Lipid droplets and lipid mediators in viral infection and immunity

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One sentence summary: Lipid droplets are key organelles in multiple cellular processes; recent advances in the field have demonstrated that they together with their lipid mediators may play pivotal roles in the outcome of viral infection.

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ABSTRACT

Lipid droplets (LDs) contribute to key pathways important for the physiology and pathophysiology of cells. In a homeostatic view, LDs regulate the storage of neutral lipids, protein sequestration, removal of toxic lipids and cellular communication; however, recent advancements in the field show these organelles as essential for various cellular stress response mechanisms, including inflammation and immunity, with LDs acting as hubs that integrate metabolic and inflammatory processes. The accumulation of LDs has become a hallmark of infection, and is often thought to be virally driven; however, recent evidence is pointing to a role for the upregulation of LDs in the production of a successful immune response to viral infection. The fatty acids housed in LDs are also gaining interest due to the role that these lipid species play during viral infection, and their link to the synthesis of bioactive lipid mediators that have been found to have a very complex role in viral infection. This review explores the role of LDs and their subsequent lipid mediators during viral infections and poses a paradigm shift in thinking in the field, whereby LDs may play pivotal roles in protecting the host against viral infection.

Keywords: lipid droplets; immunity; lipid mediators; virus; infection; antiviral

INTRODUCTION

Lipids are a major and diverse class of biomolecules that play an important role in both the physiology and pathophysiology of cells. Generally, lipids are thought to have three main functions within a cell. First, polar lipids make up the matrix of membranes and provide cellular and organelle membranes the ability for budding, tubulation, fusion and fission (reviewed in Casares, Escribá and Rosselló 2019). Specific lipids also enable the formation of highly ordered regions within the plasma membrane (lipid rafts) that serve as key platforms for receptor signaling events (reviewed in Simons and Sampaio 2011). Second, lipids can act as first and second messengers in signal transduction and molecular recognition processes and have signaling roles during infection and inflammation (reviewed in van Meer, Voelker and Feigenson 2008; Fahy et al. 2009; Subramaniam et al. 2011; Teng, Ang and Guan 2017). Lastly, fatty acids can be packaged into lipid droplets (LDs), where they can be used for energy...
storage. Triglycerides (TGs) and sterol esters (sterols esterified by a fatty acyl chain) are the major components of LDs, where they serve to store energy for a host cell in times of nutrient deprivation (reviewed in Jackson, Walch and Verbavatz 2016). TGs and sterol esters form the neutral lipid core of LDs and are surrounded by a phospholipid membrane monolayer with membrane-bound proteins (Bartz et al. 2007).

The role of lipids in viral infection has been dominated in the literature by reports of viral replication strategies that usurp LDs and alter lipidomic profiles of host cells to enhance their viral life cycles. However, in recent years, there have been many roles described for fatty acids during immune responses, such as their effects on specific immune cell subsets, their effects on intracellular calcium and MAPKs in intracellular signaling pathways, their modulation of lipid rafts, their interactions with nuclear receptors, such as PPARs, and the characterization of anti-inflammatory eicosanoids involved in the resolution of inflammatory responses. Interestingly, there is also evidence for a bidirectional relationship between the immune system and lipids (such as fatty acids and bioactive lipid mediators). There are clear links between antiviral immunity and the induction of cholesterol synthesis, with lowered cholesterol biosynthesis of sterol regulatory element-binding proteins spontaneously engaging type I interferon (IFN) signaling leading to a restriction of viral infection (reviewed in Azzam and Fessler 2012; Joss et al. 2013; O’Neill 2015; York et al. 2015). Notably 25-hydroxycholesterol is in fact an IFN-stimulated gene (ISG) known to restrict the replication of many viruses including Zika virus, rotavirus and norovirus infections (Li et al. 2017; Cîrva et al. 2018; Shawli et al. 2019). This review will focus on the latest research into the role of LDs and lipid mediators during the innate immune response, and offer insight into how both may govern the outcome of viral infection.

**WHAT IS A LIPID DROPLET?**

Since their discovery in the 1890s (Altmann 1894), LDs have shown to be highly dynamic, ubiquitous organelles, which are found in virtually all types of cells from prokaryotes to eukaryotes. They consist mainly of TGs and sterol esters, but also harbor other lipid species such as diacylglycerols, retinyl esters and ceramides (Testerinck et al. 2012; Preuss et al. 2019), and can also activate the synthesis of bioactive lipid mediators (de Almeida et al. 2018). The size, number, function and composition of LDs can differ considerably not only among different cell types but also in individual cell populations (reviewed in Pol, Gross and Parton 2014). They are involved in many cellular processes, including lipid storage and transport (Liu et al. 2004; Bartz et al. 2007), metabolism (reviewed in Xue, Zhang and Liu 2018) and protein storage and degradation (Klemm, Spooner and Ploegh 2011; Li et al. 2012). LDs have been described to be central in a number of cellular processes with the dysregulation of LDs contributing to a number of diseases, including obesity, fatty liver disease, cardiovascular disease, diabetes and also numerous cancers (reviewed in Onal et al. 2017; Cruz et al. 2020).

Typically, it was thought that the function of LDs was to fuel metabolic processes and membrane biosynthesis; however, recently other roles have emerged for LDs, such as their protection against lipotoxicity and mitochondrial damage during autophagy (reviewed in Nguyen and Olzmann 2017), and their role in immune responses.

Within a cell, LDs alternate between periods of growth and depletion. LDs are depleted either through enzymatic hydrolysis mediated by lipases (lipolysis) or through a selective form of autophagy (lipophagy) (reviewed in Olzmann and Carvalho 2019). LD depletion mainly occurs during cell starvation where two lipolytic enzymes mediate LD breakdown: adipose triacylglycerol lipase (ATGL) converts TG to diglyceride (DG) (Haemmerle et al. 2006) and hormone-sensitive lipase (HSL) converts DG to monoglyceride (MG) (Vaughan, Berger and Steinberg 1964). The enzyme phospholipase A (PLA), important for LD homeostasis, also hydrolyzes fatty acids (Kim et al. 2002; Gijas et al. 2014). Although LD depletion is fairly well described, the mechanisms underpinning LD induction appear to be more diverse, and somewhat less understood. There are currently several mechanisms described for the biogenesis of LDs, with the most well-described mechanism regulated by the accumulation of neutral lipids (most commonly TG and sterol esters) between the bilayers of the endoplasmic reticulum (ER) membrane. This accumulation of lipids within the ER membrane leads to neutral lipids producing a sphere of lipids concealed by a phospholipid monolayer of the ER, and then budding off as nascent LDs (Fig. 1) (reviewed in Jackson 2019). Proteins can be embedded into the LD during the biogenesis stage or can be later recruited/trafficked to the LD surface from the cytosol. Protoemis studies have revealed ~100–150 mammalian LD proteins (Bersuker et al. 2018), and the protein composition of LDs can vary, with cell type and metabolic state of the cell a driver of this heterogeneity. While the function of many of these proteins remains to be fully elucidated, as well as the mechanisms of protein targeting to LDs (reviewed in Dhiman et al. 2020), several have been implicated in lipid synthesis and LD biogenesis (e.g. acyl-CoA synthetase, PLIN2, DGAT2), protein degradation (e.g. AUP1 and ERAD proteins), signaling, membrane trafficking and metabolism (reviewed in Zhang and Liu 2019).

