Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Cell Host & Microbe
Short Review

Lipids in Innate Antiviral Defense

John W. Schoggins[1,*] and Glenn Randall[2,*]
[1]Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
[2]Department of Microbiology, The University of Chicago, Chicago, IL 60637, USA
*Correspondence: john.schoggins@utsouthwestern.edu (J.W.S.), grandall@bsd.uchicago.edu (G.R.)
http://dx.doi.org/10.1016/j.chom.2013.09.010

It is becoming apparent that infections by a major class of viruses, those with envelopes, can be inhibited during their entry at the step of fusion with cellular membranes. In this review, we discuss multiple innate immune mechanisms that have evolved to modify the lipid composition of cellular and viral membranes to inhibit virion fusion of enveloped viruses.

Introduction

Host cells have evolved numerous innate defenses against virus infection, including a collection of constitutively expressed antiviral restriction factors and a variety of specialized responses induced by viral infection. A major component of the induced innate immune response to viral infections is the activation of antiviral interferons (IFNs), which transcriptionally induce hundreds of interferon-stimulated genes (ISGs). ISGs have varied functions, including regulating the IFN response or, alternatively, encoding effector molecules that directly inhibit viral infection. Collectively, the ISG response facilitates clearance of virus from infected cells, establishes a protective antiviral state in uninfected cells, and promotes adaptive immune responses (Samuel, 2001).

ISGs are induced across numerous cell types and species; however, their functional characterization has been relatively limited. The ISG functions that have been characterized frequently target conserved aspects of virus infections. This includes ISGs that impact the integrity of nucleic acids, such as the 2′,5′-oligoisoadenylate synthetase (OAS) and ribonuclease (RNase) L pathway, adenosine deaminase RNA-specific (ADARs), and apiloprotein B mRNA editing enzyme catalytic polypeptides (APOBECs); protein translation, such as protein kinase R (PKR); and virion budding, such as bone marrow stromal cell antigen 2 (BST2)/Tetherin (Sadler and Williams, 2008). Functional screening approaches, including ISG overexpression and knockdown screens, have been used to catalog a broader range of putative IFN-induced antiviral effectors (Ablasser and Hornung, 2013; Fusco et al., 2013; Karki et al., 2012; Liu et al., 2012; Metz et al., 2012; Schoggins et al., 2011; Zhao et al., 2012). The respective functions of these newly defined antiviral effector ISGs will likely target an expanding repertoire of virus-host interactions.

Lipids facilitate all stages of the viral life cycle, including initial interactions of the virion with the host cell, envelope fusion, modification of cellular membranes to establish sites of replication and/or assembly, cellular metabolism, and the coordination of virion assembly and budding (reviewed in Heaton and Randall, 2011). As such, they are attractive targets for innate immune defenses and the development of therapeutics. For example, many viruses require fatty acid biosynthesis for their replication and assembly (reviewed in Chukkapalli et al., 2012). AMP kinase, which is not an ISG, limits viral replication by inhibiting the rate-limiting enzyme in fatty acid biosynthesis, acetyl coenzyme A (CoA) carboxylase (Moser et al., 2012). Indeed, one of the more extensively characterized ISGs, viperin, can modulate virus infections by impacting lipids in multiple ways. Human cytomegalovirus (HCMV) co-opts viperin to inhibit lipid β-oxidation, which in turn stimulates lipid biogenesis that supports virion envelopment. Viperin also inhibits isoprenoid synthesis, which disrupts lipid rafts and, in turn, limits virus infection that requires lipid rafts for virus entry or budding (reviewed in Seo et al., 2011).

A major lipid-dependent interaction in enveloped virus infection is the fusion of the virion lipid bilayer with a cellular membrane to release the viral genome into the cytoplasm (reviewed in Plemper, 2011). This can occur either at the plasma membrane or at distinct endosomal compartments, governed in part by the pH dependence of the viral fusion apparatus. Generically, fusion occurs when the interaction of the viral envelope protein(s) with the host cell produces conformational changes in the viral fusion protein that expose its fusion peptide and generate sufficient free energy to facilitate membrane fusion, which is inherently an energetically unfavorable event. In the case of viruses that fuse at the plasma membrane, conformational changes in the fusion protein that occur after receptor engagement are sufficient to drive fusion. For viruses that fuse in endosomal compartments, protonation of the fusion protein that occurs with acidifying endocytic pH frequently promotes fusion. Additionally, endosomal or lysosomal proteases that cleave the fusion protein or, alternatively, endosomal lipids and/or protein receptors can promote the fusion process (Plemper, 2011). Once the fusion protein has positioned the viral envelope in close proximity to the cellular membrane, hemifusion, in which the outer leaflets of the viral and cellular lipid bilayers fuse, can occur (Figure 1A). Following hemifusion, an early fusion pore forms, which then enlarges to a late fusion pore through which the viral nucelocapsid is released into the cytoplasm. Important for this review, the fusion process, and hemifusion in particular, is sensitive to the lipid composition of membranes, which affects membrane curvature and fluidity (Figure 1B). As such, the modulation of membrane lipid composition is a viable approach to inhibit virion fusion. In this review, we discuss a number of recent discoveries that characterize host restriction of viral infection, especially virion fusion, by modifying pathways that synthesize, process, or transport lipids to alter membrane composition.

