Canadian Society of Microbiologists’ Armand-Frappier Gold Medal: The emerging role of 25-hydroxycholesterol in innate immunity

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Abstract:
The metabolic interplay between hosts and viruses plays a crucial role in determining the outcome of viral infection. Viruses re-orchestrate the host’s primary metabolic gene networks, including genes associated with mevalonate and isoprenoid synthesis, to acquire the necessary energy and structural components for their viral life cycles. Recent work has demonstrated that the interferon-mediated antiviral response suppresses the sterol pathway through production of a signalling molecule, 25-hydroxycholesterol (25HC). This oxysterol has been shown to exert multiple effects, both through incorporation into host cellular membranes as well as through transcriptional control. Herein we summarize our current understanding of the multi-functional roles of 25HC in the mammalian innate antiviral response.

Keywords:
25-hydroxycholesterol, cholesterol-25-hydroxylase, metabolism, virus, immunity
Introduction:

The importance of different lipid components in physiological processes has been studied for over fifty years. In addition to acting as important structural components (Holthuis and Menon 2014), energy stores (Walther and Farese 2012), and post-translational modifications of proteins (Resh 2006), it is becoming increasingly apparent that lipids play important roles as signalling molecules in many biological processes. Since the initial discovery of the eicosanoid prostaglandin as a stimulator of vasodilation (von Euler 1936), numerous additional families of lipid metabolites have been identified as cellular signals, including phosphoinositides, sphingolipids, and fatty acids. Aberrant regulation of these lipid signalling pathways has been linked to several diseased states, such as cancer, chronic inflammation, and metabolic syndrome (reviewed in (Wymann and Schneiter 2008)). Therefore, regulation of the biogenesis, modification, and catabolism of these lipid signalling molecules plays a critical role in the maintenance of physiological processes.

Recent work has highlighted oxidized forms of cholesterol, oxysterols, as important signalling molecules. One class of oxysterols, 25-hydroxycholesterols (25HCs), has been long-established as potent modulators of cellular metabolism (Brown and Goldstein 1974; Kandutsch and Chen 1975). However, until recently, a clear physiological role has yet to be ascribed to 25HC. Recent work has highlighted a dynamic interplay between host metabolism and innate immunity (Schoggins and Randall 2013). Viruses and other pathogenic organisms (Stehr et al. 2012) perturb the metabolic flux of their host to mediate their proliferation. For example, Su, Pezacki et al. demonstrated that the early host response to hepatitis C virus (HCV) infection involves significant modulated expression of host genes associated with lipid metabolism (Su et al. 2002). Hence, cellular metabolism represents a strategic target pathway for modulation during an
antiviral response. Ghazal and coworkers observed a striking down-regulation in sterol biosynthesis pathway-associated gene expression during the innate antiviral response (Blanc et al. 2013). Subsequent work has elucidated 25HC as the key intermediate signalling molecule which is produced in response to viral infection to mediate this antiviral metabolic effect (Blanc et al. 2013; Liu et al. 2013). This review will summarize our knowledge of the oxysterol’s multi-faceted influence on cellular metabolism as it pertains to intrinsic antiviral immunity.

