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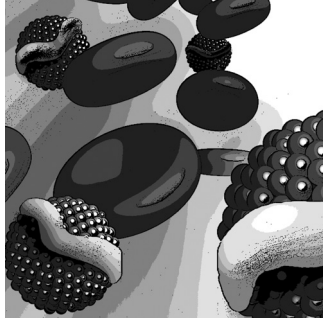

Lipid droplets in host–pathogen interactions

Increased cytoplasmic lipid droplets (also known as lipid bodies or adiposomes) in nonadipocytic cells are commonly observed pathological features of a number of infectious diseases. Although the understanding of the functional significance of lipid droplets to host–pathogen interactions and microbial pathogenesis is still very limited, great advances in this growing field have been made in the past few years. Here, we review the recent findings that are starting to uncover the molecular mechanisms that regulate lipid accumulation into lipid droplets and the functions that this organelle may play during infection, with an emphasis on the potential implications of lipid droplets on human infectious diseases.

KEYWORDS: eicosanoids ▪ inflammation ▪ intracellular pathogen ▪ lipid bodies ▪ lipid droplet ▪ LPS ▪ nuclear receptor ▪ sepsis ▪ tuberculosis

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Steatosis and lipid-laden foam cells are frequent pathological observations of a number of infectious diseases, and these reflect increased fat deposition into cytoplasmic lipid droplets. The concentration of lipids within the cytoplasm is in fact a feature of almost all cells, forming an organelle known as a lipid droplet, also termed an adiposome, lipid body or oil droplet. For a long time, lipid droplet functions in cells were considered to be limited to an inert lipid storage repository. However, accumulating evidence based on lipid droplet structure, composition, regulated biogenesis and interaction with other organelles has dramatically changed this concept. Lipid droplets are now considered dynamic and complex organelles that are composed of a triglyceride and cholesteryl ester core and a surrounding monolayer of phospholipid, cholesterol and a varied array of associated proteins with diverse functions in cell metabolism and signaling [1,2].

Among the proteins associated with the lipid droplets are the proteins that share sequence similarities and are termed the PAT family of proteins. This family includes perilipin [3], adipose differentiation-related protein (ADRP) [4,5], and tail-interacting protein of 47 kDa (TIP 47) [6]; more recently, other proteins of this family have been described [7,8]. The proteins from the PAT family are major structural proteins present at the surface of lipid droplets [9] and are often used as markers of lipid droplets in cells; they have also been implicated in lipid droplet assembly and biogenesis [4,5,10,11]. Perilipin is the most abundant protein on the adipocyte lipid droplet, and it modulates the lipase functions, while ADRP, which is ubiquitously expressed, is involved in the regulation of lipid droplet accumulation in different cells [11,12]. Aside from the accumulation of lipids, it has been shown that lipid droplets compartmentalize enzymes involved in the biosynthesis, transport and catabolism of lipids [13–19], suggesting a wide role for lipid droplets in the regulation of cellular lipid metabolism. It should be noted that proteins involved in membrane and vesicular transport, including Rab proteins and caveolin [17–25], and proteins involved in cell signaling and inflammatory mediator production, including eicosanoid-forming enzymes [26–32], phospholipases [14,33,34] and protein kinases [14,35,36], were localized to lipid droplets in different cells and activation conditions. This suggests that lipid droplets have roles as specialized, inducible cytoplasmic organelles with functions beyond the regulation of lipid metabolism in cell signaling and activation, membrane trafficking and control of the synthesis and secretion of inflammatory mediators.

Here, we review the current knowledge of the mechanisms that regulate lipid droplet formation and function during the host response to bacterial and parasite infection. The roles of lipid droplets in viral infection have been recently reviewed elsewhere and will not be addressed here [37].

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Cellular & molecular mechanisms of lipid droplet formation during infection

■ Cellular mechanisms of lipid droplet biogenesis

The biogenesis of lipid droplets is a biological process that has gained attention over the past few years. Although in the past, the presence of lipid droplets in cells has been implicated with storage and lipid trafficking, it is becoming clear that lipid droplets are highly regulated organelles involved in inflammatory and infectious processes.

The prevailing hypothesis of lipid droplet biogenesis suggests that lipid droplets are endoplasmic reticulum (ER)-derived organelles. Different models of lipid droplet biogenesis have been proposed in recent years; however, the subject is still highly controversial. The first model to be proposed is still largely accepted and is based on the formation of the hydrophobic neutral lipid core between the two leaflets of the ER bilayer owing to the accumulation of enzymes involved in lipid metabolism in this region. As a result, nascent lipid droplets loaded with proteins lacking transmembrane-spanning domains would bud off from the ER into the cytoplasm and end up surrounded by a monolayer of phospholipids directly derived from the cytoplasmic leaflet of the ER [2,38–40].

Accumulating evidence gathered by independent groups have identified the presence of membrane-associated and transmembrane spanning proteins [26,27,30–32,41–43] as well as ribosomal structures, ribosomal-associated proteins and RNA-interacting proteins [19,44–50] within lipid droplets in leukocytes and other cells, thus suggesting a greater complexity of the structure and biogenesis of lipid droplets than initially anticipated. As such, new hypothetical models of lipid droplet biogenesis have been recently proposed [1,19,39,51,52]. Accordingly, Weller and colleagues have proposed a novel model of lipid droplet biogenesis by the incorporation of multiple loops of ER membranous domains within the forming lipid droplets that may help explain the presence of membrane-associated and transmembrane-spanning proteins within lipid droplet cores [1,19]. This model is based on observations of different cell types by electron microscopy (EM) that describe membranous and membranotubular structures within the lipid droplets [19,27,53] together with the observations of immunogold EM localization of proteins with predicted membrane insertion, including caveolin and cyclooxygenase to permeate lipid droplet cores [26,27,41–43].