Interferon Modulates Sterol Biosynthesis and 25-Hydroxycholesterol

In a recent study, microarray profiling of macrophages either treated with IFN-γ or infected with murine cytomegalovirus

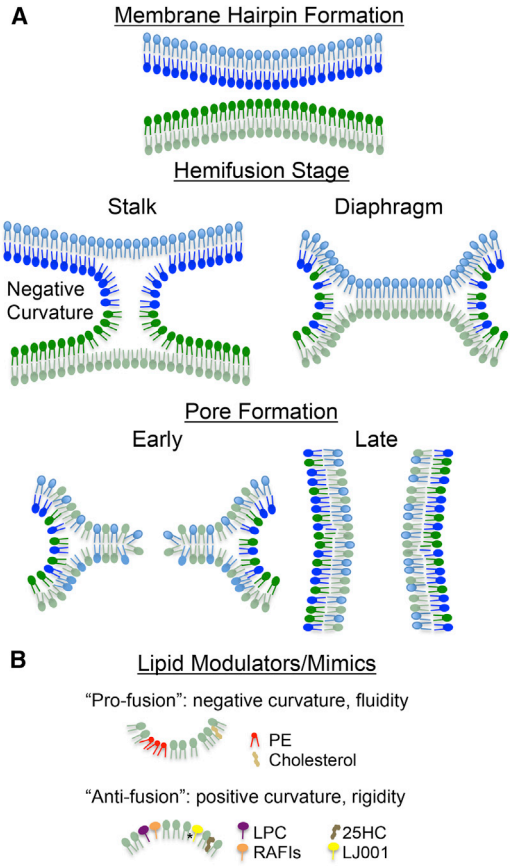

Figure 1. Model for Lipid Modulation of Membrane Fusion

(A) Viral fusion proteins (not shown) bring membranes in close apposition, thus promoting hairpin formation. Hemifusion then occurs, wherein the outer leaflets of each bilayer fuse and produce a stalk conformation induced by negative membrane curvature. This elongates into a diaphragm conformation in which the inner leaflets align, followed by pore formation, which results in the delivery of the nucleocapsid to the cytoplasm.

(B) Lipids that promote negative membrane curvature or membrane fluidity, such as PE and cholesterol, can facilitate fusion, while lipids that promote positive curvature or rigidity are inhibitory to fusion (reviewed in Teissier and Pécheur, 2007). *Fatty acid hydroxylation by LJ001.

(mCMV) identified repression of the sterol biosynthetic pathway as a significant component of IFN antiviral effects (Blanc et al., 2011). The consequence of repressed sterol biosynthetic gene expression was a reduction in levels of cellular cholesterol. The class of drugs known as statins impairs cholesterol synthesis by inhibiting HMG-CoA reductase, an enzyme required for synthesis of the cholesterol intermediate mevalonate. Cells treated with simvastatin were resistant to mCMV, and culturing cells with mevalonate, but not cholesterol, reversed this resistance. The silencing of either *Hmgcs1* or *Hmgcr*, two genes encoding enzymes in sterol biosynthesis, resulted in decreased mCMV infection. Collectively, these studies point to a role for the proximal mevalonate arm of sterol biosynthesis, but not cholesterol itself, in regulating mCMV infection (Figure 2). The authors hypothesize a requirement for the prenylation of viral and/or cellular proteins in mCMV infection. The repression of sterol biosynthesis by IFN appears to occur via the reduced expression of sterol regulatory element binding protein 2 (SREBP2), a transcriptional regulator of sterol biosynthesis. mCMV-infected mice treated with simvastatin had 1- to 1.5-log reductions in viral titers in spleen, liver, kidney, heart, and lungs.

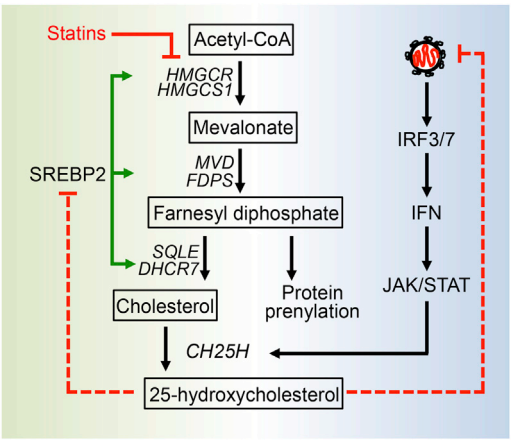

Figure 2. Intersection of Interferon Signaling and the Mevalonate Branch of Sterol Biosynthesis

Mevalonate synthesis has multiple downstream effects, including cholesterol synthesis, protein prenylation and geranylgeranylation, and synthesis of dolichols, other sterols, heme A, and ubiquinones (not shown). Cholesterol can be converted to 25-hydroxycholesterol by CH25H. Virus-mediated interferon induction leads to synthesis of CH25H in macrophages. Putative antiviral mechanisms (dashed red line) of 25HC include direct effects on virion fusion to the host cell and indirect effects mediated by modulation of SREBP2 target genes. Select SREBP2 target genes (green arrow) are shown in italics.

Further studies probing the antiviral mechanisms underlying these observations have defined a role for the oxysterol 25-hydroxycholesterol (25HC) as an antiviral sterol-lipid effector. 25HC is one of several naturally occurring oxysterols that are derived from cholesterol and contain a hydroxyl group on the side chain. Cholesterol 25-hydroxylase (*Ch25h* in mice, *CH25H* in humans) catalyzes the synthesis of 25HC from cholesterol. CH25H and 25HC are increasingly implicated in modulating host immunity at multiple levels. For example, 25HC has been shown to suppress the production of immunoglobulin A (IgA) by B cells, and *Ch25h*$^{-/-}$ mice have increased IgA levels in serum (Bauman et al., 2009). 25HC has also been implicated in suppressing the differentiation of monocytes to macrophages (Ecker et al., 2010). Dendritic cells and macrophages upregulate CH25H in response to type I IFNs, suggesting a potential role for 25HC in innate immunity (Park and Scott, 2010).

