# Chapter 12
# Metabolic Crosstalk Between Host and Parasitic Pathogens


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**Abstract** A complex network that embraces parasite–host intrinsic factors and the microenvironment regulated the interaction between a parasite and its host. Nutritional pressures exerted by both elements of this duet thus dictate this host–parasite niche. To survive and proliferate inside a host and a harsh nutritional environment, the parasites modulate different nutrient sensing pathways to subvert host metabolic pathways. Such mechanism is able to change the flux of distinct nutrients/metabolites diverting them to be used by the parasites. Apart from this nutritional strategy, the scavenging of nutrients, particularly host fatty acids, constitutes a critical mechanism to fulfil parasite nutritional requirements, ultimately defining the host metabolic

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landscape. The host metabolic alterations that result from host–parasite metabolic coupling can certainly be considered important targets to improve diagnosis and also for the development of future therapies. Metabolism is in fact considered a key element within this complex interaction, its modulation being crucial to dictate the final infection outcome.

**Keywords** Nutrient sensing pathways · Host–parasite interaction · Host metabolic pathways · Scavenging of nutrients · Host metabolic landscape

## 12.1 Host Metabolic Subversion: A Life-Saving Strategy

Parasitism is defined as an intimate, often obligatory and symbiotic relationship between two organisms during which the parasite benefits at the host's expenses. During a parasitic infection, the host cell/organism constitutes both a challenge and a crucial platform to ensure the development of the parasites. Hence, parasitic organisms need to subvert distinct host defence mechanisms for a successful colonization of the host. Most parasite organisms are believed to be metabolically dependent on the host, which has been consequently proven by the experimental demonstration of several nutritional virulence strategies adopted to survive inside the host. It is this dependency that generally makes the parasitic relationship in general obligatory. To fight against the parasites, the host can induce an indirect nutritional scarcity to the parasites through its metabolic dependence on distinct metabolites or can directly impose a metabolic restriction limiting or hijacking the access of the parasites to the host nutritional pool in a process known as nutritional immunity. As counter-attack measures in such harsh metabolic conditions, the parasites need to modulate host nutrient sensing mechanisms, altering host metabolic pathways and ultimately host nutritional requirements to fulfil the parasites' metabolic needs. The scavenging of host nutrients, particularly lipids, by the parasites also helps to outline the host metabolic landscape, being a crucial strategy to sustain parasite survival and proliferation.

This chapter covers the referred topics from a host perspective, highlighting the most significant findings in this exciting area of host–parasite metabolic coupling.

## 12.2 Host Metabolic Checkpoints Exploited During Infection

The dynamic and complex metabolic coupling established between a parasite and the host counterpart is the result of a long co-evolutionary process. Within such intricate interaction, the parasite and the host were continuously adapting and evolving up to the current host–parasite metabolic interaction, although, due to the constant and sudden alterations imposed by the host microenvironment, such metabolic coupling continues to be dynamically regulated. This constant pursuit and

competition for nutrients within the host–parasite duet will alter the host metabolic pathways with major consequences for the host nutritional reserves, affecting eventually the phenotypic and functionality of the host cell. Such host alterations are imposed by the parasite's demand for a suitable niche. The ability to adapt to a host environment, sensing their surroundings, has been attributed to the modulation of host nutrient sensing pathways. This, in fact, constitutes a major strategy to explore host metabolic pathways and manage with metabolic stress. Using such mechanisms, parasites gain access to host nutritional reserves, which are crucial for their survival and persistence inside the host. Host sensing pathways and the mechanisms used by the parasites to explore them will be extensively discussed in the current section.

### *12.2.1 Nutrient Sensing Pathways*

All organisms sense their surroundings, searching for better metabolic conditions to fulfil their nutritional requirements. In fact, nutritional deprivation has been considered the major driver for the establishment of distinct sets of nutrient sensing pathways. Such topic has been quite debated in the scientific community, with several authors proving the major impact of these pathways in times of nutritional scarcity. These pathways are able to detect extracellular and intracellular metabolic cues, switching from anabolism to catabolism in the presence or absence of nutrients availability, respectively (Efeyan 2015). More recently, the role played by nutrient sensing pathways has been addressed in the context of parasitic infections, where two major players stand out, the mechanistic target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK).