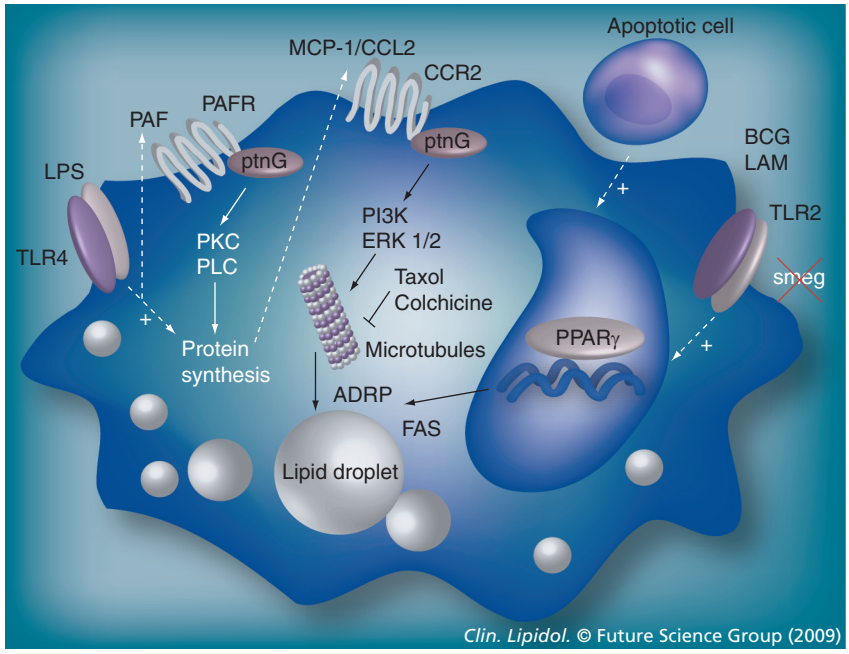

Figure 1. Molecular mechanisms regulating lipid droplet biogenesis during infection. Lipid droplet formation in leukocytes is a highly regulated process that involves receptor-mediated signaling. Lipid droplets formed during infection activate different intracellular signaling pathways, which culminates in the compartmentalization of lipids and proteins. As shown in the scheme, LPS stimulates the formation of lipid droplets through TLR4-dependent signaling that culminates in production of inflammatory mediators such as PAF and MCP-1/CCL2 that amplify the response. Apoptotic cells and pathogenic bacteria can also stimulate lipid droplets. Increased PPAR-γ expression and activation are observed during infection and could participate in the formation of lipid droplets by enhancing ADRP and FAS expression. The formation of lipid droplets is dependent on ADRP synthesis and FAS activity, as well as microtubules directing the droplet assembly.
ADRP: Adipose differentiation-related protein; BCG: Bacillus Calmette–Guérin; CCL: Chemokine ligand; FAS: Fatty acid synthase; LAM: Lipoarabinomannan; LPS: Lipopolysaccharide; MCP: Monocyte chemoattractant protein; PAF: Platelet-activating factor; PAFR: Platelet-activating factor receptor; PLC: Phospholipase C; TLR: Toll-like receptor.

■ Lipid droplet formation involves specific & well-regulated mechanisms

Different signaling pathways have been implicated in lipid droplet biogenesis in leukocytes and other cells involved in inflammatory and/or infectious reactions. Although relatively little is known regarding the molecular mechanisms that govern lipid droplet biogenesis, the observations gathered so far indicate the involvement of both pathogen and host factors **(Figure 1)**.

Lipid droplet biogenesis can be rapidly detected after a short time of stimulation. It has been demonstrated that this phenomenon depends not only on direct interaction between the pathogen and host cells but also on indirect mechanisms of

a bystander amplification-induced system through bacterial components and/or host-generated cytokines [29,54–57].

The role of cell migration in lipid droplet biogenesis has also been investigated and suggests that migration may modulate, but is not an obligatory requisite, for lipid droplet formation in leukocytes during inflammation [58]. Lipid droplet numbers are drastically increased in blood leukocytes from septic patients or rats with Chagas disease when compared with blood leukocytes from healthy subjects [29,59]. In addition, resident macrophages have a significant increase in lipid droplet numbers *in vivo* following lipopolysaccharide (LPS) stimulation [29], indicating that leukocytes that did not undergo migration can also form new lipid droplets *in vivo* during an inflammatory disease. Conversely, infection with nonpathogenic *Mycobacterium smegmatis* induced an intense recruitment of leukocytes but failed to trigger the process of lipid droplet biogenesis [58]. *In vivo* infection with *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) induced both cell recruitment and lipid droplet formation within recruited leukocytes. In this case, lipid droplet biogenesis is at least partly mediated by the phagocytosis of apoptotic neutrophils by macrophages. Indeed, BCG induced an early and huge neutrophil recruitment and activation, which was followed by apoptosis of neutrophils in the inflammatory site [60]. Treatment with the pan caspase-inhibitor zVAD-fmk peptide was able to prevent neutrophil apoptosis and reduce lipid droplet formation during BCG infection *in vivo* [60].

Role of innate immune receptors in lipid droplet formation

Members of the family of Toll-like receptors (TLRs) in vertebrates have been implicated as major pattern recognition receptors with a broad range capacity of sensing pathogen signature molecules from diverse organisms including bacteria, viruses, fungi and protozoa. These receptors have been shown to be critical triggers of host-cell signaling [61]. The involvement of membrane receptors in the mechanism of lipid droplet formation during the host response to infections has been investigated. Of the TLR family, TLR4 is expressed in a wide variety of human cells and serves as the primary recognition molecule for LPS from Gram-negative bacteria. Cells from TLR4-mutated mice (C57BL/10 ScCr) and TLR4-signaling inactive mice (C3H/HeJ) failed to form lipid droplets triggered by LPS [29,54], thus establishing that LPS-induced lipid droplet formation in macrophages occurs through a mechanism largely dependent on TLR4. Different proteins in addition to TLR4 are required to mount a sensitive cellular response to LPS, including the LPS-binding protein, CD14, CD11b/CD18 and myeloid differentiation factor (MD)-2. Accordingly, neutralization of the LPS-binding proteins CD14 or CD11b/CD18 inhibits LPS-induced lipid droplet formation [29].