LDs are highly dynamic organelles, constantly changing in both their size and location. LD size can vary (200–10 000 nm) in a range of different cell types (reviewed in Yang et al. 2012) with the size dependent on the amount of stored neutral lipid, which is the net result of TG synthesis (lipogenesis) and hydrolysis (lipolysis), and the regulation of lipolysis by external factors. Additionally, LDs are usually dispersed throughout the cell and move in small oscillations within a cell; however, they have also been demonstrated to dynamically move around cells during different stress conditions, for example, LDs cluster around microtubule organizing centers during HCV infection (Bouant et al. 2008). In mammalian cells, perilipin1 (PLIN1) expression leads to LD clustering, which is reversed upon perilipin1 phosphorylation (Marcinkiewicz et al. 2006). It is known that LDs can move around the cell environment; however, the reason behind this is not well described in mammalian cells, although recently it was discovered that DipA phosphatase was responsible for the movement and distribution of LDs in the filamentous fungus Aspergillus nidulans model (Salogiannis et al. 2020). A possible reason for LD movement within a cell is the ability of LDs to interact with other organelles, including the ER, endosomes, mitochondria and peroxisomes (Valm et al. 2017). There are several hypotheses as to why LDs may form contacts with other organelles, including the delivery of LDs to lysosomes during autophagy to generate cholesterol (Ouimet et al. 2011), channeling of FAs liberated from lipolysis to sites of oxidation (Blanchette-Mackie and Scow 1983; Binns et al. 2006) and exchange of proteins (Binns et al. 2006; Pu et al. 2011; Wang et al. 2011). The dynamic nature of LDs has been noted in a few disease pathologies, most notably their accumulation following some pathogenic infections.

Even within a cell, the size of LDs may dramatically differ under changing pathophysiological conditions. Increased LD
numbers in non-adipocytic cells are commonly observed pathological features of a number of infectious diseases. In recent years there has been a lot of research into the alternate ways in which LDs can be induced, and interestingly it has been discovered that a number of pathogens induced LDs upon infection.

**LIPID DROPLETS ARE UPREGULATED DURING VIRAL INFECTIONS**

LD formation involves specific and well-regulated mechanisms, and LD biogenesis has been rapidly detected after infection with a number of different viral and non-viral pathogens (Table 1). However, although multiple signaling pathways have been implicated in LD biogenesis, relatively little is known regarding the molecular mechanisms that govern LD biogenesis post-infection with the involvement of both pathogen and host factors usually required.

A diverse number of pathogens have been shown to upregulate LDs in numerous animal models and cell lineages following the recognition of either the pathogens themselves, or their pathogen-associated molecular patterns such as proteins, lipopolysaccharides, nucleic acids and other patterns unique from host cells (Table 1). Currently the main documented drivers for LD production following pathogen recognition by a host cell are toll-like receptors (TLRs) in mammals or TLR homologues in non-mammal models. Viral induction of LDs is a more recent phenomenon and has only been shown for a small handful of viruses. Recently, it was described that in an *Aedes aegypti* model of dengue (DENV) infection, LDs were induced following the activation of TLRs or immune deficiency pathways, with inhibition of these pathways reducing the accumulation of LDs (Barletta et al. 2016). Additionally, influenza, herpes simplex virus and Zika (ZIKV) virus have all been shown to induce LDs in both HeLa and primary immortalized astrocytes in an EGFR-dependent manner, similar to what has previously been demonstrated in a cancer model of LD induction (Penrose et al. 2016; Monson et al. 2020). Interestingly, infection with DENV and ZIKV has also been shown to deplete LDs in some cell types (Heaton and Randall 2010; Chen et al. 2020); however, it is not clear whether this is a cell-type specific response, or whether it might be a timed infection response, with LDs accumulating early as a host response following viral infection, accompanied by viral manipulation of this response, and subsequent depletion of LDs to fuel their viral life cycles.

LDs can also be upregulated following inflammasome stimulation during infection of cells with hepatitis C virus. Upon infection with hepatitis C virus, nod-like receptor protein 3 (NLRP3), a crucial mediator of the innate immune system is activated (Negash et al. 2013). The activation of NLRP3 promotes the activity of the inflammasome, which upregulates the
Table 1. LD biogenesis by different pathogens.

| Stimulation/infection type | Model | Cell type | Mechanism | Reference |
|----------------------------|-------|-----------|-----------|-----------|
| **Bacteria**                |       |           |           |           |
| Salmonella and IFN-γ        | Murine| Inflammatory monocytes and neutrophils | not determined | (Rosas-Ballina et al. 2015) |
| Leishmania sp.              | Murine| Macrophages | not determined | (Pinheiro et al. 2009; D’Avila et al. 2011; Babhi et al. 2016) |
| C. pneumoniae               | Murine| Macrophages | not determined | (Cao et al. 2007) |
| M. leprae                   | Human and murine| THP-1, peripheral and peritoneal monocytes and Schwann cells | TLR-2, TLR6, PI3K | (Tanigawa et al. 2008; Mattos et al. 2010, 2011) |
| M. bovis bacillus           | Murine| Macrophages | TLR-2 | (D’Avila et al. 2006) |
| LPS                         | Murine| Leukocytes and macrophages | CD14, CD11b/CD18, TLR-4 & MCP-1 | (Pacheco et al. 2002, 2007) |
| Acinetobacter baumannii     | Murine| J774 Macrophages | not determined | (Nicolaou, Goodall and Erridge 2012) |
| Chlamydia trachomatis       | Human| HepG2, HeLa | IncA (bacterial driven) | (Kumar, Cocchiaro and Valdivia 2006; Cocchiaro et al. 2008) |
| Chlamydia muridarum         | Murine| Epithelial cells (cervix) | not determined | (Rank et al. 2011) |
| Klebsiella pneumoniae       | Human and murine| Peripheral blood monocytes and J774 macrophages | not determined | (Nicolaou, Goodall and Erridge 2012) |
| E. coli                    | Murine| J774 macrophages | not determined | (Nicolaou, Goodall and Erridge 2012) |
| Proteus vulgaris            | Murine| J774 macrophages | not determined | (Nicolaou, Goodall and Erridge 2012) |
| Pseudomonas aeruginosa      | Murine| J774 macrophages | not determined | (Nicolaou, Goodall and Erridge 2012) |
| Pseudomonas diminuta        | Murine| J774 macrophages | not determined | (Nicolaou, Goodall and Erridge 2012) |
| Staphylococcus aureus       | Murine| J774 macrophages | not determined | (Nicolaou, Goodall and Erridge 2012) |
| Staphylococcus epidermidis  | Murine| J774 macrophages | not determined | (Nicolaou, Goodall and Erridge 2012) |
| Staphylococcus salivarius   | Human and murine| J774 macrophages and peripheral blood monocytes | not determined | (Nicolaou, Goodall and Erridge 2012) |
| Vibrio cholera              | Human| Mucosal mast cells | TLR-2 & IMD pathways | (Qadir et al. 2004) |
| Enterobacter cloacae        | Mosquito| Aag2 | Toll and IMD pathways | (Barletta et al. 2016) |
| CpG-DNA                    | Murine| J774 macrophages | not determined | (Nicolaou, Goodall and Erridge 2012) |
| Flagellin                   | Murine| J774 macrophages | not determined | (Nicolaou, Goodall and Erridge 2012) |
| **Parasites**               |       |           |           |           |
| Plasmodium berghei         | Murine| Hepatocytes, macrophages | not determined | (Rodriguez-Acosta et al. 1998; Borges et al. 2017) |
| Trypanosoma cruzi          | Rat | Inflammatory macrophages | not determined | (Melo et al. 2003, 2006; D’Avila et al. 2011) |
| Schistosoma mansoni         | Murine| Eosinophils | TLR-2 | (Magalhães et al. 2010) |
| T. gondii                   | Human| Fibroblasts | not determined | (Charron and Sibley 2002) |
| Candida albicans derivatives| Rat | Macrophages and hepatocytes | not determined | (Paraje et al. 2008) |
| **Viruses**                 |       |           |           |           |
| Hepatitis C (HCV)           | Human| CHO & HepG2 | not determined | (Barba et al. 1997) |
| Dengue (DENV)               | Hamster| BHK-21 | not determined | (Samsa et al. 2009) |
| Sindbis virus               | Mosquito| Aag2 | Toll and IMD pathways | (Barletta et al. 2016) |
| Zika (ZIKV)                 | Human| Astrocytes | EGFR | (Monson et al. 2020) |
| Herpes simplex-1 (HSV-1)    | Human| Astrocytes | EGFR | (Monson et al. 2020) |
| Influenza A (IAV)           | Human| Lung tissue | not determined | (Monson et al. 2020) |
|                           | Mouse| MDCK cell line | ROS production | (Episcopio et al. 2019) |