25HC: oxysterol regulator of sterol homeostasis

Cholesterol and its derivatives represent one of the most abundant lipid species in the human body. Sterol metabolites form a crucial component of cellular membranes and lipid droplets; in addition, farnesylpyrophosphate and geranylpyrophosphate, intermediates from the mevalonate pathway, act as substrates for protein prenylation, an important post-translational modification (Nguyen et al. 2010). Therefore, maintenance of sterol homeostasis represents a critical cellular process. The sterol regulatory element-binding proteins (SREBPs) family of transcription factors are master regulators of lipid biosynthesis. Specifically, SREBP2 promotes expression of genes associated with the mevalonate pathway. There exists a well-established negative feedback mechanism regulating cholesterol biosynthesis, including expression of HMG-CoA reductase (HMGCR), the rate-limiting enzyme (Brown and Goldstein 1980). Newly translated SREBPs bind the SREBP cleavage activating protein (SCAP) at the endoplasmic reticulum (ER). In sterol-depleted conditions, this SREBP/SCAP complex is transported to the Golgi, where SREBP is sequentially cleaved by site-1 protease (S1P) and site-2 protease (S2P) into its mature transcription factor form (Figs. 1a–c). In the presence of excess lipids, cholesterol induces a conformational change in SCAP, which causes binding to INSIGs (Adams et al. 2004), thereby anchoring the SREBP/SCAP complex in the ER and preventing maturation of the transcription
factor. This negative feedback mechanism was one of the first characterized regulatory 
mechanisms of the sterol pathway (Brown and Goldstein 1999).

Prior to 25HC’s discovery as an antiviral lipid effector, it was a well-established suppressor of 
the sterol pathway. Over 40 years ago, it was discovered that oxidized forms of cholesterol, 
such as 25HC and 7-ketocholesterol, were able to suppress cholesterol biosynthesis over 100 
times more potently than unoxidized cholesterol (Brown and Goldstein 1974; Kandutsch and 
Chen 1975). Subsequent work delineated that cholesterol and oxysterols have independent 
modes of inhibition of sterol synthesis (Adams et al. 2004) (Fig. 1d). Similar to cholesterol, 
25HC promotes ER retention of the SREBP/SCAP complex. However, in vitro analysis revealed 
that 25HC did not directly interact with SCAP (Adams et al. 2004). Given that cells 
overexpressing INSIG were more sensitive to oxysterol-mediated inhibition of the sterol 
biosynthesis pathway, this suggested that INSIGs were involved in 25HC’s mode of action. This 
was subsequently confirmed by work from Goldstein, Brown and colleagues, which revealed that 
INSIG2 directly binds 25HC and not cholesterol (Radhakrishnan et al. 2007). Binding of 25HC 
was shown to induce a conformational change in INSIG2, which promotes binding to SCAP and 
ER retention of SREBPs. Thus, 25HC has been established as a potent repressor of SREBP 
activation.

Independent of its effects on SREBPs, high cellular 25HC levels induce rapid proteasomal 
degradation of HMGCR (DeBose-Boyd 2008; Lu et al. 2015) (Figs. 1f). Russell-Boyd and 
coworkers demonstrated that 25HC triggers binding of HMGCR to INSIGs, which results in the 
accelerated degradation of HMGCR through the ubiquitin-proteasome system (Song et al. 2005). 
TRC8, MARCH6, and gp78 were identified as three key membrane-anchored E3 ubiquitin-
protein ligases involved in 25HC stimulated HMGCR degradation (Jo et al. 2011; Song et al.
Subsequently, HMGCR is extracted from the ER membrane into the cytosol in a VCP/p97-dependent manner, enabling HMGCR degradation by the 26S proteasome (Elsabrouty et al. 2013; Morris et al. 2014). Through this mechanism, 25HC inhibits the rate-limiting step of cholesterol biosynthesis.

The liver X receptors (LXRs) represent another class of transcription factors with pivotal roles in the regulation of cellular lipid homeostasis. In vitro studies suggest oxysterols, including 25HC, represent potent endogenous agonists for the LXR pathway (Janowski et al. 1996; Lehmann et al. 1997). In the presence of excess sterols, the LXR pathway is a feed-forward pathway that promotes cholesterol efflux and bile synthesis for sterol excretion (Lehmann et al. 1997; Peet et al. 1998). 25HC has also been shown to promote cholesterol esterification (Brown et al. 1975; Du et al. 2004), which may result in trafficking of cholesterol from membranes to lipid droplets. Overall, 25HC’s multiple modes of action, highlighted here, should cooperate to decrease cellular sterol content.