Besides TLR4, other TLRs are involved in the biogenesis of lipid droplets. Different members of the TLR family, including TLR1, TLR2, TLR4 and TLR6 have been implicated in mycobacterial infection recognition [62–64]. *M. bovis* BCG and the purified cell wall component lipoarabinomannan (LAM) were potent inducers of lipid droplet formation in cells from wild-type mice but failed to induce lipid droplet formation in leukocytes from mice genetically deficient in TLR2, but not TLR4 or TLR6, suggesting an important role for TLR2 in this phenomenon [54,55,65]. Interestingly, the activation of macrophages *in vitro* with *M. smegmatis*, zymosan or Pam_3Cys (which are all potent TLR2 ligands) at doses that significantly induced TNF-α production, were unable to induce lipid droplet biogenesis in macrophages under conditions where BCG infection was highly effective. This suggests that although TLR2 activation is essential for mycobacterial-induced lipid droplet formation, it is not sufficient to trigger pathways of lipid droplet formation, and other co-factors may be involved [54,65]. In addition, the induction of lipid droplet formation by *Mycobacterium leprae* was significantly inhibited by either TLR2- or TLR6-deficient macrophages [66]. However, this inhibitory effect was mainly observed in cells with no internalized bacteria in contrast to cells bearing bacteria, in which lipid droplet formation was poorly affected. Furthermore, the conditioned medium from *M. leprae*-infected wild-type macrophages was able to induce lipid droplet formation in wild-type and TLR2-deficient macrophages [66]. This suggests an important role for TLR signaling in the amplification of the paracrine/autocrine signals to form lipid droplets.

Similarly, *Chlamydia pneumonia*, a pathogen that has been implicated in human and murine macrophage foam cell formation and is a hallmark of early atherosclerosis, expresses a variety of ligands that could serve as potential TLR ligands. Accordingly, it has recently been demonstrated that chlamydial infection

can induce macrophage lipid droplet formation in the presence of LDL via TLR2-dependent mechanisms, but not those dependent on TLR4 [67]. Interestingly, *Histoplasma capsulatum,* the causative fungus of histoplasmosis, a pulmonary disease characterized by chronic granulomatous reactions, induced a dose- and time-dependent change of lipid droplet numbers in murine leukocytes in a dectin-1-, CD18- and TLR-2-dependent manner [68].

Among other responses, these data show that the activation of TLRs by pathogens and/or pathogen-derived molecules triggers signaling pathways that are involved in the formation of lipid droplets in host-infected cells and also trigger the indirect mechanism of activation of a bystander amplification-induced system through host-generated cytokines and chemokines.

Paracrine signaling involved in lipid droplet formation

The engagement of TLR proteins activates the expression of proinflammatory mediators by macrophages and has been shown to regulate host susceptibility to pathogens. Distinct lipid mediators, cytokines and chemokines have been previously described in the signaling that leads to lipid droplet formation [28,29,56,69–72]. Platelet-activating factor (PAF) and PAF-like lipids are believed to play an important role in lipid-body formation induced by LPS or by oxidized LDL *in vivo*, since pretreatment with PAF receptor antagonists significantly inhibited LPS- and sepsis-induced lipid droplet formation [29,73,74]. Although PAF may act at intracellular binding sites to induce cell activation, the results suggest that PAF is acting in a paracrine/autocrine way to induce lipid body formation, since the PAF-receptor antagonists used in those studies act preferentially at membrane receptors, and lipid droplet formation induced by LPS or oxidized LDL were inhibited by treatment with extracellular PAF-acetylhydrolase [73,74]. The downstream pathways engaged in PAF-induced lipid droplet formation involve the activation of 5-lipoxygenase (LO), protein kinase C and phospholipase C [70]. In addition, PAF-induced lipid droplet biogenesis also appears to occur, at least partly, via a new protein synthesis-dependent manner that involves cross-talk with MCP-1 [29,70,73] and is amplified by PPAR-γ activation [75].

The monocyte chemoattractant protein (also known as chemokine ligand [CCL]2) was identified as a molecule capable of triggering lipid droplet biogenesis within macrophages in a process dependent on the activation of a chemokine (C-C motif) receptor (CCR)2-elicited extracellular signal-regulated protein kinase (ERK) and phosphatidylinositol 3-kinase (PI3K) signaling, as well as on an intact and dynamic microtubular system [56]. Indeed, MCP-1-driven lipid droplet biogenesis is a highly regulated phenomenon that culminates in microtubule-dependent lipid droplet assembly and protein compartmentalization during infection-related inflammatory responses [56]. In fact, MCP-1 is centrally involved in the regulation of macrophage lipid droplet biogenesis in oxidized LDL- and LPS-induced inflammation, as well as in experimental sepsis, acting as a key endogenous mediator involved in the pathogenesis of macrophage-driven inflammation [56,72,73].

Although MCP-1 is centrally involved in the regulation of macrophage lipid droplet biogenesis in inflammatory conditions [56,72,73], studies in mice genetically deficient in MCP-1 have indicated that this chemokine is not necessary in BCG-induced lipid droplet formation during mycobacterial infection [54]. It should be noted that MCP-1 is produced in the course of BCG infection where it plays a role in leukocyte recruitment [76]. This suggests that redundant lipid droplet-triggering mechanisms may occur in the course of mycobacterial infection. Similar to this, it has been demonstrated that IFN-γ- or TNF-α-deficient mice are extremely susceptible to infections by tuberculosis-causing organisms [77]; however, both cytokines failed to modify BCG-induced lipid droplet biogenesis [54]. On the other hand, BCG-induced lipid droplet accumulation was largely dependent on TLR2-dependent, macrophage-derived, eotaxin-mediated CCR3 activation in eosinophils, but not in macrophages [55]. Other G-protein-coupled receptor agonists, including IL-8, complement component C5a and leukotriene (LT)-B_4, did not induce leukocyte lipid droplet formation, which demonstrates the requirement of specific intracellular signaling mechanisms in the process of lipid droplet biogenesis [70].

These data demonstrate that infectious stimuli lead to distinct host responses that are responsible for the amplification of the formation of lipid droplets not only in infected cells and cells in direct contact with pathogen-derived molecules but also in neighboring cells that sense the infection through inflammatory mediators secreted by the former cells.

