Significance of Cellular Lipid Metabolism for the Replication of Rotaviruses and Other RNA Viruses

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Review

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Department of Medicine, University of Cambridge Addenbrooke's Hospital, Cambridge CB2 0QQ, UK; ud207@medschl.cam.ac.uk; Tel.: +44-1223-763403; Fax: +44-1223-336846

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Abstract: The replication of species A rotaviruses (RVAs) and other RNA viruses involves recruitment of and interaction with the cellular organelles lipid droplets (LDs), both physically and functionally. Inhibition of enzymes involved in the cellular fatty acid biosynthesis pathway or of cellular lipases that degrade LDs reduces the functions of 'viral factories' (viroplasms for rotaviruses or replication compartments of other RNA viruses) and decreases the production of infectious progeny virus. Similarly, disturbance of the cellular lipid homeostasis in various ways blocks the replication of flaviviruses (hepatitis C viruses, Zikavirus, others), HIV-1, SARS-CoV-2, picornaviruses, noroviruses, influenza viruses and other negative-strand RNA viruses.

Keywords: lipid metabolism; RNA virus replication; Rotavirus; hepatitis C virus; flaviviruses; picornaviruses; noroviruses; HIV-1; SARS-CoV-2; influenza viruses

Introduction

While the replication cycles of many RNA and DNA viruses have been well researched, it has been recognized only relatively recently that various cellular lipids are involved in many of those steps (Herker, 2024; Qu et al, 2023; Farias et al, 2023; Madsen and Rossman, 2022; Li et al, 2022; Roingeard et al, 2022; Zhao et al, 2022; Theken et al, 2021; Husby and Stahelin, 2021; Crawford and Desselberger, 2016; Heaton and Randall, 2011) and also in the regulation of the host's innate immune responses (Farias et al, 2023; Theken et al, 2021). Cellular lipids, summarizingly termed the 'liposome', are classified as membrane lipids (phospholipids, sphingolipids, glycolipids), cholesterol, steroids, triacylglycerol (TAG), fatty acids, and eicosanoids (Fahy et al, 2011). Viruses interact with host cell lipids in various ways, affecting their biosynthesis and metabolism, and gaining energy for their own replication (Heaton and Randall, 2011). In the following, the significance of cellular lipids for the replication of rotaviruses will be reviewed in some detail. Analogous data have been acquired for many other RNA viruses. The fact that disturbance of the cellular lipid metabolism decreases the yield of infectious viral progeny for many viruses, makes the search for lipid-disturbing compounds an attractive aim for the development of broadly active candidate antivirals (Cesar-Silva et al, 2023; Qu et al, 2023; Farias et al, 2023; Li et al, 2022; Roingeard et al, 2022; Theken et al, 2021).

Rotaviruses

Species A rotaviruses (RVA) are a major cause of severe gastroenteritis in infants and young children worldwide and in the young of many mammalian and avian host species (Crawford et al, 2017). RVA-associated disease leads to the deaths of approximately 130.000 children under the age of 5 y annually worldwide, mainly in low-income countries (Troeger et al, 2019). Two RVA vaccines were licensed since 2006 and are now used in expanded programs of immunization (EPIs) in over 100 countries (Bergman et al, 2021). In addition, alternative RV vaccines were licensed in India, Vietnam and China (Bergman et al, 2021). RVA EPIs have significantly decreased RVA-associated disease, although at different levels in different parts of the world (Desselberger, 2017; Parker et al, 2018). The molecular biology of RVA replication has been well studied (Crawford et al, 2017). During