**Cholesterol-25-hydroxylase: an interferon stimulated gene**

25HC is synthesized by cholesterol-25-hydroxylase (CH25H), an ER-localized multi-transmembrane protein expressed in vertebrates (Lund et al. 1998). The enzyme hydroxylates cholesterol in an NADPH-dependent manner. Given the modulatory activity of 25HC in multiple lipid pathways, one would expect CH25H knockout mice to exhibit severe metabolic defects in tolerating high fat diets. While triple-knockout mice for the enzymes synthesizing 24S-HC, 27HC, and 25HC demonstrated disrupted LXR signalling (Chen et al. 2007), CH25H deficient mice displayed no aberrant metabolic phenotypes when fed high-fat diets. Collectively, this suggested that these different endogenous oxysterol species have redundant functions in
metabolism. Taken together with the observed low CH25H expression levels in lipogenic tissues (Lund et al. 1998), it also pointed to the likelihood that CH25H (and 25HC) possessed functions independent of its metabolic effects.

The first studies highlighting a potential role for CH25H in innate immunity demonstrated that ligand activation of the Toll-like receptor (TLR) family of pathogen recognition receptors induced CH25H expression and 25HC secretion in mouse macrophages and dendritic cells (DCs) (Bauman et al. 2009; Diczfalusy et al. 2009; Park and Scott 2010). Similar increases in CH25H expression were observed in influenza virus infected human airway epithelial cells (Gold et al. 2014). TLR activation results in the expression of a family of antiviral cytokines, interferons (IFN), which promotes antiviral gene programming through the Jak/STAT pathway. CH25H expression was demonstrated to be induced by IFN-α and IFN-γ in a Jak1- and STAT1-dependent manner (Blanc et al. 2013; Liu et al. 2013; Park and Scott 2010). Subsequent work demonstrated that interferon regulatory factor 1 (IRF1) cooperates with STAT1 to positively regulate CH25H transcription in mouse primary macrophages (Mboko et al. 2014). Conversely, activating transcription factor 3 (ATF3) (Gold et al. 2012), a known inhibitor of TLR-induced cytokine production, represses CH25H expression. Collectively, these studies pointed towards a role for CH25H-catalyzed 25HC production in the intrinsic pathogen response.

**25HC: broad antiviral lipid effector**

There is a broad viral requirement for specific lipid microenvironments to facilitate various stages of viral life cycles (Chukkapalli et al. 2012). Several viruses require altered membrane lipid composition for the formation of replication complexes and lipid envelopes, as well as efficient viral entry and egress. This broad dependence on lipid metabolism is further
exemplified by the fact that small molecule inhibitors of both cholesterol and fatty acid biosynthesis have been demonstrated to elicit antiviral effects against several classes of virus (Blanc et al. 2011; Lyn et al. 2014; Mazièrel et al. 1994; Moser et al. 2012; Munger et al. 2008; Pezacki et al. 2009; Sagan et al. 2006; Su et al. 2002). Prior to the discovery of its role in innate immunity, 25HC was similarly demonstrated to possess broad antiviral activity. The first evidence of 25HC’s antiviral activity derived from the observation that 25-HC and cholesterol treated cells had decreased permissiveness to rhinovirus entry due to down-regulated low density lipoprotein receptor (LDLR) expression (Hofer et al. 1994). Subsequent in vitro studies displayed 25HC’s antiviral effect against viral replication in human immunodeficiency virus (HIV) (Moog et al. 1998), HCV (Pezacki et al. 2009; Su et al. 2002), and West Nile virus (WNV) (Mackenzie et al. 2007) models. Collectively, this work established 25HC as a broad antiviral oxysterol.