Modulation of gene expression by nuclear receptors is involved in lipid droplet formation

Besides identification of the activation stimuli involved in the formation of lipid droplets, progress has been made in understanding how cells respond to these stimuli to form these organelles. Recent studies have demonstrated that bacterial components may regulate PPAR-γ expression and function. PPAR-γ is a member of the lipid-activated nuclear receptor family and has been demonstrated to function as a key transcriptional regulator of cell differentiation, inflammation and lipid metabolism in macrophages and dendritic cells (DCs) [78]. Indeed, PPAR-γ is highly expressed in macrophage-derived foam cells within atherosclerotic lesions, where it plays an important role in lipid homeostasis and metabolism [79–82]. Almeida and colleagues have demonstrated that TLR2 activation is involved in the regulation of PPAR-γ expression and/or activation during experimental infection by *M. bovis* BCG. BCG-induced PPAR-γ expression, lipid droplet formation and TNF-α generation were drastically inhibited in TLR2-deficient mice, demonstrating a requisite role for TLR2 in BCG-mediated macrophage upregulation of PPAR-γ protein content. Interestingly, activation of macrophages *in vitro* with *M. smegmatis* also failed to induce PPAR-γ expression and lipid droplet formation, although it did induce TLR2-dependent TNF-α [65].

How does PPAR-γ modulate lipid droplet formation? The PPAR transcription factor directly regulates the expression of several genes participating in fatty acid uptake, lipid storage and the inflammatory response by binding to specific DNA response elements in target genes as heterodimers with the retinoid X receptors (RXR), including fatty acid synthase and ADRP [83–85]. It should be noted that treatment with the fatty acid synthase inhibitor C75 has been shown to significantly inhibit lipid droplet formation in macrophages induced by apoptotic cells with or without infection, confirming the role of new lipid synthesis in lipid droplet biogenesis [60]. In addition, increased expression of scavenger receptors including macrophage receptor with collagenous structure, macrophage scavenger receptor and CD36, has been observed in mycobacterial infection leading to increased uptake and accumulation of host-derived oxidized lipids in the infected cells [86]. Conversely, enhancing cholesterol efflux by liver X receptor (LXR) activation by the synthetic agonist GW3965 significantly decreased the cholesterol ester content of cells triggered by the TLR-dependent mechanisms, including cells exposed to *C. pneumonia* and LPS [67]. Of note, viral- and bacterial-derived TLR-ligands have been shown to inhibit LXR transcriptional activity and cholesterol efflux via activation of TLR3 or TLR4 signaling pathways [87]. Collectively, these data suggest that mechanisms of increased lipogenesis, as well as regulation of the influx and efflux of lipids, operate synergistically with lipid droplet accumulation during infection.

The PPAR-γ-regulated expression of ADRP has been shown in different cells and conditions [88,89]. As previously discussed, ADRP is a member of the PAT family of proteins and plays an important role in adipocyte differentiation, lipolysis modulation, lipid body assembly and biogenesis [11]. Increased ADRP expression has been demonstrated to directly promote triglyceride and cholesterol storage and to reduce cholesterol efflux [90–92]. ADRP may also act as a nucleation center for the assembly of lipids to form nascent lipid bodies and to enhance droplet stability upon lipolytic conditions [93,94]. In addition to transcriptional regulation, ADRP may also be regulated at the translational level or by post-transcriptional mechanisms [95–98].

PPAR-γ has been shown to participate in the formation of lipid droplets during infection at distinct levels. Further studies to identify other transcription factors and mechanisms involved in the modulation of the cell response that lead to lipid droplet biogenesis would contribute to a better understanding of the role of these organelles during infection.

Lipid droplet roles in the pathogenesis of infectious diseases

■ Lipid droplets in sepsis

Lipid droplets are involved in many inflammatory diseases. Previous studies have demonstrated the presence of lipid droplets in bacterial infections, including clinical and experimental bacterial sepsis [29,56,74], endotoxic shock [29,56], septic arthritis [99] and bronchoalveolar fluids of animals and patients with adult respiratory distress syndrome (ADRS) [100,101]. Sepsis is a systemic inflammatory response associated with infection. The development of sepsis involves the deregulated production of inflammatory mediators with a complex interaction of lipids, carbohydrates and protein mediators. At present, great attention is being devoted to the role of lipid metabolism and lipid-derived mediator functions in sepsis. In addition, studies

are beginning to shed light on the importance of lipid domains, including lipid rafts, caveolae and lipid droplets, in the signaling compartmentalization and regulation of the innate immune response [58,102,103]. Here, we review the current evidence that identifies lipid droplets as critical regulators of inflammatory mediator production in sepsis and in the proinflammatory amplification loop during endotoxic shock or sepsis.