replication, 'viroplasms' form, which are intracytoplasmic inclusion bodies utilized for early RVA morphogenesis and viral RNA replication take place; they have been termed 'viral factories' and been recognized as essential (Crawford et al, 2017). The rotavirus-encoded components of viroplasms, NSP2 and NSP5, are subject to liquid-liquid phase separation (Geiger et al, 2021; Papa et al, 2020). In 2010 rotavirus viroplasms (containing VP1, VP2, VP3, VP6, viral RNA segments) were discovered to associate with components of the cellular organelles lipid droplets (LDs) (Cheung et al, 2010) [Figure 1]. LDs of various sizes (0.05-200 um in diameter) are the storage sites of sterol esters and neutral lipids which serve as the major cellular energy source and are in close contact with mitochondria and the endoplasmic reticulum (ER) from [PLINs], diacylglycerol acyl transferases 1 and 2 [DGAT1, DGAT2], Rab18 [transport], and CCT [CTP-phosphocholine cytidylyltransferase, the rate-limiting enzyme of phosphatidylcholine synthesis] (Farese and Walther, 2009). The LDs also contain acyl-CoA cholesterol acyltransferases 1 and 2 (ACAT1, ACAT2) which catalyse cholesterol esterification. A genome-wide, siRNA-based screen identified 550/18000 genes of human macrophages (THP-1) as being involved in modulating lipid storage according to number, size and cellular localization and functions such as proteasome activity, intracellular transport, transcription activity, E3 ligase activity and lipid modifying enzymes (Mejhert et al, 2021). In summary, LDs are central to the regulation of cellular lipid homeostasis (Mejhert et al, 2021). Fluorescence resonance energy transfer (FRET) has shown to occur between perilipin A and NSP2, proving the close spatial proximity of LDs and viroplasms (Cheung et al, 2010).

Complexes of viroplasms and lipid droplets in RV-infected MA104 cells at 8h p.i.
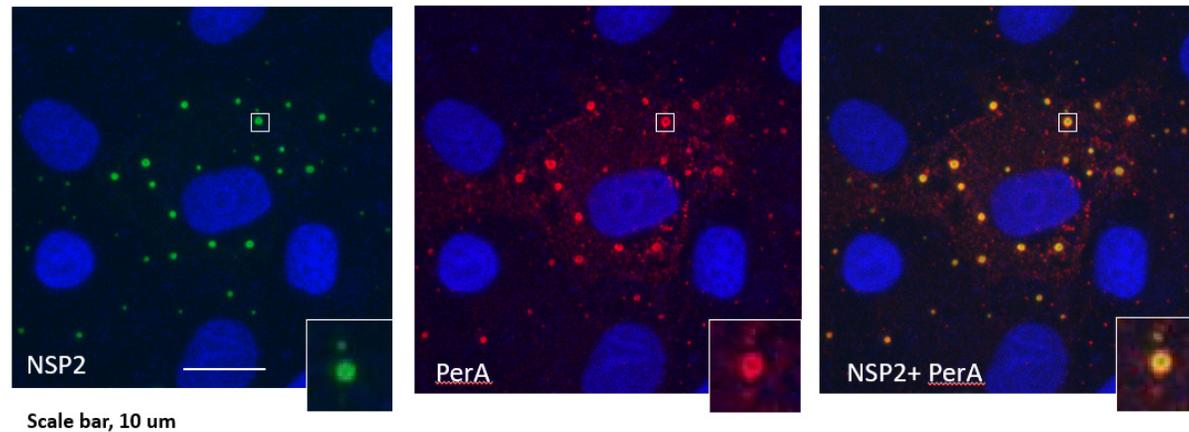
Modified from: Cheung W et al, 2010.

Figure 1. RV-infected cells grown on coverslips were processed for confocal microscopy (CM) as described by Nejmeddine et al, 2000. Cells were blocked with 1%BSA-0.1%triton X-100. Primary antibodies against perilipin A (rabbit polyclonal) were from Abcam, those against NSP2 (mouse monoclonal) were a kind gift of Dr Oscar Burrone, ICGEB, Trieste IT. Secondary antibodies were goat anti-rabbit IgG conjugated with Alexafluor 633 and goat anti-mouse IgG conjugated with Alexafluor 488, both from Invitrogen. Staining with secondary antibodies was in the presence of 2ug/ml of Hoechst33342 (Sigma). Coverslips were mounted on glass slides with Prolong bold Antifade mounting medium (Molecular Probes) and observed by CM using a Leica DM Libre TCS SP instrument. An individual viroplasm-LD complex is magnified in the inserts. From: Cheung W et al, 2010.