expression of sterol regulatory element-binding proteins (SRBPs) (Im et al. 2011). This cascade enhances the upregulation of lipogenic genes that may result in the accumulation of LDs (Ji, Chan and Kaplowitz 2006). Although LD numbers are now considered an inflammatory hallmark following innate immune activation, as can be seen in Table 1, there are still a lot of unknowns regarding the mechanism of LD induction in an infection setting. This further highlights the importance of understanding these mechanisms and pathways as a potential target of microbial infection. It is also poorly understood if the biogenesis of LDs is host driven, or a mechanism that pathogens have acquired to manipulate natural LD metabolism to promote their survival, or to promote replication and virion maturation to avoid immune clearance from the host innate immune response.

THE ROLE OF LIPID DROPLETS DURING VIRAL INFECTION

Several bacterial, protozoan and viral pathogens are known to exploit host LDs to promote infection, thus emphasizing the importance of LDs at the host–pathogen interface. In particular, associations between LDs and ssRNA viruses, which exhibit intimate relationships with the host ER, are constantly emerging at varying stages of the virus life cycle. From the Flaviviridae family, WNV, DENV, JEV and ZIKV capsid proteins all co-localize with LDs, and the residues involved in LD binding appear to be conserved amongst flaviviruses (Samsa et al. 2009; Carvalho et al. 2012; Martins et al. 2012, 2019; Shang et al. 2018; Ishida et al. 2019). The association between DENV capsid protein and LDs is particularly strong, and it has been proposed that LDs...
Lipid droplet localization with immune proteins

Many of the known LD resident proteins have central roles in lipid metabolism; however, recent work has demonstrated that several LD-resident and transient proteins have known functions outside of lipid homeostasis, having profound impacts on protein abundance and function, as well as roles in innate immune functions. The LD resident proteome is vast, but also contains many documented proteins of a transient nature depending on cellular conditions (Rösch et al. 2016; Menon et al. 2019).

It is well described that LDs are a place of viral assembly for some viruses, mainly members of the Flaviviridae and Reoviridae families, with viral proteins from members of these families being shown to localize with LDs or LD associated proteins during infection (Samsa et al. 2009; Cheung et al. 2010; Herker et al. 2010; Carvalho et al. 2012; Li et al. 2012; Camus et al. 2013). We now know that multiple viral families are able to induce the formation of LDs during infection (Table 1); however, up until recently this was thought to be induced solely by the virus for replication, and did not have a link with host immunity. When cells are infected, they produce and secrete antiviral cytokines, mainly IFNs, and subsequently, a collection of ISGs are expressed (reviewed in Schoggins and Rice 2011). Interestingly, several ISGs are also found to be localized to LDs, a phenomenon that could be a host immune strategy to combat viral propagation.

One of the most well-described ISG proteins to localize to the LD is viperin. Viperin localizes to the LD via its N-terminal amphipathic α-helix and was first found to be antiviral against human cytomegalovirus, but since then has been found to have antiviral properties against multiple viral families (Hinson and Cresswell 2009; Peña Cárcamo et al. 2018; Crosse et al. 2019; Li et al. 2019a). Interestingly, human cytomegalovirus blocks fatty acid β-oxidation by forcing the redistribution of viperin from the LD to the mitochondria resulting in an increase in LD numbers and viral envelope assembly (Seo and Cresswell 2013). This led the field to hypothesize that viperin requires its LD localization to restrict some of these viral families (Seo and Cresswell 2013; Peña Cárcamo et al. 2018; Bai et al. 2019) and also perform important post-translational modifications during antiviral immune pathways (Bai et al. 2019).

Viperin is not the only ISG that has been found on the LD, with members of the immunity related GTPase (IRG) family (IRGM1 and IRGM3) also localizing to this organelle. There is less known about why members of the IRG family of proteins localize to LDs. A few instances have shown they require the LD to bind lipids, mediate host responses and regulate autophagy and lipophagy (Grégoire, Raboudrin-Combe and Faure 2012; Haldar et al. 2013; Lin et al. 2016). Interestingly, the IRGM family of proteins are a common target of RNA viruses, with interactions with 12 viral proteins belonging to five different viruses [Chikungunya virus (ChikV), Mumps virus (MuV), Hepatitis C Virus (HCV), Measles virus (MeV) and HIV-1] making it the most targeted autophagy-associated protein by these viruses (Grégoire et al. 2011). A member of this family, IRGM1 is induced by IFNs in a...
Incubation of IAV infected cells with histone H4 results in a reduction of LD numbers. Although these histones inhibited influenza growth in vitro, there has not been a mechanism of how this modulates the host immune response (Bougères et al. 2009). Lastly, members of the helicase family are known to localize to LDs during HCV infection (Rosch et al. 2016). Of these helicase family members, DDX3X and DDX1 are involved in innate host responses, particularly the upregulation of IFN-β (Soulat et al. 2008; Li et al. 2015; Zhou et al. 2017; Niu et al. 2019; Xue et al. 2019); however, there are currently no studies demonstrating their reliance on their LD localization to impart their antiviral effects, unlike viperin and IRG family members. Although not in a viral setting, a very recent study has now demonstrated that LDs serve as hubs of innate immune responses to bacterial LPS stimulation. Proteomic analyses of LDs derived from the livers of LPS treated mice demonstrated that multiple proteins involved in a successful antibacterial response, including CAMP and viperin were able to localize to LDs as a function of innate immune activation (Bosch et al. 2020). This recent work paves the way for a better understanding of the role of the LD as a potential functional hub for innate immune proteins.