An overexpression screen designed to identify IFN-induced antiviral effectors uncovered an antiviral role for CH25H (Liu et al., 2012). Ectopic expression of CH25H in 293T cells conferred a strong antiviral effect against two enveloped viruses: the DNA virus murine gamma herpesvirus 6 (MHV68) and the RNA virus vesicular stomatitis virus (VSV). In a follow-up study, the same group characterized the mechanisms of CH25H antiviral activity (Liu et al., 2013). They used either shRNA-mediated knockdown of *Ch25h* or cells from *Ch25h*$^{-/-}$ mice to show that optimal control of VSV replication required *Ch25h*. In a series of media transfer experiments, it was demonstrated that CH25H expression produces a soluble antiviral factor that was not type I IFN. Thus, the authors examined the natural product of CH25H activity, 25HC. Indeed, exogenous addition of 25HC to cells suppressed VSV infection. Remarkably, a pan-antiviral

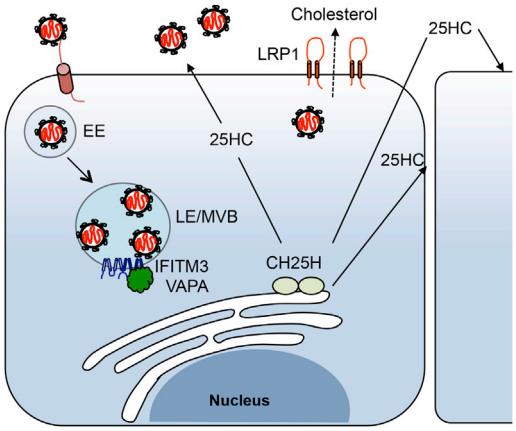

Figure 3. Model for Innate Antiviral Responses that Modify Lipid Compostition

Virus infection triggers multiple innate immune defenses modulating membrane composition, including IFITM3 homodimer formation and IFITM3 targeting of VAPA to LE and MVB to increase their cholesterol and LBPA levels, CH25H expression at the ER for 25HC synthesis and secretion, and plasma membrane accumulation of LRP1 to decrease intracellular cholesterol. This prevents virion hemifusion in endosomal compartments by modulating the lipid composition of viral and cellular membranes. EE, early endosome.

effect of 25HC was demonstrated against a panel of enveloped viruses including HIV-1, MHV68, herpes simplex virus 1 (HSV-1), ebolavirus, Rift Valley fever virus, and Nipah virus. 25HC had no effect on the nonenveloped adenovirus, suggesting that the antiviral effects may be specific to enveloped viruses, although other nonenveloped viruses remain to be characterized. The antiviral potency of 25HC was variable depending on the virus, with effects on viral titers ranging from 2-fold to more than 10-fold. The antiviral effects of 25HC on VSV infection were selective relative to other oxysterols and independent of other known 25HC functions, including the regulation of SREBP2, mevalonate production, or protein prenylation (Liu et al., 2013).

25HC did not impair VSV or HIV binding, but rather inhibited virus-cell fusion by affecting some property of the cellular membrane. In a cell-cell membrane fusion assay using only the Nipah virus fusion (F) and attachment (G) proteins, 25HC was shown to reduce syncytia formation. This surrogate assay suggests that similar mechanisms may be operating at the viral-host membrane interface. The antiviral effect of 25HC on VSV could be competed by incubating the 25HC-containing VSV inoculum with artificial liposomes containing lipid content similar to cellular membranes. These rescue experiments further suggest that 25HC directly alters cellular membranes, thereby interfering with virus entry (Figure 3). Lastly, 25HC inhibited HIV in a humanized mouse model, and *Ch25h*$^{-/-}$ mice were more susceptible to MHV68 than wild-type mice were, thereby solidifying the importance of this oxysterol pathway in innate antiviral immunity in vivo (Liu et al., 2013).

25HC was also uncovered as an IFN-induced antiviral molecule using a distinct metabolomics approach (Blanc et al., 2013). Liquid chromatography-mass spectrometry (LC-MS) analysis of oxysterols selectively identified 25HC as upregulated by mouse macrophages after either viral infection or IFN treatment. Similar to the results by Liu et al. (2013), the authors demonstrated that exogenous 25HC had antiviral effects against a panel of enveloped viruses, but no effect on adenovirus. To dissect the selectivity of 25HC, an enantiomer of 25HC, *ent*-25HC, was tested for effects on viral growth. This compound was also able to suppress viral infection, but only at high concentrations. The authors conclude that the enantioselectivity of 25HC antiviral action may suggest additional membrane-independent functions of 25HC. Although HSV-1 entry was inhibited by 25HC, other enveloped viruses were inhibited by 25HC at postentry steps. 25HC treatment of cells infected with mCMV, influenza A virus (IAV), or MHV68 resulted in a small plaque phenotype, whereas the total number of plaques was not impacted by 25HC. Thus, 25HC likely does not affect the infectivity of the viral inoculum, but rather impairs the ability of the virus to spread. This result is indicative of a postentry inhibition of these virus infections, possibly at the stage of cell-cell spread, as was shown by the authors for varicella zoster virus infection. The somewhat different conclusions of the two studies with regards to 25HC mechanism of action may represent differences in experimental conditions. For example, Blanc et al. (2013) used delipidated media in their studies, while Liu et al. (2013) used normal serum-complemented media. The differential effects of these media formulations in the context of antiviral mechanisms are unclear. Nonetheless, it is likely that 25HC can exert antiviral activity at multiple stages of the viral life cycle. In the case of mCMV infection, evidence was presented for both SREBP-dependent and SREBP-independent modes of 25HC antiviral action. Both studies implicate additional postentry roles for 25HC, including inhibition of virus-induced cell-cell fusion (syncytia formation) and virus spread. Additionally, 25HC has been found to interfere with the replication of HCV, possibly by altering the lipid composition of membranous replication compartments or protein prenylation (Pezacki et al., 2009). Similar mechanisms may be responsible for 25HC effects on viruses such as mCMV. Thus, 25HC has the attributes to modulate virus infections at many levels.