Iodixanol gradient velocity ultracentrifugation of RV-infected cell extracts resulted in co-sedimentation of viral dsRNA, NSP2 and perilipin A in low-density fractions (1.11-1.15 g/ml). By contrast, rotavirus dual-layered particles (DLPs; of density 1.38 g/ml), spiked into mock-infected lysates, sedimented to the bottom of the gradient (Cheung et al, 2010). Iodixanol gradient fractions of low density, containing peaks of the RV dsRNA genome and LD- and viroplasm-associated proteins, were analysed for 14 different classes of lipids by mass spectrometry and found to contain maximum amounts of lipids as typical components of LDs, confirming the close interaction of LDs with viroplasms (Gaunt et al, 2013b).

The complex formation of viroplasms and LDs has functional consequences. LD formation depends critically on the presence of long chain fatty acids. Their biosynthesis is catalysed by acetyl-CoA cocarboxylase (ACC-1), fatty acid synthase (FASN), long chain acyl-CoA synthetase (HCSL) and various glycerol-acyl transferases (GATs), the latter converting long chain fatty acids into triacyl esters (neutral fats) which are incorporated into the precursors of LDs in the endoplasmic reticulum (ER). The ER-localized enzymes DGAT1 and DGAT2, ACAT1 and ACAT2 synthesize TAGs from fatty acids and sterol esters from cholesterol, respectively. Lipid droplets bud from the lipid bilayer of the ER into the cytoplasm and acquire various lipid droplet-associated proteins (PLINs 1-5 and many others) (Crawford and Desselberger, 2016).

Key inhibitors of these pathways are: TOFA (inhibiting ACC-1), C75 (anti-FASN), triacsin C (anti-ACSL) and A922500 (anti-DGAT-1) (Kim et al, 2012; Crawford and Desselberger, 2016). Application of these inhibitors to RV-infected cells significantly reduce the size and number of viroplasms formed, the production of viral dsRNA and the titers of infectious viral progeny to various extent. Similarly, the treatment of cells with a combination of [isoproterenol + IBMX], which fragments LDs into smaller microdroplets (Marcinkiewicz et al, 2006) also reduces RV progeny production. Representative data for inhibition with TOFA, triacsin C and [Isoproterenol + IBMX) are presented in Table 1 (Cheung et al, 2010; Gaunt et al, 2013a).

Table 1. Comparison of inhibitory effects of different compounds affecting lipid droplet homeostasis on rotavirus replication.

Treatment of cells		Viral dsRNA		Infectivity of progeny	
		Relative Value[a]	*Difference*[b]	*log TCID$_{50}$/ml ± S.E. (n)*	*Difference*[b]
Isoproterenol+IBMX[c]	-	1.00		8.2 ± 0.3 (3)	
	+	0.25	4.0-fold	6.5 ± 0.1 (3)	50-fold
Triacsin C[c]	-	1.00		7.5 ± 0.1 (3)	
	+	0.26	3.8-fold	6.2 ± 0.2 (3)	20-fold
TOFA[d]	-	1.00		8.4 ± 0.5 (6)	
	+	0.17	5.9-fold	6.7 ± 0.5 (6)	50-fold

[a] Calculated from densitometric values of RNA gels (Cheung et al, 2010). [b] Underlining indicates statistical difference ($P<0.05$). [c] From: Cheung et al, 2010. [d] From: Gaunt et al, 2013a.

Furthermore, TOFA-treated and RV-infected cells were analysed for the production of DLPs and TLPs (purified by CsCl gradient equilibrium ultracentrifugation). In the presence of TOFA the production of DLPs is decreased by a factor of 2, but that of TLPs by a factor of 20, compared to DLP and TLP production in untreated cells. These suggested that inhibition of fatty acid biosynthesis affects not only the recruitment of LDs by viroplasms but is also involved in interfering with the later step of RV maturation (Cheung et al, 2016).