It is clear that LDs are protein sequestration sites for proteins that have clear roles outside LD function (Cermelli et al. 2006; Welte 2007). LDs have been found to sequester large amounts of histones, particularly in early Drosophila embryos (Cermelli et al. 2006) but also in C. elegans, silkworm and human LD proteomes (Yang et al. 2010; Liu et al. 2012; Wang et al. 2013). Proteomic analysis of LDs isolated from Drosophila embryos confirmed the presence of histone localization via a protein known as jabba (Cermelli et al. 2006; Li et al. 2012). Interestingly, Drosophila embryos lacking jabba and hence histone sequestration were more susceptible to infections than embryos that had no disruption in their ability to sequester histones (Anand et al. 2012). There have not been many reports of histones having antiviral mechanisms; however, viral proteins have been demonstrated to drive the depletion of histones in vitro (Irwin et al. 2018). Interestingly, the M1 protein of influenza A and B virus selectively binds histones H2A, H2B, H3 and H4 (Zhirmov and Klenk 1997), with these histones showing anti-influenza activity against both seasonal H3N2 and H1N1, but not pandemic H1N1 (Hoeksema et al. 2015). Incubation of IAV infected cells with histone H4 results in decreased viral replication via direct effects on viral particles. Although these histones inhibited influenza growth in vitro indicating a possible role for histones in the innate immune response against IAV, in vivo treatment with histones did not yield antiviral effects and instead exacerbated lung pathology. (Ashar et al. 2018) Clearly, more work is needed in this area to elucidate the role of these histones during infection, and uncover the role LDs play in this relationship.

It has also been observed that LDs are vital to protecting the developing zebrafish embryo from pathogens, prior to development of a functional immune system. Zebrafish only develop an immune system after 4–6 weeks of embryo development, with an intact innate immune system present at fertilization (Lam et al. 2004). LDs have been shown to play a vital role in protecting the zebrafish embryo from bacterial pathogens, with increased LD numbers being highly protective against invading bacterial species (Dutta, Banerjee and Sinha 2018). LDs were found to secrete antimicrobial compounds to afford embryo protection from bacteria, and interestingly, injected exogenous LDs also increased the life expectancy of the embryos strengthening this correlation (Dutta, Banerjee and Sinha 2018). The role of LDs in viral protection of the zebrafish embryo has not been demonstrated to date; however, recent work has shown that LD depletion in the mammalian HeLa and Huh-7 cell lines diminishes the production of type I and III IFN mRNA species following both activation of RNA and DNA PRRs as well as viral infection (Monson et al. 2018).

The ability of LDs themselves, and perhaps the proteins they house to impact viral infection as well as infection of other pathogens, is very interesting; however, the role LDs play in this is not entirely known. One potential hypothesis is that LDs may act as an organelle platform during host defense pathways, hence the requirement for their upregulation during pathogen infection. However, recent evidence suggests that their role may be multi-pronged, and that they may contribute to amplification of an antiviral state in more ways than providing a platform for anti-viral proteins.

**Lipid droplets enhance innate immune pathways**

Recently, LDs have been described to contribute to an effective immune response. It is now known that LDs play a role in the antiviral response in the mosquito, where viral infection was shown to induce LD formation in the cells of the midgut (Barletta et al. 2016). This LD accumulation was mimicked via synthetic activation of the antiviral innate pathways, Toll and IMD, indicating that accumulation of LDs may be an important antiviral response in the mosquito, independently of viperin expression (Barletta et al. 2016).

The induction of LDs following viral infection has also been shown to drive enhanced interferon output. In an in vitro model, both ZIKV and Herpes simplex virus 1 infected cells promoted an increase in LD accumulation. An enhanced number of LDs resulted in decreased viral replication with heightened interferon production in the early stages of viral replication (Monson et al. 2020). Importantly, this increase in interferon was also abolished when LD induction was inhibited, suggesting that LD induction can drive an effective interferon response following viral infection. Additionally, LD upregulation following viral infection was shown to be regulated by EGFR and subsequent PI3K activation, in an interferon-independent manner, which is an alternative mechanism to what has previously been demonstrated for alternate pathogen induction of LDs. However, the regulation of interferon output and LD communication is a recent finding, with the full mechanisms responsible still unknown, yet it appears there is a clear relationship between LD regulation and orchestrating a successful immune response following infection.

Interestingly, the presence of LDs has been demonstrated to be crucial for dendritic cell cross-presentation, an essential step in the antigen-specific activation of CD8+ T cells during viral infection (Bougères et al. 2009). These authors were able to demonstrate that dendritic cells with increased LDs are more proficient at antigen cross-presentation, and when LD biogenesis was blocked, this process was inhibited. Genetic removal of LD-localized proteins, IRG3M or ADRP, from dendritic cells lead to defects in LD formation, which severely impaired cross-presentation (Bougères et al. 2009). Type I IFN has also been shown to impact cross-presentation by DCs, and the recent finding that LDs may play a role in the production of type I IFN...
Figure 2. Lipid droplets are sites for lipid mediator synthesis. Polyunsaturated fatty acids (PUFAs; mainly from TGs) such as arachidonic acid can be sequestered into LDs where they can undergo synthesis within the COX/LOX pathway. This synthesis yields bioactive lipid mediators such as prostaglandins, leukotrienes and lipoxins. Sphingosine-1-phosphate (S1P) has been demonstrated to interact with LDs through the promotion of ceramide synthesis and storage as well as platelet-activating factor (PAF), which has been demonstrated to promote LPS- and sepsis-induced LD formation.

The recent literature surrounding the contribution of the LD to an effective antiviral immune response is very understudied and still in its infancy. To further investigate how these organelles may contribute to an effective antiviral response, it is important to look at the make-up of LDs. To date, there are no studies looking at the dynamic exchange of lipids within LDs during an antiviral response; however, it has been demonstrated that this does occur during stress responses (Chitraju et al. 2012; Hartler et al. 2014; Bailey et al. 2015). Interestingly, many lipid classes as well as bioactive lipid signaling mediators are known to play pivotal roles in an immune response and are either packaged within LDs, or directly synthesized via LDs; demonstrating the importance of understanding their role and relationship to LDs to gain a better understanding of the role of LDs during this response (Fig. 2).