Interferon-Inducible Transmembrane Proteins 1–3

Interferon-inducible transmembrane proteins (IFITMs) 1–3 are part of a family of structurally related proteins that have basal expression in many cell types but are induced to higher levels of expression by IFNs. The various IFITMs exhibit inhibitory activity against multiple enveloped viruses prior to fusion, with their commonality being that the subset of inhibited viruses appears to share the same cellular location for virion fusion (reviewed in Diamond and Farzan, 2013). For example, IFITM3 inhibits the fusion of influenza A virus (IAV), dengue virus, and West Nile viruse, which fuse at the late endosome (LE), while IFITM1 preferentially inhibits ebolavirus and severe acute respiratory syndrome (SARS) coronavirus, which fuse at the lysosome (Brass et al., 2009; Feeley et al., 2011; Huang et al., 2011). IFITM3 is particularly important for the control of IAV in vivo. *IFITM3* knockout mice have enhanced pathology following IAV infection (Bailey et al., 2012). In humans, a rare *IFITM3* allele with reduced anti-iAV efficacy has been identified in hospitalized patients presenting with severe influenza (Bailey et al., 2012; Everitt et al., 2012). IFITMs generally do not restrict viral fusion at the plasma membrane or recycling endosome. In support of a model wherein the site of fusion determines IFITM antiviral activity, SARS coronavirus, which is typically restricted by IFITM1,

becomes resistant to restriction if it is pretreated with trypsin to enable plasma membrane fusion (Huang et al., 2011). IFITM proteins primarily localize to cytosolic vesicles, including LEs. These data have led to the hypothesis that IFITMs may modify either the pH or lipid composition of LEs and lysosomes to render them resistant to fusion. IFITMs, particularly IFITM1, can also localize to the plasma membrane following overexpression or interferon induction. Thus, IFITM1 can also efficiently inhibit cell-cell fusion (syncytia formation), HCV coreceptor interactions, and HIV replication at a postentry step (Li et al., 2013; Lu et al., 2011; Wilkins et al., 2013).

It was recently proposed that IFITMs inhibit viral hemifusion (Li et al., 2013). IFITM1, IFITM2, or IFITM3 expression blocked cell membrane hemifusion induced by all classes of viral fusion proteins. Altering the membrane composition with oleic acid, which induces negative membrane curvature favorable to hemifusion, overcame IFITM-mediated restriction of cell fusion. IFITM expression alters the biophysical properties of membranes. The membranes of IFITM-expressing cells have increased lipid packing (and thus decreased fluid dynamics), as assessed by fluorescence polarization approaches (Li et al., 2013). This alteration in membrane fluidity would inhibit hemifusion by increasing the free energy required to induce negative membrane curvature.

The mechanism by which IFITM3 prevents hemifusion has been proposed to require both homo- and heteromeric interactions. Mapping of IFITM3 topology using sites for posttranslational modifications suggests a model wherein the N and C termini are cytosolic and flank two intramembranous domains that are separated by a conserved cytosolic loop (Yount et al., 2012). The first intramembranous domain plus the conserved cytosolic loop compromise a CD225 domain, which is present in over 300 proteins. Mutagenesis studies show that the N-terminal cytosolic domain and the CD225 domain are essential for IFITM3 function (John et al., 2013). Amino acids in the intramembranous portion of the CD225 domain are required for IFITM self-association, leading to a model wherein IFITM3 homomeric interactions alter endosomal membrane fluidity and curvature (Figure 3).

Additionally, IFITM mechanism of action involves perturbation of intracellular lipid transport (Amini-Bavil-Olyaee et al., 2013). Vesicle-associated membrane protein (VAMP)-associated protein A (VAPA) was identified as an IFITM3-interacting protein using the yeast two-hybrid system. VAPA interacts with oxysterol-binding protein (OSBP) to transfer cholesterol from the endoplasmic reticulum (ER) to organelles and vesicles. IFITM1–IFITM3 all bind VAPA via the second transmembranous domain, and the binding of IFITM3 to VAPA interferes with the VAPA-OSBP interaction. IFITM3 overexpression altered cellular cholesterol levels, with increased accumulation of cholesterol and lysobisphosphatidic acid (LBPA) in LE and multivesicular body (MVB) compartments. Increased endosomal cholesterol and LBPA can perturb virion fusion (Chevallier et al., 2008). The IFITM3-VAPA interaction appeared to be important for inhibition of VSV and IAV entry. In the case of VSV infection, IFITM3 expression resulted in the accumulation of VSV in MVBs during the entry process, suggestive of a block in fusion. The authors propose a model wherein IFITMs bind VAPA and recruit it to endosomal compartments to transfer cholesterol and LBPA, creating a lipid environment that is resistant to virion fusion (Figure 3).