Two recent companion studies elegantly established CH25H-mediated 25HC production as a critical arm of the mammalian innate antiviral response. Blanc et al. performed supernatant oxysterol profiling of interferon-stimulated and virus-infected mouse macrophages – revealing 25HC as the only secreted and intracellular oxysterol that was up-regulated (Blanc et al. 2013). In parallel, Liu et al. also confirmed 25HC as a soluble antiviral factor produced during IFN stimulation of macrophages (Liu et al. 2013). Both studies confirmed broad antiviral activity of 25HC in vitro against different classes of lipid enveloped RNA and DNA viruses. This was subsequently reaffirmed by several independent studies (Arita et al. 2013; Chen et al. 2014; Mboko et al. 2014), and the antiviral activity was additionally extended to non-enveloped viruses (Civra et al. 2014). Table 1 summarizes the viruses whose susceptibility to 25HC’s antiviral
effects has been validated. Macrophage and dendritic cell secretion of 25HC should enable both paracrine and autocrine effects, suggesting a more systemic antiviral role for 25HC.

Limited *in vivo* studies have been performed examining 25HC’s antiviral role. To date, only two studies have examined the oxysterol’s relevance to innate immunity *in vivo*. CH25H *^/-* mice were demonstrated to possess increased susceptibility to MHV68 infection (Liu et al. 2013). The same study demonstrated that 25HC treatment of humanized mice prior to infection inhibits HIV replication (Liu et al. 2013). In a separate conflicting report, deletion of CH25H resulted in no significant change in influenza A viral loads (Gold et al. 2014). These contradictory results suggest a potential virus-specific antiviral effect of 25HC *in vivo*. They also highlight the need for further small animal studies to delineate 25HC’s physiological relevance in the innate antiviral response.

**25HC: multiple potential modes of action**

25HC appears to exert effects in a multitude of signalling pathways, including the aforementioned SREBP and LXR signalling pathways. While SREBP signalling appeared to contribute to 25HC’s viral inhibition, these antiviral effects appear to occur in an LXR-independent manner (Blanc et al. 2013). Below, we discuss the contribution of repressed SREBP signalling and other pathways to 25HC’s effect on the host response to viral infection.

**Figure 2** summarizes these effects in the context of HCV infection. The relevance of each of these modes of inhibition appears to be context-specific, depending on the virus as well as on the pre-existing cellular lipid environment.

*Modification of cellular membranes*
While previous work described an antiviral effect for 25HC at the stage of viral replication, Liu and coworkers demonstrated that 25HC also broadly inhibits viral entry (Liu et al. 2013). The authors demonstrated that longer pre-treatment of cells with 25HC results in strong inhibition of vesicular stomatitis virus (VSV) infection. This suggested that 25HC induces an antiviral cellular state prior to infection. After showing a lack of effect on virion attachment, 25HC was determined to inhibit VSV virion fusion to the host cell. This was attributed to changes in the cellular membrane properties, as 25HC treatment also inhibited Nipah virus (NiV) F and G protein-mediated cell-cell fusion. These results were also extended to HIV entry, demonstrating that 25HC modulates membrane properties to inhibit both pH-dependent (VSV) and pH-independent (HIV and NiV) modes of viral fusion. The efficiency of virion fusion is sensitive to host membrane lipid composition and fluidity. For example, HIV has been shown to require cholesterol-rich microdomains to facilitate viral entry (Carter et al. 2009). 25HC-mediated suppression of SREBP activation and induction of HMGCR degradation could, therefore, contribute to decreased rates of viral entry.

Furthermore, 25HC has been shown to modulate membrane fluidity and cholesterol accessibility (Bielska et al. 2014; Richert et al. 1984), which may contribute to the observed decrease in membrane fluidity in interferon treated cells (Chatterjee et al. 1982). Given that small molecule inhibitors of fusion demonstrate broad antiviral activity against enveloped viruses (St.Vincent et al. 2010), 25HC’s effect on the plasma and endosomal membranes represents a potential contributing mechanism to its antiviral effects. Similarly, modulation of intracellular membranes should result in an antiviral effect against positive RNA viruses, which are known to induce significant ER, Golgi, lysosome, or endosome membrane remodelling to create scaffolds that serve as sites of viral replication (Miller and Krijnse-Locker 2008). Direct integration of 25HC
into these intracellular membranes could restrict the ability of viruses to properly house their replication complexes, while indirect 25HC-induced changes in lipid components of both host membranes and virion envelopes could impair the viruses’ ability to infect bystander cells.