THE ROLE OF LIPID SIGNALING MEDIATORS DURING A VIRAL INFECTION

The concept that lipids not only function as structural components in membranes, or act as a source of energy for a cell, but also function directly as intercellular and intracellular signaling mediators has only gained interest in the last 20 years. Lipid mediators have several characteristics that distinguish them from other signaling molecules. First, they are synthesized on demand from precursor membrane lipids. They also have relatively short half-lives (seconds to minutes) and are degraded both enzymatically and non-enzymatically (Corey et al. 1975; Sala et al. 1990; Jones et al. 2005). Lipid mediators can be structurally divided into three categories. Class 1 lipid mediators include well-described arachidonic acid (AA) derived eicosanoids, which include prostaglandins (PGs) and leukotrienes (LTs) and their relatives (Narumiya and FitzGerald 2001; Frank Austen 2008). Class 2 lipid mediators are phospholipids and sphingolipids, and consist of lysophospholipids such as platelet-activating factor (PAF), lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) and their derivatives (Ishii, Nagase and Shimizu 2002; Takabe et al. 2008; Choi et al. 2010). Class 3 lipid mediators are primarily anti-inflammatory and are derived from ω-3 polyunsaturated fatty acids (PUFAs) such as resolvins and protectins and the AA-derived eicosanoid lipoxin (Ariel and Serhan 2007). Interestingly, members of all three classes of lipid mediators have roles in the effective progression of viruses and/or are involved in an effective host response (Table 2). While it has been known for a long time that viral infections and IFNs interfere with lipid metabolism, including fatty acid (FA) and cholesterol synthesis, it is not yet well established how lipids can positively contribute to an effective antiviral host response. Interestingly, some of the lipid mediators described below have been associated with LDs, or LD biogenesis pathways; however, a more detailed lipidomic analysis, especially at early stages of infection is required to fully understand the relationship between signaling lipid mediators and LDs, and their subsequent effects on host immunity.
| Mediator | Virus | Prohost/provirus | Mechanism | Reference |
|---------|-------|------------------|-----------|-----------|
| **Class 1** | | | | |
| PGE₂ᵃ | HSV-1 | Provirus | Increase viral replication  
- PGE2 contributes to immunosuppressive effect  
- PGE2 increases plaque formation and viral DNA copy number  
- PGE2 plays a role in direct cell-to-cell spreading | (Harbour, Blyth and Hill 1978; Nokta et al. 1996; Hooks et al. 2006; Schröer and Shenk 2008) |
| CMV | CMV Provirus | - PGE2 contributes to immunosuppressive effect  
- PGE2 increases plaque formation and viral DNA copy number  
- PGE2 plays a role in direct cell-to-cell spreading | (Nokta et al. 1996; Hooks et al. 2006; Schröer and Shenk 2008) |
| EBV | Provirus | Lytic reaction via EP signaling pathways  
Might be required for early infection, i.e. attachment | (Gandhi et al. 2015) |
| Enterovirus 71 | Provirus | PGE2 might be required for replication | (Tung, Hsieh and Yang 2010; Tung et al. 2011; Wang et al. 2015) |
| Sapovirus | Provirus | PGE2 decreases the production of nitric oxide, leading to an increase in PSAV | (Alfajaro et al. 2017) |
| VSV | Provirus | - COX inhibitors/antagonist reduced viral production, but the effect is overcome by exogenous PGE2  
- COX-2 antagonist decreased viral titers | (Chen, Warner and Reiss 2000; Chen, Restivo and Reiss 2002) |
| LCMV | Provirus | PGE2i inhibits the survival and effector functions of Tc | (Chen et al. 2015) |
| HTLV-1 | Provirus | PGE2 causes an increased production of virus | (Kuno et al. 1986) |
| MDV | Provirus | Promotes virus replication | (Boodhoo et al. 2019) |
| IAV | Provirus | PGE2 inhibits type-I IFNs and apoptosis pathways | (Coulombe et al. 2014; Park et al. 2016) |
| RSV | Provirus | PGE2 causes a delayed protective RSV specific immune response | (Bartz et al. 2002) |
| Coxsackie virus | Prohost | Decrease viral titers | (Xie et al. 2012) |
| HPIV-3 | Prohost | PGE2 inhibits viral replication | (Luczak et al. 1975) |
| HBV | Prohost | - PGE2 results in loss of viral replication  
- PGE2 decreases viral antigen | (Flowers et al. 1994; Hyman et al. 1999) |
| HIV | Prohost | - PGE2 decreases virion penetration by suppressing expression of CCR5  
- PGE2 inhibits virus replication by protein kinase A-dependent mechanism  
- PGE2 reduces cell-to-cell spreading  
- PGE2 enhances HIV-1 long terminal repeat mediated reporter gene activation | (Thivierge et al. 1998; Hayes et al. 2002; Clemente et al. 2014) |
| HIV | Provirus | - PGE2 has an immunosuppressive effect when co-infected with HPV  
- PGE2 could play a role in pathogenicity via TH17 cell regulation | (Hughes-Fulford et al. 1992) |
| dmPGA₁ | HSV-1 | Prohost | Significantly inhibited viral replication | (Hughes-Fulford et al. 1992) |
| dmPE₂ | HSV | Prohost | Significantly inhibited viral replication | (Hughes-Fulford et al. 1992) |
| dmPGA₂ | HSV | Provirus | Up to a 60% increase in HSV replication compared with that in untreated virus-infected cells | (Hughes-Fulford et al. 1992) |
| LTE₄ | RSV | Provirus | Urinary LTE₄ was 8-fold higher in infants with bronchiolitis than in controls | (Piedimonte et al. 2005) |
| LTE₄ᵃ | HTLV-1 | Provirus | HTLV-1 infection dysregulates the LT pathway to promote viral replication | (Trindade et al. 2012; Percher et al. 2017) |
| CMV | Prohost | Significant reduction in viral titers | (Gaudreault and Gosselin 2007) |
| IAV | Prohost | Significant decrease in lung viral loads | (Gaudreault and Gosselin 2008) |
| HIV | Prohost | LT₄ induces the secretion alpha defensins and MIP-1 beta to activate neutrophils  
LT₄ can induce T-cell activation that inhibits the EBV-induced proliferation of B lymphocytes | (Liu et al. 2008) |
| EBV | Prohost | Significant reduction in viral titers | (Widegren et al. 2011) |
| Influenza B | Prohost | Significant reduction in viral titers | (Widegren et al. 2011) |
| Coronavirus | Prohost | Significant reduction in viral titers | (Widegren et al. 2011) |
| RSV | Prohost | Increase in antimicrobial proteins to restrict infection | (Flamand, Tremblay and Borgeat 2007) |
Table 2. Continued