Low-Density Lipoprotein-Related Receptor 1

Low-density lipoprotein-related receptor 1 (LRP1) is an antiviral host protein that modulates virion lipid composition to impact the efficiency of viral fusion (Gudleski-O'Regan et al., 2012). It is a ubiquitously expressed plasma membrane protein that regulates cholesterol efflux and import. Its accumulation at the plasma membrane is transiently increased following HCMV infection and is associated with decreased intracellular cholesterol. Inhibition of LRP1 expression or function elevated intracellular cholesterol and enhanced infectious HCMV production (Gudleski-O'Regan et al., 2012). This effect is due to increased cholesterol in HCMV virions, leading to an enhanced specific infectivity. It was speculated that this increased infectivity is due to enhanced fusion, since HCMV fusion requires cholesterol. Taken together, the data suggest that LRP1 is induced shortly after infection to decrease intracellular cholesterol, thus altering the lipid composition of viral envelopes and decreasing their subsequent fusion capacity in a second round of infection (Figure 3).

Lipid Modulation as an Approach for Broad-Spectrum Antivirals

A number of compounds have been identified as potential broad-spectrum inhibitors of enveloped viruses. The proposed mechanisms of action for these compounds target different aspects of the fusion process. One of the better studied compounds is Arbidol, which is approved for influenza A and B virus treatment in Russia and China and has been proposed to modulate fusion protein interaction with phospholipids to prevent protein conformational changes required for fusion (Teissier et al., 2011). Squalamine, a cationic amphipathic sterol isolated from the dogfish shark, has antiviral activity in vitro and in vivo. It also has affinity for phospholipids and may perturb the fusion process (Zasloff et al., 2011).

The inhibition of viral fusion by modulating membrane lipid composition underscores the importance of the biophysical properties of virion envelopes for fusion. One antiviral approach is to design small molecules that resemble fusion inhibitory lysophospholipids. Synthetic compounds called rigid amphipathic fusion inhibitors (RAFIs), which are designed to physically resemble lysophospholipids, also show promise in inhibiting fusion with improved toxicity and pharmacokinetic profiles (St Vincent et al., 2010). These compounds insert into membranes and are predicted to produce positive membrane curvature in the outer leaflets, thereby inhibiting hemifusion. LJ001, a rhodanine derivative that is chemically distinct from RAFIs, also inhibits the replication of enveloped viruses by targeting the membrane component (Wolf et al., 2010). Interestingly, its mechanism of action is not simply based on mimicking a lipid shape, such as lysophosphatidylcholine (LPC). It intercalates lipid bilayers and acts as a photosensitizer, hydroxylating the fatty acid chains of unsaturated phospholipids (Vigant et al., 2013). This alters the physical properties of membranes, resulting in the inhibition of virion fusion.

Yet another approach to alter lipid composition for viral entry inhibition is to target cellular lipid synthesis or uptake pathways.

Neimann Pick C1 (NPC)-like 1 (NPCL1) is a cholesterol uptake receptor expressed on hepatocytes and enterocytes. Inhibition of its cholesterol uptake activity with the FDA-approved drug ezetimibe blocks HCV and HBV infection (Lucifora et al., 2013; Sainz et al., 2012). In the case of HCV, it inhibits entry prior to completion of fusion, in a cholesterol-dependent manner, although the specific mechanism of action is undefined (Sainz et al., 2012).

The inhibition of sterol biosynthesis also has potential for antiviral therapy. In particular, statins are generally well tolerated in patients and have broad-spectrum antiviral activity in vitro. Accumulating evidence suggests that they have some efficacy in treating HCV and IAV infections, either via antiviral activity or immunomodulatory effects (reviewed in Sheridan et al., 2013; Walsh, 2012).

Remaining Questions and New Roles for Lipids in the Antiviral Response

As the discoveries of these antiviral mechanisms that primarily target fusion are quite recent, many questions and exciting avenues for research remain. In the case of 25HC, at least four major questions remain. (i) As a secreted oxysterol that can permeate membranes, what is the primary target membrane to inhibit virion fusion? Since entry has already occurred in the infected cell, two nonexclusive possibilities remain: modification of neighboring cellular membranes in a paracrine manner or, alternatively, directly neutralizing intra- or extracellular virions. (ii) What is the chemical basis of 25HC fusion inhibition? Options include the exclusion of cholesterol and alterations of membrane fluid dynamics by 25HC lipid packing properties. (iii) What are the mechanisms of postfusion 25HC antiviral activity? As lipids are required at virtually every stage of the viral life cycle, there are multiple potential intervention points. Additionally, 25HC regulates sterol biosynthesis at multiple stages, suggesting potential indirect roles in antiviral regulation. (iv) 25HC has been shown to regulate both the innate and adaptive immune responses. How does 25HC integrate the two arms of the immune system to orchestrate an effective antiviral response? Given that LJ001 functions by hydroxylating fatty acid chains, one wonders whether increasing the hydroxylated lipid content in membranes is a more general approach to inhibiting viral fusion.