The mechanism of interaction of viroplasms with LDs was explored further. Following cellular infection with a RVA mutant with delayed viroplasm formation (rRV NSP2 S313D, engineered by a RVA-specific plasmid only-based reverse genetics system; Kanai et al, 2017), an early interaction of viroplasm-bound NSP2 with phospho-perilipin 1 (leading to lipolysis; Tansey et al, 2003) was observed (Criglar et al, 2022), thus exploiting the lipid metabolism.

Recently, it was found that DGAT1, required for triacylglycerol and LD biosynthesis, is degraded in RV-infected MA104 cells and in human intestinal enteroids (HIEs) (Liu, Smith et al, 2023). In the uninfected cell, DGAT1 synthesizes TAGs from ER-resident DAGs and cytoplasmic acyl-CoAs, and TAGs are deposited in the ER lipid bilayer. Upon RV infection, NSP2 is expressed which interacts with DGAT1. The viroplasm-DGAT1 complexes are then tagged with ubiquitin and degraded in proteasomes. The loss of DGAT1 induces the budding of TAGs and LD formation by a still not fully understood mechanism (Liu, Smith et al, 2023). Suppression of DGAT1 by rotavirus infection leads to an increase in the number of viroplasms and of the infectivity titer of viral progeny in MA104 cells (Liu, Smith et al, 2023).

Other RNA Viruses

Besides for rotaviruses, the significance of cellular lipid metabolism has been recognized for the replication of many other RNA viruses, and some of the relevant information is summarized in Table 2. Members of the *Flaviviridae* (hepatitis C virus [HCV], Dengue virus (DENV), GB virus-B, Zika virus and others), *Picornaviridae* (poliovirus, enterovirus), *Caliciviridae* (norovirus), *Coronaviridae* (SARS-CoV-2), *Retroviridae* (HIV-1), *Orthomyxoviridae* (influenza virus) and *Rhabdoviridae* (lyssa virus) families utilize components and pathways of the cellular lipidome in various ways for their replication, such as in the formation of double membrane vesicles in contact with LDs (potentially acting as viral assembly platforms), the stimulation of cellular lipases, and the requirement of cellular lipids and membranes for viral envelope formation.

Table 2. Lipid metabolism involved in the replication of RNA viruses (other than rotaviruses).

Virus	*Mechanism*	*References*
Hepatitis C virus (HCV)	Formation of double membrane vesicles (DMVs) for viral replication, interacting with LDs via viral core protein D2 domain and NS5A; role of apolipoproteins; dynamic profile of lipidome-DMV complexes during HCV infection	Miyanari et al, 2007; Shavinskaya et al, 2007; Boulant et al, 2007; Chatel-Chaix and Bartenschlager,2014; Vieyres and Pietschmann, 2019; Islam et al, 2023
GB virus-B	similar to HCV	Hope et al, 2002
Dengue virus	Viral capsid protein-LD interactions; similar to HCV	Samsa et al, 2009; Chatel-Chaix and Bartenschlager, 2014
Zika virus	Disturbance of lipid homeostasis; induction of mitochondrial dysfunction	Chen Q et al, 2020
Picornaviruses	Phosphatidylcholine synthesis to support membranous replication complexes; hydrolysis of TAGs in LDs, lipolysis	Victorova et al, 2018; Laufman et al, 2019; Belov and von Kuppeveld, 2019
Norovirus	Multimembrane vesicle clusters replication organelles, as in picornaviruses, containing NoV NS proteins;	Doerflinger et al, 2017
SARS-CoV-2	DMVs as replication complexes; viral reprograming of lipid synthesis pathways; LDs as assembly platforms?	Dias et al, 2020; Roingeard et al, 2022; Cesar-Silva et al, 2023
HIV-1	Requirement of ceramide for viral capsid maturation and full infectivity	Waheed et al, 2023; Yoo et al, 2023
Influenza virus	Upregulation of LDs upon infection; viral replication depending on cellular lipase activity; elevation of lipid metabolites in IV-infected cells	Baek et al, 2022; Chen X et al, 2023