| Mediator | Virus | Prohost/provirus | Mechanism | Reference |
|----------|-------|------------------|-----------|-----------|
| Class 2  |       |                  |           |           |
| PAF<sup>a</sup> | HIV | Proivirus | Enhances replication | (Lima et al. 2006) |
|          | RSV  | Proivirus | Enhances the inflammatory environment | (Villani et al. 1991) |
|          | DENV | Proivirus | Induces an increase in vascular permeability, hypotension, and production of cytokines | (Souza et al. 2009) |
|          | HCV  | Proivirus | A higher amount of plasma PAF level was observed in infected patients | (Caini et al. 2007) |
| LPA      | HIV  | Proirus | Inhibiting LPA signaling reduced HCV replication | (Farquhar et al. 2017) |
|          | HSV-1 | Proivirus | Role in HSV filament formation and virus-induced cell-to-cell fusion | (Gower et al. 2005) |
|          | RSV  | Proivirus | Overexpression of S1P heightened the cells’ susceptibility to influenza virus infection | (Seo et al. 2010; Zhao et al. 2019) |
|          | IAV  | Proivirus | Facilitates successful HSV-1 replication | (Graber et al. 2020) |
|          | DENV | Prohost | Low S1P levels correlated with exaggerated DENV replication | (Gomes et al. 2014; Michels et al. 2015) |
| Class 3  |       |                  |           |           |
| PD1      | IAV (HSN1) | Prohost | PD1 treatment improved the survival and pathology of severe influenza in mice, decreases in TNF-α and IFN-γ | (Morita et al. 2013) |
| Lipoxin<sup>a</sup> | IAV (HSN1) | Prohost | NPD1 significant reduction in the severity and incidence of HSV-induced stromal keratitis | (Rajasagi et al. 2013) |
|          | RSV  | Prohost | TNFα and interferon-γ were found to be upregulated | (Cillonez et al. 2010) |
|          | KSHV | Prohost | Promotes differentiation of alternatively activated macrophages and helps resolve airway hyperreactivity | (Richardson et al. 2005; Shirey et al. 2014) |
|          | HSV-1 | Prohost | Creates an anti-inflammatory environment by decreasing the levels of NF-κB, AKT, ERK1/2, COX-2 and 5-lipoxygenase | (Chandrakekharan et al. 2016) |
|          | RSV  | Prohost | Restores the alternatively activated macrophage phenotype | (Rajasagi et al. 2011) |

<sup>a</sup>Found to be produced from LDs or influence LD biogenesis

Abbreviations: HIV-1: Human immunodeficiency virus 1; CMV: Cytomegalovirus; EBV: Epstein–Barr virus; VSV: Vesicular stomatitis virus; LCMV: Lymphocytic choriomeningitis virus; HTLV-1: Human T-lymphotropic virus 1; MDV: Marek’s Disease Virus; IAV: Influenza A virus; RSV: Respiratory syncytial virus; HPIV-3: Human parainfluenza virus-3; HBV: Hepatitis B; DENV: Dengue virus; and KSHV: Kaposi’s sarcoma-associated herpesvirus.

**Class 1 lipid mediators**

Class 1 lipid mediators are the most distinguished family of lipid mediators involved in immunity and inflammation with their main role being the amplification or reduction of inflammation, coordination of immune cell recruitment, cytokine and chemokine production, antibody formation, cell proliferation and migration, and antigen presentation (Funk 2001; Harizi, Corcuff and Gualde 2008) (reviewed in Chiurchiu and Maccarrone 2016). One of the best characterized functions of LDs in cells of the immune response are their capacity to act as platforms for heightened eicosanoid synthesis of leukotrienes and prostaglandins (Bozza, Magalhães and Weller 2009), as well as house enzymes that generate eicosanoids, such as prostaglandin H2 synthase/cyclooxygenase (PGH2 synthase/COX) and lipoxygenases (LO) (Dvorak et al. 1992; Accioly et al. 2008; Weibel et al. 2009) (Fig. 2). Eicosanoids are non-storable, but are promptly synthesized signaling molecules that are synthesized de novo from the enzymatic oxygenation of arachidonic acid, which is released from the LD phospholipid monolayer by PLÅ2 after cell activation (Nicolaou, Goodall and Erridge 2012; Melo and Weller 2016). It has been well described that LDs release FAs that in turn regulate important signaling pathways such as the PPAR transcriptional network (Zechnier et al. 2012) and also store FAs that can be used for conversion into eicosanoids (Weller et al. 1991; Triggiani et al. 1995; Bozza et al. 2011); however, it was only recently discovered that TGs stored in LDs are in fact sources of FA precursors for lipid mediator production and that their availability is controlled by neutral lipases (Dichiberger et al. 2014; Schlagter et al. 2015; Gartung et al. 2016; Kuo et al. 2018; Sohn et al. 2018) (Fig. 2).

Class 1 lipid mediators are the most widely studied lipid mediators during a viral infection, and interestingly, have been described to be both beneficial to the virus, and/or beneficial for the host during viral infection (Table 2 and Fig. 3). Of the class 1 mediators, Prostaglandin E2 (PGE<sub>2</sub>) is the most well described. It has been shown to act in a proviral manner in aiding the progression of multiple viruses such as HSV-1, CMV, EBV, IAV, RSV, and rotavirus and others (Table 2). The effect of PGE<sub>2</sub> on viral infection and replication is cell type dependent and virus family dependent, with it mainly affecting the modulation of immune pathways via the regulation of cytokines (Rowan 2005; Schröer and Shenk 2008; Chen et al. 2015). Interestingly, there are multiple mechanisms in which PGE<sub>2</sub> works to modulate these infections with PGE<sub>2</sub> working to dampen the host response in different ways and through multiple signaling pathways. For example, during IAV infection, upregulation of PGE<sub>2</sub> leads to the inhibition of type I interferon production and apoptosis in macrophages, with antigen presentation and T cell mediated immunity also suppressed, ultimately causing an increase in virus replication (Coulombe et al. 2014). However, during HSV-1 infection, PGE<sub>2</sub> ...
increases the spread of HSV-1 between cells, but the exact mechanism is unknown (Harbour, Blyth and Hill 1978). Interestingly, PGE<sub>2</sub> has strong suppressive effects on LPS-induced IFN-γ production at both the mRNA and protein levels in macrophages; however, the role PGE<sub>2</sub> plays on the regulation of IFN-γ during viral infection is currently not known (Xu et al. 2008). PGE<sub>2</sub> provides a proinflammatory environment in which some viruses such as rotavirus thrive in, thus supporting and promoting their replication (Rossen et al. 2004). Both the innate and adaptive immunity can also be regulated by PGE<sub>2</sub>, which can either have adverse or beneficial effects on the immune system’s ability to fend off pathogens (Kalinski 2012). One of the most well-described effects of PGE<sub>2</sub> on the immune system is the effect it has on regulation and activity of T cells, thus affecting the interplay between innate and adaptive immunity. It is well described that PGE<sub>2</sub> modulates proliferation, apoptosis and cytokine production of CD4<sup>+</sup> T cells; however, it was not until recently that PGE<sub>2</sub> was implicated in the enhancement of T helper 2 (Th2) responses via cAMP, promoting infection of Enterovirus 71 (Wang et al. 2015).