In the case of IFITMs, a remaining question is the basis for the differing antiviral properties of IFITM1–IFITM3. Do they individually recruit VAPA to distinct endosomal compartments? Do ISGs modulate lipid composition at other locations, such as the plasma membrane, to inhibit viral fusion? Finally, although most IFITM studies have focused on fusion, it remains possible that they may also inhibit subsequent events in the viral life cycle. The lipid composition of intracellular organelles is likely important for membrane remodeling to establish sites of replication for positive strand RNA viruses. HCV interacts with VAPA and requires VAPA and OSBP for its replication. Additionally, multiple viruses access MVBs as part of their egress and may be impacted by IFITMs. The inhibition of later stages of the viral life cycle would provide an antiviral role for IFN induction of IFITMs within the infected cell and not just in establishing an environment that is resistant to viral entry in naive cells.

Multiple questions remain regarding the role of LRP1 in viral infections. It is not clear what elevates LRP1 plasma membrane levels following HCMV infection and whether this is regulated by the IFN response. Although it was not investigated whether LRP1 alters the infectivity of other enveloped viruses, LRP1 is upregulated by HIV infection of T cells and in monocytes of long-term nonprogressors, suggesting that it may have broad-spectrum antiviral activity (Rasheed et al., 2008; Stebbing et al., 2003).

Finally, we anticipate an expanding appreciation of the roles of lipids in the antiviral response to infection, in terms of both directly inhibiting viral infection and regulating the adaptive and inflammatory responses. As an example, bioactive lipids from the 12/15 lipoxygenase pathway have recently been discovered to modulate IAV infection. Omega-3 polyunsaturated fatty acid (PUFA) lipid mediator D1 (PD1) inhibits IAV infection in cell culture (Morita et al., 2013). It acts at the stage of early gene expression, preventing the export of viral messenger and genomic RNAs by inhibiting their interaction with the nuclear export factor NXF1 by an unknown mechanism. PD1 levels are decreased during IAV infection in vivo, and its expression inversely correlates with disease severity. Tam et al. (2013) also identified 12/15 lipoxygenase-derived metabolites that differentially accumulate in response to IAV infection in vivo. Importantly, their accumulation correlates with both antiviral activity and resolution of inflammation. PD1 administration in IAV lung infection in mice reduced viral titers and improved survival, suggesting therapeutic potential. Thus, lipids have multiple roles in antiviral innate and adaptive responses, in control of inflammation, and potentially in the development of antiviral therapeutics.

ACKNOWLEDGMENTS

We thank David Russell and Vineela Chukkapalli for helpful discussions. Unfortunately, due to space limitations, we were unable to cite all relevant literature and apologize to those omitted. G.R. is supported by NIAID (AI080703 and AI102236), the American Cancer Society (118676-RSG-10-059-01-MPC), and Susan and David Sherman. J.W.S. is supported by NIDDK (DK095031).

REFERENCES

Ablasser, A., and Hornung, V. (2013). DNA sensing unchained. Cell Res. *23*, 585–587.

Amini-Bavil-Olyaee, S., Choi, Y.J., Lee, J.H., Shi, M., Huang, I.C., Farzan, M., and Jung, J.U. (2013). The antiviral effector IFITM3 disrupts intracellular cholesterol homeostasis to block viral entry. Cell Host Microbe *13*, 452–464.

Bailey, C.C., Huang, I.C., Kam, C., and Farzan, M. (2012). Ifitm3 limits the severity of acute influenza in mice. PLoS Pathog. *8*, e1002909.

Bauman, D.R., Bitmansour, A.D., McDonald, J.G., Thompson, B.M., Liang, G., and Russell, D.W. (2009). 25-Hydroxycholesterol secreted by macrophages in response to Toll-like receptor activation suppresses immunoglobulin A production. Proc. Natl. Acad. Sci. USA *106*, 16764–16769.

Blanc, M., Hsieh, W.Y., Robertson, K.A., Watterson, S., Shui, G., Lacaze, P., Khondoker, M., Dickinson, P., Sing, G., Rodríguez-Martín, S., et al. (2011). Host defense against viral infection involves interferon mediated down-regulation of sterol biosynthesis. PLoS Biol. *9*, e1000598.

Blanc, M., Hsieh, W.Y., Robertson, K.A., Kropp, K.A., Forster, T., Shui, G., Lacaze, P., Watterson, S., Griffiths, S.J., Spann, N.J., et al. (2013). The transcription factor STAT-1 couples macrophage synthesis of 25-hydroxycholesterol to the interferon antiviral response. Immunity *38*, 106–118.

Brass, A.L., Huang, I.C., Benita, Y., John, S.P., Krishnan, M.N., Feeley, E.M., Ryan, B.J., Weyer, J.L., van der Weyden, L., Fikrig, E., et al. (2009). The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus. Cell *139*, 1243–1254.

Chevallier, J., Chamoun, Z., Jiang, G., Prestwich, G., Sakai, N., Matile, S., Parton, R.G., and Gruenberg, J. (2008). Lysobisphosphatidic acid controls endosomal cholesterol levels. J. Biol. Chem. *283*, 27871–27880.

Chukkapalli, V., Heaton, N.S., and Randall, G. (2012). Lipids at the interface of virus-host interactions. Curr. Opin. Microbiol. *15*, 512–518.

Diamond, M.S., and Farzan, M. (2013). The broad-spectrum antiviral functions of IFIT and IFITM proteins. Nat. Rev. Immunol. *13*, 46–57.