Arachidonic acid (AA) esterified within phospholipids and neutral lipids that can be metabolized into eicosanoids in several inflammatory processes have been demonstrated within lipid droplets. Eicosanoids, including leukotrienes and prostaglandins, are a family of potent signaling lipids derived from the enzymatic oxygenation of AA. Eicosanoids may control key processes involved in cell–cell communication, including cell activation, recruitment and metabolism [102,104], and their increased production and effects have been described in the pathophysiology of sepsis. The highly regulated generation of eicosanoids is dependent on the activation of phospholipases and specific eicosanoid-synthesizing enzymes and activation-dependent localization of enzymes in discrete compartments within cells [1,105–109]. The intracellular compartmentalization of eicosanoid synthesis has emerged as a key feature that not only regulates the amount of eicosanoid generated, but may also have implications for the type of eicosanoid produced and may regulate the effects they exert. In agreement with the hypothesis that the cellular responses leading to lipid droplet biogenesis may participate in heightened eicosanoid synthesis during inflammation and sepsis, lipid droplets have been shown to be enriched in LO and cyclooxygenase eicosanoid-forming enzymes in peripheral leukocytes from septic patients [29]. The lipid droplet compartmentalization of key eicosanoid-forming enzymes has been validated by using a variety of techniques, cells and stimulatory conditions. The major enzymes, 5-LO, 15-LO, 5-LO-activating protein and COX, which are involved in the enzymatic conversion of AA into eicosanoids, were shown by immunocytochemistry/immunofluorescence, ultrastructural postembedding immunogold EM and/or western blotting from subcellular fractions to localize within lipid droplets stimulated *in vitro* [26–28,41,110,111] or obtained from *in vivo* inflammatory responses [29,54,56,71,75,95,112]. Indeed, increased lipid droplet numbers correlate with an increased capacity of the cells to produce eicosanoids, thus suggesting that the compartmentalization of eicosanoid-synthetic machinery within lipid droplets may have a role in the cellular capacity to generate eicosanoids [29,56,113]. Recently, we have provided direct evidence of the role of lipid droplets in leukotriene generation during infection-driven cell activation. Using a technique to immobilize the leukotriene at its site of synthesis, we demonstrated that LPS-induced lipid droplets were major locales of LTB_4 synthesis within macrophages and neutrophils in a mechanism that was requisitely dependent on MCP-1 and microtubule-dependent lipid droplet assembly and protein compartmentalization [56]. Collectively, these data add support to the role of lipid droplets as dynamic organelles involved in inflammatory mediator production and amplification of the inflammatory response in sepsis. It should be noted that endotoxin-induced lipid droplet formation and the subsequent effect on lipid mediator production were modulated by dietary fatty acid intake. Dietary intake of extra virgin olive oil for 6 weeks led to the inhibition of LPS-induced lipid droplet formation and decreased the generation of LTB_4 [114]. Inhibition of lipid droplet formation and inflammatory mediator production by an olive oil-enriched diet also protected mice from death associated with endotoxic shock [114]. Similarly, dietary fatty acids also have modifying roles on membrane-associated lipid domains [102], as the consumption of the long chain n-3 polyunsaturated fatty acids has been demonstrated to modify the composition of lipid rafts and caveolae, impacting their functions in signaling [115–118]. Recently, the effects of four commercial lipid emulsions with diverse fatty acid compositions were evaluated for their capacities to modulate lipid droplet formation and eicosanoid production by human mononuclear cells and neutrophils [119]. Exposure of leukocytes to lipid emulsions led to increased lipid droplet formation and potentiated lipid droplet formation induced by LPS in the *in vitro* system [119]. Notably, there may be differences in the effects and modes of action of fatty acids, whether the fatty acids are *in vitro*-administered or ingested during prolonged dietary intake. Further studies to address the role of the dietary intake of different fatty acids on lipid droplet formation and function would be of interest.

Pseudomonas aeruginosa, which commonly infects the airways, is one of the major pathogens associated with hospital-acquired pneumonia, acute lung injury and sepsis in hospitalized

patients. One of its multiple virulence factors is the type III secretion toxin ExoU, which has cytosolic PLA_2 effects [120] and has been implicated in the increased prostaglandin production observed during *P. aeruginosa* infection *in vivo* and *in vitro* [121,122]. Cytosolic PLA_2 plays important roles in cellular signaling and prostanoid metabolism. Human airway epithelial cells infected with *P. aeruginosa* potently released IL-6, IL-8, AA and PGE_2 via a mechanism that was highly dependent of the PLA_2 activity of ExoU [121,122]. In sharp contrast to the observation following LPS administration or other bacterial infections, which showed increased formation of lipid droplets, the airway cells exposed to *P. aeruginosa* ExoU exhibited significantly decreased lipid droplet contents. Notably, this ExoU-induced mobilization of lipid droplets culminated with the release of free AA as well as their conversion to PGE_2 and was reduced by previous treatment of bacteria with a cytosolic PLA_2 inhibitor [122]. This suggests that as part of their pathogenic mechanism, toxins from *P. aeruginosa* may mobilize host lipids from lipid droplets.

Curiously, lipid droplets are also sites of cytokine storage. Cytokines are a family of glycoproteins that have a variety of biological activities, including cell growth, inflammation, immunity, differentiation and repair, and have key functions in sepsis. Cytokines and chemokines are produced and secreted by a variety of activated leukocytes as well as by other cells participating in the inflammatory response, such as endothelial and epithelial cells. It has been demonstrated that cytokines/chemokines and growth factors can be localized to lipid droplets formed in activated leukocytes through mechanisms that are currently unknown [123–125]. Notably, two key cytokines in sepsis pathophysiology, TNF and macrophage migration inhibitory factor, were localized within leukocyte lipid droplets formed after LPS stimulation *in vivo* and within lipid droplets in peripheral blood neutrophils and monocytes from septic patients [29,126]. What is the functional significance of cytokine storage in lipid droplets? If lipid droplets represent additional subcellular storage compartments for cytokines within leukocytes, routes for lipid droplet-derived cytokine-mediated secretion may exist and still need to be characterized. Alternatively, lipid droplet-stored cytokines may function locally as intracrine-signaling mediators. Future studies will be required to characterize this issue.

■ Lipid droplets & intracellular bacterial infection

Lipid droplet formation has been observed in distinct types of cells in the course of both intracellular and extracellular bacterial infections under clinical and experimental conditions. During intracellular bacterial infections, the presence of foamy lipid-laden differentiated cells is often observed in human and experimental infections, as shown in *M. bovis BCG* [54,60,65], *Mycobacterium tuberculosis* [127,128], *Mycobacterium leprae* [66], *C. pneumonia* [67] and *Chlamydia trachomatis* [129] infections. There is evidence to show that lipid droplet accumulation during infections is a well-regulated phenomenon that may have implications for microbial survival inside leukocytes and epithelial cells [54,57,60,65,129–131].

The capacity of pathogenic versus nonpathogenic species of mycobacteria to induce lipid droplet accumulation has been analyzed. Pathogenic mycobacteria, such as *M. tuberculosis, M. avium* and *M. leprae*, were able to induce lipid droplet accumulation in human cells during granulomatous [57] and lepromatous responses [66,132]. In addition, *M. bovis* BCG [54,55,60,65,133] and *M. leprae* [66] triggered lipid droplet formation in murine leukocytes. In contrast, poorly virulent or avirulent mycobacteria, such as *M. smegmatis*, are not able to induce lipid droplet formation in either human or murine cells [54,57,65].

In this context, components from bacterial cell walls, including *M. bovis* BCG- or *M. tuberculosis*-derived LAM, can mimic the pathogen and induce lipid droplet accumulation in a time- and dose-dependent manner [D'Avila H, Unpublished Data] [54,65]. In addition, Peyron and colleagues have demonstrated a direct role of *M. tuberculosis*-derived oxygenated mycolic acids, which are involved in virulence mechanisms, in foamy macrophage formation [57]. Interestingly, this effect is induced by both whole bacilli and isolated lipids, suggesting that oxygenated mycolic acids and LAM are either secreted by the bacilli or exposed at the cell wall surface, enabling their bioactivity.

