynlg120lg1g (obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH, from Eun-Chung Park and Brian Seed). The expression and purification of the DG3.5-Fc fusion protein were performed by GenScript (Piscataway, NJ). Briefly, the expression plasmid was transiently transfected into 293 cells. The fusion protein was captured from the culture supernatant by production of 25-hydroxycholesterol as a novel host restriction factor and a part of the primary innate immune responses against hepatitis C virus infection. J Virol 84:573–584. http://dx.doi.org/10.1128/JVI.01697-09.

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