A kinetic study on lipidome changes in HCV (strain 96/JFH-1)-infected human hepatoma Huh-7 cells using mass spectrometry discovered a dynamic profile, with phosphatidyl phospholipids, triacylglycerol, phosphatidyl choline and ceramide derivatives being enriched early in infection, and free fatty acids, phosphatidylethanolamine peaking at late stages of the replication cycle. This profile could be correlated with different lipidome functions during viral replication (Islam et al, 2023; Qu et al, 2023).

For DENVs it was observed that a LD-associated membrane protein, the acyl transferase AUP1, associates with the viral NS4A protein to be relocalized to autophagosomes where the complexes are degraded; this triggers lipophagy and a decrease in virus production (Zhang et al, 2018).

For coronaviruses (mainly SARS-CoV-2), ceramide plays a central role during virus entry, and inhibitors of acid sphingomyelinase, the rate-limiting enzyme of ceramide biosynthesis, suppresses viral replication. Various drugs inhibiting enzymes of the sphingolipid metabolism, are under investigation as potential antivirals (Cesar-Silva et al, 2023, and text and Table 2 therein). Similarly, various drugs reducing cholesterol biosynthesis and esterification (and LD formation) are being explored (Cesar-Silva et al, 2023; D'Avila et al, 2024) or are already under clinical investigation (Theken et al, 2021, and Table 1 therein).

The double-membrane vesicles (DMVs), in which coronaviruses replicate, have similarities with analogous replication structures of HCV (Roingeard et al, 2021; Tabata et al, 2021).

Lipid rafts, located in proximity of cellular membranes, are rich in cholesterol and are involved in influenza A virus cell entry, particle maturation and budding (Madsen and Rossman, 2023; Li YJ et al, 2022). Influenza viruses and other negative-strand, non-segmented RNA viruses obtain their envelope during the budding step from the host cell's plasma membrane where cholesterol forms microdomains (rafts) which act as scaffold for viral 'envelope' proteins (Husby and Stahelin, 2021; Madsen and Rossman, 2023). Statins which act as inhibitors of cholesterol biosynthesis, may have a role in blocking the replication of influenza viruses and of members of the *Mononegavirales* (Li et al, 2022).

[The significance of cellular lipid metabolism for DNA viruses has been reviewed by Farias et al, 2022].

Compounds Interacting with the Cellular Lipidome as Potential Candidate Antivirals

A list of compounds interfering with the cellular lipidome to reduce viral replication is presented in Table 3. The compounds inhibit fatty acid biosynthesis and neutral fat production, disturb LD stability and metabolism, inhibit cellular lipases, interfere with neutral sphingomyelinases and the activity of inflammatory metabolites, or decrease the concentration of cholesterol. The development of such compounds to clinically applicable antivirals is still in its infancy.

Table 3. Compounds interacting with the cellular lipidome as potential candidate antivirals.

Virus	*Compound*
Rotavirus	TOFA; [isoproterenol + IBMX]
Hepatitis C virus	Lipid-specific compounds targeting the interaction of viral core proteins with LDs
Zikavirus	DGAT-1 inhibitor A922500
Enterovirus	Adipose triglyceride lipase inhibitor atglistatin
SARS-CoV-2	Inhibitors of sphingolipid metabolism; inhibitors of inflammatory compounds; lipase inhibitors; statins
HIV-1	Neutral sphingomyelinase 2 inhibitor PDDC, preventing ceramide biosynthesis
Influenza A virus	methyl-beta-cytodextrin; sphingomyelinase inhibitors; lipase inhibitors; statins

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