It has been demonstrated that enhanced lipid droplet formation during intracellular pathogen infection is associated with an increased generation of eicosanoids [54,55,60,65,68,133]. Evidence supports the hypothesis that the formation of new lipid droplets elicited by bacterial infection are a distinct cytoplasmic domain for regulated eicosanoid production. First, lipid droplet

accumulation induced by bacterial and fungal infections are sites of localization of 5-LO in BCG [54] and *Histoplasma capsulatum* infections [68] and COX-2 in BCG infection [54]. Second, increased lipid droplet formation in leukocytes has been correlated with a significantly enhanced capacity of leukocytes to generate PGE_2 or LTB_4, suggesting that lipid droplets are early response structures involved in the production of lipid mediators in bacterial and fungal infections [29,54,56,65,68,133].

Eicosacell, a technique that enables the immobilization of newly formed eicosanoids at their intracellular site of synthesis [134], has been used to directly localize prostaglandins derived from lipid droplets induced by BCG infection [54]. Macrophages are the main prostaglandin-producing cells in the BCG-induced inflammatory reaction. Macrophages from BCG-stimulated, but not vehicle-stimulated, mice exhibited a strong, localized punctate immunofluorescent staining for PGE_2 that perfectly matched ADRP-stained lipid droplets [54]. It should be noted that prostaglandins downmodulate many macrophage functions. Indeed, prostaglandins lead to decreased proinflammatory cytokine secretion, decreased antigen presentation and decreased production of free radicals in macrophages and other leukocytes. Altogether, these data point to a central role for lipid droplets as compartmentalization sites of inflammatory mediator production, favoring increased production of eicosanoids in cells involved in infectious processes. In addition, according to the cell and stimuli, lipid droplets may compartmentalize different sets of proteins that may lead to either proinflammatory amplification or the down-modulation of leukocyte functions.

Moreover, Almeida and colleagues have offered evidence that *M. bovis* BCG is able to increase macrophage lipid accumulation and PGE_2 formation through the increased expression and activity of PPAR-γ [65]. It was observed that the PPAR-γ agonist BRL49653 potentiated lipid droplet formation and PGE_2 production induced by suboptimal doses of BCG and that pretreatment with the selective PPAR-γ antagonist GW9662 significantly inhibited lipid droplet PGE_2 production induced by BCG, thus indicating a required role for PPAR-γ signaling activation in lipid droplet biogenesis and further prostanoid production during BCG infection. In high concentrations, PGE_2 is a potent inhibitor of the Th1-type response and TNF and nitric oxide (NO) production. In experimental BCG infection, treatment with aspirin or NS-398 led to an enhancement of TNF production and a drastic reduction of IL-10 generation induced by BCG, which paralleled the inhibitory effects of these NSAIDs on PGE_2 and lipid droplet formation. In agreement with these results, increased levels of PGE_2 favored intracellular pathogen growth, a phenomenon that could be reverted by treatment with COX-2 inhibitors [54,135,136]. Current findings support the hypothesis that lipid droplet-derived endogenous PGE_2 downmodulates the macrophage response by inhibiting BCG-induced TNF production and increasing the levels of the anti-inflammatory cytokine IL-10, and as such, pharmacological inhibition of either prostaglandin production or lipid droplet formation would have beneficial effects to the host to control the infection. Accordingly, PPAR-γ inhibition in macrophages not only leads to decreased lipid droplet biogenesis but also enhances macrophage mycobacterial killing, supporting the hypothesis that lipid droplets may have implications in the pathogenesis of mycobacterial infection via PPAR-γ expression and activation [65]. Future studies in animal models as well as in *M. tuberculosis* infection will be necessary to further characterize the role of PPAR-γ and lipid droplets in the pathogenesis of tuberculosis and as targets for therapeutic intervention.

Besides the impact of lipid droplets in regulating the host response to infection by modulating inflammatory mediator production, lipid droplets may serve as rich sources of nutrients for intracellular pathogens, thus favoring bacterial replication. Some evidence suggests that adipose tissue or foamy cells within the granuloma might constitute one important mycobacterial reservoir, in which the *M. tuberculosis* could persist in a dormancy-like state and avoid antimycobacterial drugs and host defense mechanisms [57,130,131]. Although there is still no direct evidence that this mechanism occurs, a number of findings suggest that lipid droplet interaction with pathogen-containing compartments occurs. In mycobacterial infections, for example, lipid droplets observed in experimental tuberculosis models have frequently been found in close proximity to phagosomes with suggestive images of organelle interactions [54,130,131] **(Figure 2)**. In addition, Luo and colleagues demonstrated that the mycobactin (the lipophilic siderophore of mycobacteria) and iron complex accumulated with high selectivity in macrophage lipid