*Interactions with oxysterol binding proteins*

The oxysterol binding protein (OSBP) and OSBP related proteins (ORPs) represent another family of proteins which can interact with 25HC. This family of proteins bind and transfer sterols between cellular organelles, such as the Golgi and ER membranes (Mesmin et al. 2013). Interestingly, RNA viruses have been shown to co-opt OSBP and ORPs to facilitate assembly (Amako et al. 2009) and/or to shuttle and enrich cholesterol at sites of viral replication (Barajas et al. 2014; Strating et al. 2015; Wang et al. 2014). In fact, small molecule targeting of OSBP has shown broad-range efficacy in inhibition of enterovirus replication (Strating et al. 2015). In the absence of viral infection, 25HC relocalizes OSBP from the cytoplasm to the Golgi (Ridgway et al. 1992). This has been linked to 25HC’s antiviral effect towards rhinoviruses, where 25HC prevents OSBP-mediated shuttling of cholesterol to the membranous scaffolds of viral replication (Roulin et al. 2014). It remains unknown whether OSBP or any of the ORPs are capable of mediating transfer of 25HC directly to the membranes modified by viruses for replication and particle assembly.

*Repression of protein prenylation*

Prenylated host and viral proteins have also been implicated in the replication of viruses, including HCV, hepatitis delta virus, pseudorabies virus, and respiratory syncytial virus (Bordier et al. 2003; Gower and Graham 2001; Kapadia and Chisari 2005; Wang et al. 2005). Since repression of SREBP signalling should result in down-regulation of cellular farnesyl and
geranylgeranyl pyrophosphate levels, 25HC should elicit alterations in protein prenylation status, which may convey an alternative mechanism of viral inhibition. This is consistent with recent work demonstrating that supplementation with geranylgeraniol can rescue the antiviral effects of lower concentrations of 25HC against murine cytomegalovirus (Blanc et al. 2013).

Integrated stress response

Transcriptome profiling of 25HC-treated macrophages revealed that, independent of its effect on LXR and SREBP signalling, 25HC could activate genes associated with the integrated stress response (ISR) in macrophages (Shibata et al. 2014). Activation of this pathway was attributed to altered amino acid metabolism and increased oxidative stress and results in suppression of translation, which may contribute to 25HC’s broad antiviral activity.

Potentiation of interferon signalling

A recent study demonstrated that 25HC potentiates IFN-β expression in primary human bronchial epithelial cells stimulated with poly(I:C), a viral RNA analogue (Koarai et al. 2012). This was partially attributed to increased nuclear translocation of a key transcription factor, IFN regulatory factor 3 (IRF3). In addition, the study demonstrated that 25HC increased nuclear factor-kappa B (NF-κB) signalling, another key regulator of type I and III IFN expression (Ichikawa et al. 2013; Onoguchi et al. 2007). It remains to be seen whether this IFN/25HC-mediated feed-forward loop is conserved in other cell types and in vivo.

Effects on inflammation and adaptive immunity

In addition to its effects on innate immunity, recent studies have proposed a role for 25HC in the regulation of inflammation and adaptive immunity. Gold and colleagues identified pro-
inflammatory effects of CH25H-catalyzed 25HC production in mouse macrophages (Gold et al. 2014). This activation is mediated, in part, by increased recruitment of activator protein 1 (AP-1) transcription factor complex to the promoters of TLR-responsive genes. Accordingly, influenza A virus-infected CH25H knockout mice presented with lower morbidity due to decreased inflammation. Another 25HC-mediated pro-inflammatory effect may result from its activation of NF-kB signalling, resulting in increased release of interleukin (IL)-6 and IL-8 (Koarai et al. 2012; Rydberg et al. 2003). Type I IFN signalling has previously been linked to decreased inflammasome activity and production of the pro-inflammatory cytokine IL-1β; however, the ISG responsible for this effect on IL-1β signalling was unclear until a recent study observed increased IL-1β expression levels in CH25H knockout mice (Reboldi et al. 2014). The authors identified IL-1β expression as a SREBP target gene in macrophages, and CH25H as the key ISG responsible for IFN-mediated repression of IL-1β inflammasome activation. 25HC-mediated activation of LXR signalling may also negatively influence inflammation via LXR-mediated trans-repression (Joseph et al. 2003; Ogawa et al. 2005); however, it remains to be seen if this represents a physiologically relevant mechanism of action in vivo for 25HC. Collectively, these studies illustrate a complex role for 25HC in the regulation of inflammation.