#### 12.2.1.1 mTOR Sensing Pathway

mTOR is defined as a protein kinase anchored to a signalling network that by responding to diverse environmental cues will promote anabolic metabolism generating energy and nutrients. mTOR signalling has a broad impact in terms of proliferation and cell growth (mass accumulation). This protein is an atypical serine/threonine kinase that belongs to the phosphoinositide 3-kinase (PI3K)-related kinase family interacting with a distinct set of proteins, forming two major complexes defined as mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). Yet, while mTORC1 and mTORC2 share several components, such as the catalytic mTOR subunit, the mammalian lethal with sec-13 protein 8 (mLST8), DEP domain containing mTOR-interacting protein (DEPTOR) and the Tti/1/Tel2 complex (Jacinto et al. 2004; Kaizuka et al. 2010; Kim et al. 2003; Peterson et al. 2009), the regulatory-associated protein of mechanistic target of rapamycin (raptor) is only present in the mTORC1 subunit (Hara et al. 2002; Kim et al. 2003). Conversely, rapamycin-insensitive companion of mTOR (rictor) is only part of mTORC2

signalling pathway (Jacinto et al. 2004; Sarbassov et al. 2004). The distinct composition between mTORC1 and mTORC2 may explain the different sensibilities to the classical mTOR inhibitor rapamycin (Laplante and Sabatini 2012).

mTORC1 integrates inputs from extracellular and intracellular cues, such as growth factors, stress, energy status, oxygen and amino acids. In that sense, the major metabolic pathways are regulated by mTORC1, inducing and inhibiting anabolic and catabolic processes, respectively, through the enhancement of protein and lipid synthesis while decreasing autophagic processes (Laplante and Sabatini 2012). A heterodimer protein composed of the tuberous sclerosis 1 (TSC1; also known as hamartin) and TSC2 (also known as tuberin) is considered a central regulator of the mTORC1 pathway. This protein complex functions as a GTPase-activating protein (GAP) for the Ras homologue enriched in brain (Rheb) GTPase. Such mechanism impairs the direct interaction between the GTP-bound form of Rheb with mTORC1 that occurs specifically at the lysosomal surface through the conversion of Rheb into its inactive GDP-bound state (Inoki et al. 2002; Tee et al. 2003). The TSC1/2 complex is also regulated upstream by protein kinase B (Akt/PKB), extracellular-signal-regulated kinase 1/2 (ERK1/2) and ribosomal S6 kinase (RS6K), which directly inactivate the TSC1/TSC2 complex by phosphorylation leading to mTORC1 activation (Potter et al. 2002; Roux et al. 2004). Besides these, other factors are involved in mTORC1 activation, such as Akt, TNF-α and Wnt pathway as well as increasing levels of amino acids, specifically leucine and arginine. Both amino acids are mandatory signals; they must be present for any upstream signal, including growth factors, to activate mTORC1 (Laplante and Sabatini 2012). This amino acid-dependent activation mechanism requires the interaction between Rag GTPases with the raptor component of mTORC1. After this complex interplay, mTORC1 is able to translocate from the cytoplasm to the lysosomal surface, leading to the docking of Rag GTPases on a complex known as Ragulator, which is essential for the activation of mTORC1 (Sancak et al. 2010). Ragulator functions as a lysosomal scaffold for other protein complexes such as GATOR1 and GATOR2, which are a negative and positive regulator of mTORC1 pathway, respectively (Bar-Peled et al. 2013) The cytosolic sensor for leucine was recently described as Sestrin2. This protein can interact with GATOR2, consequently inhibiting mTORC1 pathway in the absence of leucine. In terms of arginine, so far two sensors were defined: SLC38A9 and the CASTOR1. The former constitutes a lysosomal sensor, forming a supercomplex with Ragulator that is key for the transmission of arginine levels to mTORC1 pathway. The latter seems to function in parallel with SLC38A9 sensor. CASTOR1 forms a homodimer and heterodimerizes with CASTOR2. Both complexes interact with GATOR2 regulating negatively mTORC1 activity. Interestingly, the disruption of such interaction is accomplished only in the presence of arginine, permitting the direct binding of this amino acid to CASTOR1 and the consequent activation of mTORC1 pathway (Chantranupong et al. 2016; Wolfson et al. 2016). More recently, cholesterol was identified as a nutrient input that drives mTORC1 recruitment and activation at the lysosome surface through the complex SLC38A9-Niemann-Pick C1 (NPC1) (Castellano et al. 2017). SLC38A9 amino acid transporter has a conserved cholesterol-

responsive element which may facilitate the mTORC1 activation by cholesterol, independently of its arginine-sensing function. Additionally, NPC1 constitutes the major lysosomal cholesterol transporter, which makes the SLC38A9-NPC1 complex key for the sensing of dietary lipids by mTORC1 pathway. Lysosomes have emerged as an important cellular platform to sustain mTORC1 activation and also as a key nutrient gateway, sensing and communicating cholesterol availability to mTORC1 (Castellano et al. 2017).