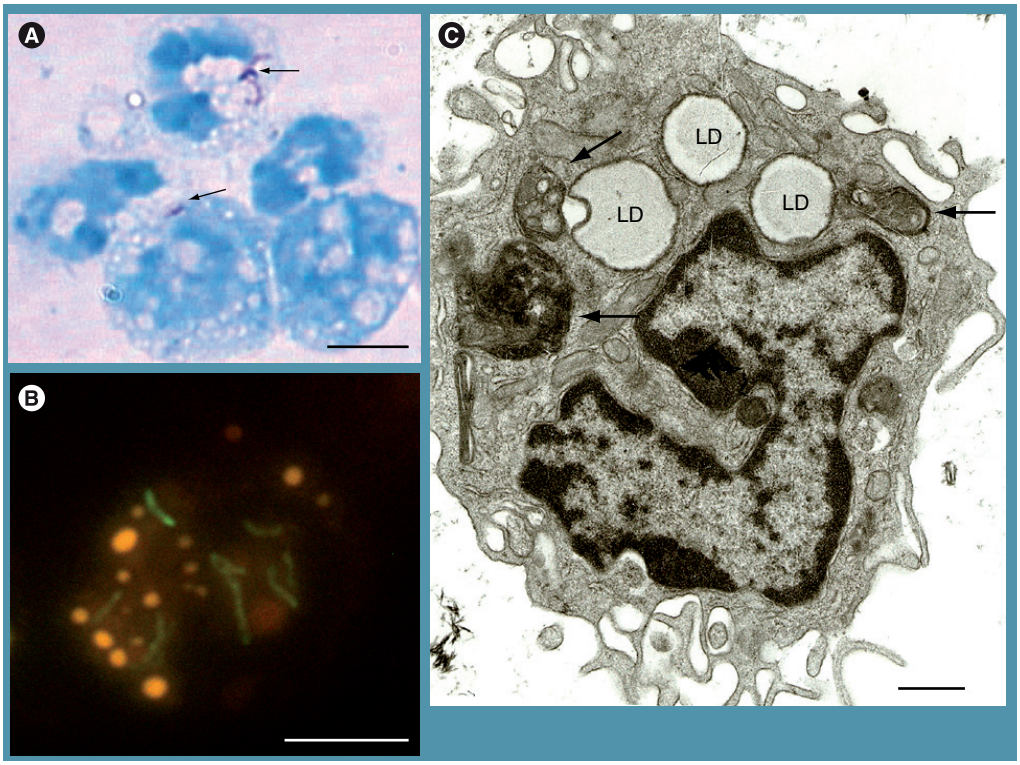

Figure 2. Leukocytes from bacillus Calmette–Guérin-infected mice exhibiting cytoplasmic lipid droplets. (A) Foam cells containing phagocytosed bacillus Calmette–Guérin (arrows) after Kinyoun's staining. **(B)** Fluorescent Nile Red-labeled lipid droplets were visualized as cytoplasmic orange punctate inclusions, while phagocytosed fluorescently labeled bacillus Calmette–Guérin is imaged in green in one infected macrophage. Scale = 10 µm. **(C)** Ultrastructural features of a macrophage presenting LDs and phagosomes with amorphous material (arrows). Scale = 1 µm. LD: Lipid droplet.
(C) Taken with permission from [54] © 2006 The American Association of Immunologists, Inc.

droplets [137]. Ferric mycobactin-enriched lipid droplets were found in close contact with phagosomes, suggesting that this organelle association could enable iron delivery to mycobacteria [137]. It has recently been shown that infection with *Chlamydia trachomatis*, the causative agent of trachoma and many sexually transmitted diseases, leads to increased lipid droplet formation. Moreover, chlamydial infection targets host lipid droplets into proximity with phagosomes, and lipid droplets are often observed intact inside *Chlamydia*-containing phagosomes, which is suggestive of a fusion of host lipid droplets with the phagosome [129,138]. Moreover, the inhibition of lipid droplet formation by triacsin C greatly reduced chlamydial proliferation, supporting evidence that *C. trachomatis* targets lipid droplets to enhance its survival and replication in host cells [129]. Although the significance of lipid droplet–phagosome interactions remains to be elucidated, it raises intriguing possibilities that lipid droplets may serve as a carbon source for intracellular pathogen growth.

■ Lipid droplets & parasite infection

Lipid droplet accumulation has been shown in the cytoplasm of distinct cell types after infection with various parasites, such as *Plasmodium berguei* [139], *Tripanosoma cruzi* [59,140], *Toxoplasma gondii* [141] and *Leishmania amazonensis* [142]. Parasites evolved complex strategies to infect hosts and escape the immune system of the host. Interestingly, many biological responses developed by the host against parasite infection are exploited by these microorganisms for their survival and growth.

Analogous to mycobacterial infections, *T. cruzi* infection in rats leads to the generation of lipid droplets in macrophages, which is paralleled by an increase in PGE_2 levels [59]. PGE_2 is thought to be involved in distinct mechanisms that favor parasite growth [135,142]. *T. cruzi* infection is associated with T-cell apoptosis and apoptosis exacerbates parasite growth [135]. Phagocytosis of apoptotic cells stimulates macrophages toward an anti-inflammatory profile, characterized by PGE_2 and TGF-β generation.

TGF-β shifts the macrophage response of NO production to arginine metabolism, which has been shown to fuel *T. cruzi* replication. Inhibition of COX-2 ablates TGF-β production and, as a consequence, reverts the enhancement of parasitemia induced by the phagocytosis of apoptotic cells [135]. Notably, the uptake of apoptotic bodies by macrophages has been shown to induce lipid droplet formation and PGE_2 generation [60], but whether and how it participates in the enhancement of parasitemia is currently under investigation.

Although autophagy has been shown to participate in parasite elimination through targeting entrapped parasites to lysosomes [143], starvation-induced autophagy in Balb-c mouse macrophages is accompanied by increases in lipid droplet numbers and facilitation of *Leishmania* parasite growth [142]. This effect has been correlated to the induction of lipid droplet formation, an increase of the PGE_2 level, and reduction of NO production [142]. Furthermore, PGE_2 enhances the *Leishmania amazonensis* load in macrophages of Balb/c mice, and COX-2 inhibition improves parasite killing [142]. The interplay between lipid droplets and autophagy is starting to be investigated [144,145], and such studies suggest that lipid droplets are in close contact and act in concert with the proteasomal and autophagic pathways of protein degradation [144,145].

Eicosanoid synthesis is also correlated to immune evasion of *T. gondii* [146]. The expression of 5-LO and the production of lipoxin are induced in the host after *T. gondii* infection. Lipoxin is a potent anti-inflammatory lipid mediator, and it is thought to work as a break that attenuates exacerbated inflammatory responses. Thus, lipoxin appears to have an important role in host survival and parasite infection. The enzymes responsible for lipoxin synthesis, 5-LO and 15-LO, have been localized to lipid droplets in macrophages and other cells [27,28,72,111]. However, the subcellular location of lipoxin synthesis within infected cells is currently unknown and deserves further investigation. It would be of interest to investigate whether lipid droplets are also involved in lipoxin-mediated mechanisms of pathogen evasion.