There have also been a few studies investigating 25HC’s influence on adaptive immunity that have highlighted a repressive role for the oxysterol. Macrophage-secreted 25HC suppressed both immunoglobulin A (IgA) class switching in B cells as well as IL-2-stimulated B cell proliferation (Bauman et al. 2009). This repressive role for 25HC in adaptive immunity is also consistent with SREBP signalling’s critical role in CD8+ T cell clonal expansion during viral infection (Bensinger et al. 2008; Kidani et al. 2013).

**Future Outlook**
It should be noted that 25HC is metabolized by several other host enzymes; therefore, it is important to consider the roles of these other enzymes and modified oxysterols in the antiviral response. For example, CYP7B1 hydroxylates 25HC to form 7α25HC, an oxysterol which also plays an important role as a guidance cue for B cells through interaction with Epstein-Barr virus induced receptor 2 (EBI2) (reviewed in (Daugvilaite et al. 2014)). Recent work has also identified hydroxysterol sulfotransferase 2B1b (SULT2B1b) as the enzyme which catalyzes sulfation of 25HC to 25-hydroxycholesterol-3-sulfate (25HC3S) (Ren and Ning 2014). Studies have generally found the sulfated oxysterol to have effects opposing to 25HC on both inflammation and metabolism (Ren and Ning 2014). While metabolomic profiling revealed no significant increase in sulfated oxysterol levels during interferon stimulation of macrophages (Blanc et al. 2013), it remains to be seen if 25HC3S plays a role in the antiviral response in other cell types and tissues, such as the liver, where 25HC3S has been shown to be localized in the nuclei and mitochondria (Ren et al. 2006). Future work should consider the importance of 25HC as an intermediate metabolite for other oxysterols which may be key players in immunity.

In summary, our understanding of 25HC has rapidly evolved from its initial use as a research tool to its discovery as a natural product. To date, a large body of evidence has supported a key role for 25HC in regulating the crosstalk between metabolism and immunity, however, several questions remain. This IFN-regulated oxysterol has been clearly demonstrated to possess antiviral activity against several classes of viruses, however, we are only beginning to understand how 25HC elicits these broad antiviral effects. The physiological contribution of each of the 25HC linked pathways (LXR, SREBP, AP1, OSBP and ISR) to the oxysterol’s role in the antiviral response should be analyzed in vivo. The effect of 25HC on transcription has, thus far, been focused on coding genes. It remains to be seen whether the oxysterol elicits any antiviral
effects through modulation of the non-coding transcriptome. Furthermore, the majority of experiments looking at 25HC have been performed in both in vitro and in vivo mouse models. Patients with HCV chronic infection possess elevated serum levels of 25HC and other oxysterols (Ikegami et al. 2014), however, it is unclear if this was a direct effect of the virus, or a product of autoxidation or the metabolic disease associated with HCV infection (Ikegami et al. 2012). Future work should determine the relevance of CH25H and 25HC to human viral immunity.