Ecker, J., Liebisch, G., Englmaier, M., Grandl, M., Robenek, H., and Schmitz, G. (2010). Induction of fatty acid synthesis is a key requirement for phagocytic differentiation of human monocytes. Proc. Natl. Acad. Sci. USA *107*, 7817–7822.

Everitt, A.R., Clare, S., Pertel, T., John, S.P., Wash, R.S., Smith, S.E., Chin, C.R., Feeley, E.M., Sims, J.S., Adams, D.J., et al.; GenISIS Investigators; MOSAIC Investigators. (2012). IFITM3 restricts the morbidity and mortality associated with influenza. Nature *484*, 519–523.

Feeley, E.M., Sims, J.S., John, S.P., Chin, C.R., Pertel, T., Chen, L.M., Gaiha, G.D., Ryan, B.J., Donis, R.O., Elledge, S.J., and Brass, A.L. (2011). IFITM3 inhibits influenza A virus infection by preventing cytosolic entry. PLoS Pathog. *7*, e1002337.

Fusco, D.N., Brisac, C., John, S.P., Huang, Y.W., Chin, C.R., Xie, T., Zhao, H., Jilg, N., Zhang, L., Chevaliez, S., et al. (2013). A genetic screen identifies interferon-α effector genes required to suppress hepatitis C virus replication. Gastroenterology *144*, 1438–1449, e1–e9.

Gudleski-O'Regan, N., Greco, T.M., Cristea, I.M., and Shenk, T. (2012). Increased expression of LDL receptor-related protein 1 during human cytomegalovirus infection reduces virion cholesterol and infectivity. Cell Host Microbe *12*, 86–96.

Heaton, N.S., and Randall, G. (2011). Multifaceted roles for lipids in viral infection. Trends Microbiol. *19*, 368–375.

Huang, I.C., Bailey, C.C., Weyer, J.L., Radoshitzky, S.R., Becker, M.M., Chiang, J.J., Brass, A.L., Ahmed, A.A., Chi, X., Dong, L., et al. (2011). Distinct patterns of IFITM-mediated restriction of filoviruses, SARS coronavirus, and influenza A virus. PLoS Pathog. *7*, e1001258.

John, S.P., Chin, C.R., Perreira, J.M., Feeley, E.M., Aker, A.M., Savidis, G., Smith, S.E., Elia, A.E., Everitt, A.R., Vora, M., et al. (2013). The CD225 domain of IFITM3 is required for both IFITM protein association and inhibition of influenza A virus and dengue virus replication. J. Virol. *87*, 7837–7852.

Karki, S., Li, M.M., Schoggins, J.W., Tian, S., Rice, C.M., and MacDonald, M.R. (2012). Multiple interferon stimulated genes synergize with the zinc finger antiviral protein to mediate anti-alphavirus activity. PLoS ONE *7*, e37398.

Li, K., Markosyan, R.M., Zheng, Y.M., Golfetto, O., Bungart, B., Li, M., Ding, S., He, Y., Liang, C., Lee, J.C., et al. (2013). IFITM proteins restrict viral membrane hemifusion. PLoS Pathog. *9*, e1003124.

Liu, S.Y., Sanchez, D.J., Aliyari, R., Lu, S., and Cheng, G. (2012). Systematic identification of type I and type II interferon-induced antiviral factors. Proc. Natl. Acad. Sci. USA *109*, 4239–4244.

Liu, S.Y., Aliyari, R., Chikere, K., Li, G., Marsden, M.D., Smith, J.K., Pernet, O., Guo, H., Nusbaum, R., Zack, J.A., et al. (2013). Interferon-inducible cholesterol-25-hydroxylase broadly inhibits viral entry by production of 25-hydroxycholesterol. Immunity *38*, 92–105.

Lu, J., Pan, Q., Rong, L., He, W., Liu, S.L., and Liang, C. (2011). The IFITM proteins inhibit HIV-1 infection. J. Virol. *85*, 2126–2137.

Lucifora, J., Esser, K., and Protzer, U. (2013). Ezetimibe blocks hepatitis B virus infection after virus uptake into hepatocytes. Antiviral Res. *97*, 195–197.

Metz, P., Dazert, E., Ruggieri, A., Mazur, J., Kaderali, L., Kaul, A., Zeuge, U., Windisch, M.P., Trippler, M., Lohmann, V., et al. (2012). Identification of type I and type II interferon-induced effectors controlling hepatitis C virus replication. Hepatology *56*, 2082–2093.

Morita, M., Kuba, K., Ichikawa, A., Nakayama, M., Katahira, J., Iwamoto, R., Watanebe, T., Sakabe, S., Daidoji, T., Nakamura, S., et al. (2013). The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza. Cell *153*, 112–125.

Moser, T.S., Schieffer, D., and Cherry, S. (2012). AMP-activated kinase restricts Rift Valley fever virus infection by inhibiting fatty acid synthesis. PLoS Pathog. *8*, e1002661.

Park, K., and Scott, A.L. (2010). Cholesterol 25-hydroxylase production by dendritic cells and macrophages is regulated by type I interferons. J. Leukoc. Biol. *88*, 1081–1087.

Pezacki, J.P., Sagan, S.M., Tonary, A.M., Rouleau, Y., Bélanger, S., Supekova, L., and Su, A.I. (2009). Transcriptional profiling of the effects of 25-hydroxycholesterol on human hepatocyte metabolism and the antiviral state it conveys against the hepatitis C virus. BMC Chem. Biol. *9*, 2.