Besides being sites of production of inflammatory mediators that alter the immune response against infection, lipid droplets may also be a nutritional source for parasites. Notably, lipid droplets were observed in close apposition and even inside parasite-containing phagosomes during *T. cruzi* infection [59]. Moreover, adipocytes are a target of *T. cruzi*, and numerous amastigotes are found clustered around lipid droplets [147]. This association is suggestive that the parasites exploit the lipids generated by the lipolysis machinery of the host for its growth [59,147]. *T. gondii* resides in a parasitophorous vacuole, and it depends on cholesterol trafficking to parasitophorous vesicles for survival. The LDL receptor has been reported to mediate cholesterol mobilization [141]. *T. gondii* is able to scavenge and metabolize host lipids [148]. Moreover, enzymes involved in cholesterol esther synthesis have been identified in *T. gondii* [149,150]. Lipid droplets are found in the parasitophorous vacuole, and it should be noted that lipid droplets are also found inside parasites [141]. Accordingly, it has been proposed that parasites are able to divert host lipids for membrane building and possibly for lipid metabolism.

A prominent production of lipid droplets was observed in the hepatocytes of mice infected with *P. berghei* and in the parasitophorous vacuole and in the food vacuole of erythrocytes infected with *Plasmodium falciparum* [139,151,152]. Intraerythrocytic *P. falciparum* is able to synthesize neutral lipids, such as triacylglycerol, and store them in lipid droplets present in the parasite cytoplasm [153]. Various lipid bodies have been observed in the intraerythrocytic *P. falciparum* and determine the localization of amphipathic and neutral lipids in these cells [153]. Moreover, it has been suggested that the lipid droplets associated with the food vacuole may play roles in the detoxification of heme in the parasite [153,154]. Collectively, these data indicate that these organelles may exert a functional role in parasite lipid homeostasis.

Parasites are able to produce lipid droplets in their cytoplasm as well. Distinct stages of *Schistosoma mansoni*, cercaria and schistosomula exhibit lipid droplets [155]. Lipid-like droplets have also been reported below the muscle layer of the parenchymal region of male *Schistosoma japonicum* [156]. *S. mansoni* is not able to synthesize *de novo* sterols and fatty acids, but it does have the capacity to synthesize all of its complex lipids when supplied with dietary sources [157]. Although the mechanisms for the accumulation of neutral lipids are not clear, one can reason that *Schistosoma* developed a strategy to hijack lipids of the host for its own requirements.

Besides being able to induce lipid droplet formation in the host, it has also been reported that parasite infection can decrease lipid droplet

accumulation in the host. *S. mansoni* infection is associated with decreased levels of circulating cholesterol and decreased lipid droplets in the liver of ApoE$^{-/-}$ mice fed with a high-fat diet. It also provokes a reduction of adipose tissue around the liver, heart and blood vessels. This effect was attributed to parasite eggs instead of the adult parasite [158]. Further studies are necessary to understand the mechanism that underlies this effect. The authors suggested that it may be related to an egg-induced granulomatous response. Interestingly, parasite lipid droplets may also be secreted by *S. mansoni*. Numerous lipid droplets have been found in the gut of parasites, and this finding was attributed to a mechanism of heme detoxification during parasite feeding of host blood [159].

In conclusion, parasite–host interactions modulate the lipid metabolism of both organisms. Lipid droplets are important sites for the generation of lipid mediators involved in the host immune responses. Parasites evolved to take advantage of host immune responses, so that they may be able to survive and establish chronic infections. Lipid droplets of host cells play a relevant role in this scenario. Parasites have also adapted to exploit lipid droplets as a source of lipids for membrane building, a crucial step for parasite growth. Finally, parasites have lipid droplets of their own, proving that the ability to form lipid droplets is evolutionarily conserved among organisms.

Conclusion & future perspective

A number of key recent studies have expanded the range of the role of lipid droplets from its original description as a lipid storage compartment to a full-range cellular organelle that actively participates in innate immunity and inflammation.

Studies of lipid droplet composition and structural features have revealed that lipid droplets contain a diverse array of proteins in addition to lipids. Those studies have indicated that lipid droplets have a much more complex structure than initially anticipated. Moreover, according to the cell and stimulatory condition, lipid droplets may compartmentalize a distinct set of proteins and the heterogeneity of lipid droplet composition may determine different cellular functions for lipid droplets. Our contemporary view of lipid droplets places this organelle as an important regulator of different inflammatory and infectious diseases and a key marker of leukocyte activation. In leukocytes and other cells involved in infectious conditions, lipid droplets have been shown to have central roles in compartmentalizing the synthesis of inflammatory mediators leading to the heightened production of prostaglandins and leukotrienes and participating in the amplification of the inflammatory response. Notably, lipid droplet biogenesis is highly induced during intracellular pathogen infection, where lipid droplets exhibit roles in downregulating the host immune response and are often found in close proximity to the phagosomes, suggesting that pathogens may highjack lipid droplets for exploitation as a nutrient source.

Although great advances in the understanding of the mechanisms of lipid droplet biogenesis and its roles in lipid metabolism and inflammatory mediator production have been achieved, critical questions remain with regard to the formation and the functions that lipid droplets play in infectious diseases. In conclusion, recent studies have identified lipid bodies as multifunctional organelles with key functions in lipid storage and cell signaling in inflammation, and as such, they are emerging as attractive target candidates for therapeutic intervention. Future studies will be necessary to characterize the role of lipid droplets as targets for therapeutic intervention in infectious diseases that progress with increased lipid droplet accumulation. These will need to include the development of selective lipid droplet inhibitors. Moreover, the safety characterization of lipid droplet inhibition is required, as lipid accumulation within lipid droplets may act as a protective mechanism in lipid homeostasis against cellular lipotoxicity.

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Executive summary

Lipid droplet biogenesis & structure

- Lipid droplet biogenesis is a highly regulated cell- and stimulus-specific biological process.
- Both the lipid and protein compositions of lipid droplets may vary according to cellular conditions.
- Identification of key pathways, molecules and functions of lipid droplets may enable the development of therapeutic targets for future intervention.

Lipid droplets in infection

- Increased numbers of lipid droplets in leukocytes and other cells are observed in bacterial, viral, fungal and parasitic infections.
- Innate immune receptors, including Toll-like receptor 2 and 4, and nuclear receptors play important roles in infection-driven lipid droplet biogenesis.
- Lipid droplets compartmentalize the eicosanoid enzymatic machinery and are sites of eicosanoid production during infection and participate in the amplification of the inflammatory response.
- Lipid droplets interact with a variety of intracellular components, including phagosomes, suggesting interplay of lipid droplets with the endocytic pathway and possibly enabling the exchange of content between lipid droplets and pathogen-containing phagosomes.

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