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### Table 1: Summary of viruses responding to 25HC treatment

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<td>Gammaherpesvirus</td>
<td>(Mboko et al. 2014)</td>
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<td>Hepatitis C virus</td>
<td>(Chen et al.; Pezacki et al. 2009; Sagan et al.</td>
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<td>Herpes Simplex Virus-1</td>
<td>(Blanc et al. 2013; Liu et al. 2013)</td>
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<td>Human immunodeficiency virus</td>
<td>(Liu et al. 2013; Moog et al. 1998)</td>
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<td>Human papillomavirus-16</td>
<td>(Civra et al. 2014)</td>
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<tr>
<td>Human rotavirus</td>
<td>(Civra et al. 2014)</td>
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<td>Influenza A virus (H1N1)</td>
<td>(Blanc et al. 2013)</td>
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<td>Mouse cytomegalovirus</td>
<td>(Blanc et al. 2013)</td>
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<tr>
<td>Murine gamma herpes virus 68</td>
<td>(Blanc et al. 2013; Liu et al. 2013)</td>
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<td>Nipah virus</td>
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<td>Poliovirus</td>
<td>(Arita et al. 2013)</td>
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<td>Rhinovirus</td>
<td>(Civra et al.; Hofer et al. 1994; Roulin et al.)</td>
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<td>Rift Valley Fever Virus</td>
<td>(Liu et al. 2013)</td>
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<tr>
<td>Russian Spring-Summer Encephalitis Virus</td>
<td>(Liu et al. 2013)</td>
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<tr>
<td>Varicell Zoster Virus</td>
<td>(Blanc et al. 2013)</td>
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<td>Vesicular stomatitis virus</td>
<td>(Liu et al. 2013)</td>
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<tr>
<td>West Nile virus</td>
<td>(Mackenzie et al. 2007)</td>
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**Figure 1: Summary of 25HC’s effects on sterol homeostasis.** An overview of 25HC’s multiple modes of action in the sterol pathway. (A,B) In low sterol and oxysterol conditions, the SREBP/SCAP complex is transported to the Golgi, where SREBP is cleavage into its mature from cleavage by two proteases (S1P and S2P). (C) This mature form translocates to the nucleus, where it regulates genes associated with lipid metabolism. (D) In sterol- and oxysterol-rich conditions, cholesterol binds to SCAP, or 25HC binds to INSIG, inducing conformational changes which promote binding between SCAP and INSIG, retaining the SREBP/SCAP complex at the ER, preventing SREBP activation. (E) Lastly, high oxysterol levels acts as an agonist for LXR signalling, which regulates genes associated with cholesterol efflux and bile synthesis. (F) Lastly, 25HC also promotes association of INSIGs with HMGCR, the enzyme catalyzing the rate limiting step of cholesterol biosynthesis, and promotes its degradation via the ubiquitin-proteasome system.

**Figure 2: Overview of 25HC’s antiviral effects on HCV life cycle.** IFN-stimulated 25HC production has the potential to impair HCV infection at several stages of its viral life cycle. An overview of the HCV lifecycle is shown in the top left image. 25-HC has the potential to influence the virus’ life cycle at three main stages: entry and fusion, replication, and assembly. (A) At the stage of entry, 25HC decreases SREBP-regulated LDLR expression, a proposed entry receptor for the virus (Molina et al. 2007). Also, 25HC modulates the lipid composition of the plasma membrane, potentially modulating fluidity and impairing subsequent viral fusion. (B) Similarly, modulation of cellular membranes will influence HCV replication complex formation, which relies on significant ER membrane remodeling. The virus also depends on OSBP proteins...
as a key host factor for viral replication and virion maturation (Amako et al. 2009; Wang et al.).

25HC’s interaction with OSBP could interfere with the virus’ shuttling of cholesterol to replication complexes. HCV hijacks the geranylgeranylated host factor FBL2 for replication; however, this may be impaired due to 25HC’s effects on protein prenylation. The virus also relies on lipid droplets as a platform for assembly. There should be an overall decrease in cellular lipid content (and lipid droplets) due to 25HC’s inhibition of the SREBP signalling. (C)

25HC should also exert broad antiviral effects due to potentiation of IFN signalling and activation of the integrated stress response.