Very recently, a study has linked a role for TG synthesis within LDs to the effective production of PGE<sub>2</sub> (Castoldi et al. 2020). This study has therefore placed LDs, in a position of central importance in inflammatory macrophage activation. The failure of inflammatory macrophages to make PGE<sub>2</sub> when TG synthesis is inhibited is critical for this phenotype, as addition of exogenous PGE<sub>2</sub> is able to reverse the anti-inflammatory effects of TG synthesis inhibition (Castoldi et al. 2020). These findings suggest that the mechanism by which PGE<sub>2</sub> enhances these inflammatory responses to viruses may directly be due to this accumulation of TGs within LDs, therefore, a more detailed analysis of the relationship of lipid classes within LDs during infection will provide insight into this relationship.

Although PGE<sub>2</sub> is the most well-described prostaglandin in the context of viral infections, Dimethyl Prostaglandin A<sub>1</sub> (dmPGA<sub>1</sub>) dmPGA<sub>1</sub> has interestingly been shown to have unusual broad-spectrum antiviral activity against both HSV and HIV-1 (Hughes-Fulford et al. 1992). Interestingly, closely related prostaglandins dmPGE<sub>2</sub> and dmPGA<sub>2</sub> did not have the same effect on the replication of HSV and their treatment on HSV-1 infected cells resulted in a 60% increase in replication compared with that in untreated virus-infected cells. Another well-studied member of class 1 lipid mediators are the leukotrienes, which are eicosanoid inflammatory mediators secreted to recruit immune cells, mostly leukocytes and eosinophils (Woodward et al. 1991; Medeiros et al. 1999). In particular, LTE<sub>4</sub> is a potent chemoattractant that favors the recruitment of leukocytes to inflammatory sites (Canetti et al. 2003; Grespan et al. 2008; Afonso et al. 2012; Lämmerrmann et al. 2013). LTE<sub>4</sub> has been found to inhibit the replication of a number of viruses including Influenza A and B, RSV and Coronavirus; however, LTE<sub>4</sub> secretion was recently demonstrated to facilitate the recruitment of HTLV-1-infected T cells and virus transmission in vitro (Percher et al. 2017) (Table 2).

### Class 2 lipid mediators

In comparison to class 1, class 2 lipid mediators have only been found to contribute to a proviral environment (Table 2). Class 2 lipid mediators are mainly membrane phospholipids (particularly phosphatidic acid, phosphatidylcholine, phosphatidylserine and phosphatidylinositol) and sphingolipids (ceramide and sphingosine-1-phosphate) (Hannun and Obeid 2008). They are reservoirs of bioactive metabolites of profound importance to a myriad of cell signaling and cellular processes that are important in immunity, inflammation and inflammatory disorders, including cell growth, survival, immune cell trafficking, and vascular and epithelial integrity (reviewed in Maceyka and Spiegel 2014). They are also known to exert pleiotropic effects such as inflammation, vesicular trafficking, endocytosis, cell cycle and senescence, survival and apoptosis, cell migration and cell-stress responses (El Alwani et al. 2006). Class 2 lipid mediators have been shown to be proviral, with members of this class exacerbating a number of viral infections (Table 2).

The phospholipid platelet-activating factor (PAF) is a proinflammatory class 2 lipid mediator, which has been found to enhance the replication of multiple viral infections such as HIV-1, RSV, DENV and HCV (Table 2). Endogenous release of PAF is related to a number of acute inflammation effects in DENV infection, such as increased vascular permeability, altered leukocyte numbers, thrombocytopenia and degrees of bleeding (Souza et al. 2009).

Class 2 lipid mediators are also made up of sphingolipids, a class of lipids that have several significant roles in regulating cellular functions. The sphingolipid family is made up of diverse members, including sphingomyelin, ceramide, ceramide 1-phosphate, sphingosine and sphingosine 1-phosphate (S1P). Member of class 1 lipid mediators are the leukotrienes, which rely on sphingosine kinase activity and S1P-S1PR signaling for their action. A potential subtype of class 2 lipid mediator, which has been found to be proviral, with members of this class exacerbating a number of viral infections (Table 2).

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Lipid mediators in viral infection. Lipid mediators can act in both a prohost and proviral sense during infection. Lipid mediators [prostaglandin E2 (PGE2), dimethyl prostaglandin A1 (dmPGA1), leukotriene B4 (LTB4), sphingosine-1-phosphate (S1P), platelet-activating factor (PAF) and lysophosphatidic acid (LPA)] all act to either restrict or foster viral replication. Lipid mediators can also act in a proviral manner to increase the production of inflammatory cytokines, restrict type-1 IFN signaling and increase cell–cell fusion [resolvin E1 (RvE1), protectin D1 (PD1) and lipoxins]. On the other hand, lipid mediators can decrease inflammatory cytokines and promote macrophage differentiation.

Class 3 lipid mediators

Class 3 lipid mediators are primarily derived from omega-6 fatty acids (lipoxins), and omega-3 fatty acids (resolvins, protectins and maresins) are produced during an inflammatory response, and act in reducing and/or resolving the inflammatory process in infection by stimulating inflammation resolution pathways (reviewed in Mittal, Ranganath and Nichani 2010; Molfino et al. 2011). As part of the neutrophil-monocyte sequence, the lipoxin signals promote the blocking of the acute inflammatory response. Lipoxins and resolvins stimulate the recruitment of immune cells and regulate the actions of the classic proinflammatory class 1 lipid mediators, prostaglandins and leukotrienes (Colas et al. 2014), reducing the duration of inflammation, and stimulating re-epithelialization, wound healing and tissue regeneration as signs of resolution (reviewed in Serhan 2014). Interestingly, unlike the other two classes of lipid mediators, class 3 lipid mediators have only been demonstrated to have prohost activities against viral infections (Table 2).