Plemper, R.K. (2011). Cell entry of enveloped viruses. Curr Opin Virol *1*, 92–100.

Rasheed, S., Yan, J.S., Lau, A., and Chan, A.S. (2008). HIV replication enhances production of free fatty acids, low density lipoproteins and many key proteins involved in lipid metabolism: a proteomics study. PLoS ONE *3*, e3003.

Sadler, A.J., and Williams, B.R. (2008). Interferon-inducible antiviral effectors. Nat. Rev. Immunol. *8*, 559–568.

Sainz, B., Jr., Barretto, N., Martin, D.N., Hiraga, N., Imamura, M., Hussain, S., Marsh, K.A., Yu, X., Chayama, K., Alrefai, W.A., and Uprichard, S.L. (2012). Identification of the Niemann-Pick C1-like 1 cholesterol absorption receptor as a new hepatitis C virus entry factor. Nat. Med. *18*, 281–285.

Samuel, C.E. (2001). Antiviral actions of interferons. Clin. Microbiol. Rev. *14*, 778–809.

Schoggins, J.W., Wilson, S.J., Panis, M., Murphy, M.Y., Jones, C.T., Bieniasz, P., and Rice, C.M. (2011). A diverse range of gene products are effectors of the type I interferon antiviral response. Nature *472*, 481–485.

Seo, J.Y., Yaneva, R., and Cresswell, P. (2011). Viperin: a multifunctional, interferon-inducible protein that regulates virus replication. Cell Host Microbe *10*, 534–539.

Sheridan, D.A., Neely, R.D., and Bassendine, M.F. (2013). Hepatitis C virus and lipids in the era of direct acting antivirals (DAAs). Clin Res Hepatol Gastroenterol *37*, 10–16.

St Vincent, M.R., Colpitts, C.C., Ustinov, A.V., Muqadas, M., Joyce, M.A., Barsby, N.L., Epand, R.F., Epand, R.M., Khramyshev, S.A., Valueva, O.A., et al. (2010). Rigid amphipathic fusion inhibitors, small molecule antiviral compounds against enveloped viruses. Proc. Natl. Acad. Sci. USA *107*, 17339–17344.

Stebbing, J., Gazzard, B., Kim, L., Portsmouth, S., Wildfire, A., Teo, I., Nelson, M., Bower, M., Gotch, F., Shaunak, S., et al. (2003). The heat-shock protein receptor CD91 is up-regulated in monocytes of HIV-1-infected "true" long-term nonprogressors. Blood *101*, 4000–4004.

Tam, V.C., Quehenberger, O., Oshansky, C.M., Suen, R., Armando, A.M., Treuting, P.M., Thomas, P.G., Dennis, E.A., and Aderem, A. (2013). Lipidomic profiling of influenza infection identifies mediators that induce and resolve inflammation. Cell *154*, 213–227.

Teissier, E., and Pécheur, E.I. (2007). Lipids as modulators of membrane fusion mediated by viral fusion proteins. Eur. Biophys. J. *36*, 887–899.

Teissier, E., Zandomeneghi, G., Loquet, A., Lavillette, D., Lavergne, J.P., Montserret, R., Cosset, F.L., Böckmann, A., Meier, B.H., Penin, F., and Pécheur, E.I. (2011). Mechanism of inhibition of enveloped virus membrane fusion by the antiviral drug arbidol. PLoS ONE *6*, e15874.

Vigant, F., Lee, J., Hollmann, A., Tanner, L.B., Akyol Ataman, Z., Yun, T., Shui, G., Aguilar, H.C., Zhang, D., Meriwether, D., et al. (2013). A mechanistic paradigm for broad-spectrum antivirals that target virus-cell fusion. PLoS Pathog. *9*, e1003297.

Walsh, E.E. (2012). Statins and influenza: can we move forward? J. Infect. Dis. *205*, 1–3.

Wilkins, C., Woodward, J., Lau, D.T., Barnes, A., Joyce, M., McFarlane, N., McKeating, J.A., Tyrrell, D.L., and Gale, M., Jr. (2013). IFITM1 is a tight junction protein that inhibits hepatitis C virus entry. Hepatology *57*, 461–469.

Wolf, M.C., Freiberg, A.N., Zhang, T., Akyol-Ataman, Z., Grock, A., Hong, P.W., Li, J., Watson, N.F., Fang, A.Q., Aguilar, H.C., et al. (2010). A broad-spectrum antiviral targeting entry of enveloped viruses. Proc. Natl. Acad. Sci. USA *107*, 3157–3162.

Yount, J.S., Karssemeijer, R.A., and Hang, H.C. (2012). S-palmitoylation and ubiquitination differentially regulate interferon-induced transmembrane protein 3 (IFITM3)-mediated resistance to influenza virus. J. Biol. Chem. *287*, 19631–19641.

Zasloff, M., Adams, A.P., Beckerman, B., Campbell, A., Han, Z., Luijten, E., Meza, I., Julander, J., Mishra, A., Qu, W., et al. (2011). Squalamine as a broad-spectrum systemic antiviral agent with therapeutic potential. Proc. Natl. Acad. Sci. USA *108*, 15978–15983.

Zhao, H., Lin, W., Kumthip, K., Cheng, D., Fusco, D.N., Hofmann, O., Jilg, N., Tai, A.W., Goto, K., Zhang, L., et al. (2012). A functional genomic screen reveals novel host genes that mediate interferon-alpha's effects against hepatitis C virus. J. Hepatol. *56*, 326–333.