Lipoxins are anti-inflammatory metabolites of the arachidonic acid pathway, which are synthesized from arachidonic acid by the action of a series of lipoxygenases (Serhan, Hamberg and Samuelsson 1984). Lipoxins have been shown to alter levels of various transcription factors such as NF-κB, AP-1, PPARγ and Nrf-2, as well as various cytoplasmic signaling molecules such as phosphatidylinositol 3-kinase, AKT, mTOR, Ras, JAK and STAT to create an anti-inflammatory environment (Qiu et al. 2001; Weinberger et al. 2008; Prieto et al. 2010). As lipoxins are eicosanoids like prostaglandins and leukotrienes, they can be synthesized from LDs during host responses; however, a direct link between lipoxins and LDs during a viral infection has not been identified. As lipoxins are anti-inflammatory lipid mediators, they have been found to protect host cells against viral infections such as IAV (H5N1), RSV and Kaposi’s sarcoma-associated herpes virus (KSHV) by mainly regulating the production of anti-inflammatory cytokines and decreasing the levels of proinflammatory NF-κB, AKT, ERK1/2, COX-2 and 5-lipoxygenase (Chandrasekharan et al. 2016) (Table 2). Although IAV, RSV and KSHV are the only viruses for which lipoxins have shown an antiviral capacity for, interestingly they have been shown to be upregulated in a range of respiratory viruses.
POTENTIAL ROLES OF LIPIDS AND LIPID DROPLETS IN THE IMMUNE RESPONSE

The role of lipids and LDs in the viral immunity field is understudied; however, there are many lessons that can be learnt from other recent fields of research. The main constituents of LDs are neutral lipids, specifically triacylglycerols and sterol esters, both lipids that we are only beginning to realize as instrumental in orchestrating pathways in the immune responses. A small and growing body of literature has focused on the role of different lipid species during cellular pathways, such as the immune response. It has also been demonstrated that the combination of neutral lipids packaged into LDs can have differing immune functions. Recently, there has been more attention being paid to LDs, and research has started to find links in the function they have within a cell, however, there are still a lot of unanswered questions around their role in an infection, and whether their role may have a protective mechanism for the host, rather than dominate the driving of inflammation as has been suggested in some other pathologies.

LDs can have both direct and indirect effects on cell signaling and immune pathways. They can affect signaling pathways by actively removing signaling lipids from their bioactive pool to a storage pool, or by regulating their release and production from stored precursors (Coleman and Mashek 2011; Zechner et al. 2012). In addition, by regulating the storage and release of sterols, retinoids and ceramides, LDs also affect various signaling pathways that are directly or indirectly modified by these lipids and their metabolites. Sequestration of these various lipids into the LD core thus reduces their availability for the activation of signaling pathways involved in cellular stress and inflammation, including the activation of PPAR signaling, TLR and NF-κB inflammatory pathways by saturated FAs, the conversion of PUFAs into eicosanoids, the regulation of SREBP signaling by cholesterol and FAs, the activation of protein phosphatase 2A (PP2A) by ceramides, or of protein kinase C by membrane-resident DAG (Shen, Azhar and Kraemer 2016; Yu et al. 2018; Shmarakov et al. 2019; Stith, Velazquez and Obeid 2019). It is also possible that following TLR activation the dramatic increase in de novo lipogenesis and/or lipid uptake increases LDs as a way to protect cells against elevated free fatty acid levels and the toxic effects that this may have on the cells. Another relatively unexplored role of LDs during an infection, is how they contribute to the protection of important fatty acids during an infection. LDs have been demonstrated to be induced by reactive oxygen species (ROS) generated by environmental exposure to hypoxia and other prooxidants in a developing CNS in a Drosophila model (Bailey et al. 2015). LDs formed in the glia of the stem cell (neuroblast) niche during oxidative stress and were shown to limit ROS levels to inhibit the peroxidation of polyunsaturated fatty acids (PUFAs) via the diversion of PUFAs, away from membranes to the core of LDs, where they are less vulnerable to peroxidation. It is well established that activation of PRRs can trigger a ROS response (reviewed in Li et al. 2019b), and it is possible that this protective mechanism is also at play following PRR activation by viral infection, although this has not been explored to date. LDs could thus limit cell damage, prevent cell death and reduce the inflammatory response by sequestering PUFAs and reducing their availability for oxygenation and action as danger signals.

Recently, lipids directly derived from LDs, and separate to the lipid mediators described above, have been involved in the activation of a number of immune cells. Externally acquired fatty acids that are transiently stored in LDs have been demonstrated to be converted into phospholipids to promote the proliferation of innate lymphoid cells (Karagiannis et al. 2020). Innate lymphoid cells are big drivers of inflammation in the lung, with this metabolic program being imprinted by interleukin-33 and regulated by the genes Ppara and Dgat1, which are both controlled by glucose availability and mTOR signaling (Karagiannis et al. 2020). There is also evidence of lipids derived from LDs being packaged into exosomes and resulting in activation of a population of macrophages in adipocytes (Flaherty et al. 2019). In this case, LDs provide specific lipid species to bone marrow progenitor cells promoting their differentiation into macrophages, similar to adipose tissue macrophages (Flaherty et al. 2019). The higher numbers of circulating macrophages increase the likelihood of an enhanced immune response, thus highlighting the immune function of both LDs and lipid species within promoting an efficient immune response following infection. This example also highlights the ability of the lipid content of a LD to act externally to its local environment. Neurons have also been demonstrated to secrete excess FAs, which are taken up by astrocytic LDs during times of enhanced activity (Ioannou et al. 2019). The
accumulation of lipids in hyperactive neurons is not only toxic due to the susceptibility of these lipids to peroxidation, but also an overabundance of FAs may enter non-oxidative metabolic pathways triggering excessive ceramide production that is toxic to cells (Guenther et al. 2008). This coordinated mechanism for metabolizing FAs between cell types in the brain could potentially provide a protective mechanism by which cells are able to signal to nearby cells during viral infection, however, needs to be explored in more detail. While LDs are readily detected intracellularly, their extra-cellular presence is not widely reported; however, it has been noted (Eyster and Van Camp 2003; Corrêa Soares et al. 2007). We now know that there are several cases of lipids being secreted and taken up by neighboring cells, but if whole LDs are able to be released and potentially taken up by neighboring cells, this could have implications for not only the delivery of FAs but also potentially important proteins during infection. To our knowledge, this is something that has not been examined; however, it could be potentially important for cell-to-cell communication.

CONCLUSIONS/INSIGHTS

The role that lipids play in a viral infection is very complex, and although it is well understood that lipids are essential for cell survival by providing energy, underpinning the integrity and flexibility of cellular structures and contributing to signal transduction, we also know that many viruses are able to alter the cellular lipidome to benefit their own viral life cycles. Excess lipids are stored within LDs in cells, and over the last 15 years the accumulation of LDs has become a hallmark of general infection. The mechanisms driving accumulation of LDs in this sense have traditionally been presumed to be pathogen driven for their own benefit; however, recent work has now identified that LDs can also have a positive role in respect to the antiviral response, as well as influence the synthesis of many bioactive lipid mediators. Additionally, there are still questions that need to be addressed to fully understand why there seems to be such a differing role for lipids and their bioactive mediators during the host response to infection. As lipid synthesis machinery is induced very early following infection, it may be that analysis of the role of lipids at earlier time points following viral infection could provide us with a better understanding of the role of lipids for the host response. There also seems to be a balancing act in the fact that an early induction of lipids may help the host initially, but may lead to an exaggerated inflammatory response causing the production of cytokine storms, particularly in the lungs during respiratory viral infections, and a better understanding of what tips this scale may also underpin novel treatment strategies for viral infections moving forward.

It is clear that the field of LDs and their cargo is an emerging field of discovery with many unanswered questions; however, we believe that a better understanding of the roles LDs play in communicating with various immune pathways may assist in informing potential advanced therapeutic approaches to combat pathogenic microorganisms.

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