Upon activation, mTORC1 induces protein synthesis mainly by direct phosphorylation of the translational regulators eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1) and S6 kinase 1 (S6K1). S6K1 is responsible for an increase of mRNA biogenesis and also for the translational initiation and elongation. Such mechanism is indeed highly relevant since the inhibition of mTORC1 significantly reduces the overall rates of protein synthesis in proliferating cells (Laplante and Sabatini 2012). mTORC1 activation also modulates as well the synthesis of lipids acting through the sterol regulatory element-binding protein 1/2 (SREBP1/2) transcription factors that control the expression of numerous genes involved in fatty acid and cholesterol synthesis (Laplante and Sabatini 2009). mTORC1 can regulate SREBP function through S6K1, at least in some cell types, and also by Lipin-1 phosphorylation (Peterson et al. 2011). The expression and activity of peroxisome proliferator-activated receptor γ (PPAR-γ), the master regulator of adipogenesis, is also controlled by the mTORC1 pathway (Zhang et al. 2009). Energy consumption is a hallmark of anabolic metabolism that is in fact regulated by mTORC1 protein. In such context, mTORC1 enhances the glycolytic flux through the increased transcription and translation of hypoxia-inducible factor 1 α (HIF-1α), which is a positive regulator of glycolytic genes (Düvel et al. 2010). Conversely, mTORC1 also promotes growth by negatively regulating autophagy, through direct phosphorylation and suppression of ULK1/Atg13/FIP200 (unc-51-like kinase 1/mammalian autophagy-related gene 13/focal adhesion kinase family-interacting protein of 200 kDa), required to initiate autophagy (Ganley et al. 2009; Hosokawa et al. 2009). Inversely, mTORC2 complex is insensitive to nutrients but does respond to growth factors such as insulin through a mechanism that might be dependent on PI3K signalling. mTORC2 after being activated regulates cytoskeletal organization and cell survival/metabolism. It can also control several members of the AGC subfamily of kinases including Akt, serum- and glucocorticoid-induced protein kinase 1 (SGK1) and protein kinase C-α (PKC-α) (Laplante and Sabatini 2012).

#### 12.2.1.2 AMPK Sensing Pathway

AMPK protein is defined as an energetic sensor, responding to unbalanced energy levels with the upregulation of catabolic and the downregulation of anabolic pathways. In response to an energetic/nutritional stress, AMPK upregulates glucose uptake, mitochondria biogenesis and fatty acid oxidation pathways and simultaneously downregulates protein, cholesterol and fatty acid synthesis. Ultimately, in such context, AMPK will restore metabolic homeostasis ensuring cell survival and

growth. AMPK becomes activated when the levels of AMP or ADP increase and subsequently the ATP levels are diminished (Hardie et al. 2012). Many physiological and pathological conditions are capable of activating AMPK such as lack of nutrients, exercise and hormones (adiponectin and ghrelin) besides a diverse set of diseases (Steinberg et al. 2009).

From a structural point of view, the mammalian AMPK is a heterotrimeric protein, composed of a catalytic domain (α) and two regulatory subunits (β and γ). The catalytic domain presents a Thr172 residue that is mainly phosphorylated by the tumour suppressor liver kinase B1 (LKB1), the pseudokinase STRAD and the scaffold protein MO25. Thr172 conserved residue can also be phosphorylated by calcium/calmodulin-dependent protein kinase kinase β (CAMKKβ) that is activated by higher levels of intracellular calcium (Hardie et al. 2012) and also by the transforming growth factor-β-activated kinase 1 (TAK1), while its role still needs to be clearly defined. Moreover, the binding of AMP and/or ADP to the γ subunit is also required for AMPK activation. This alters the structure and subsequent phosphorylation of the conserved residue Thr172, within the activation loop (Moreira et al. 2015a). Interestingly, an intriguing mechanism demonstrated showing that AMP binding causes AMPK-LKB1 interaction at the lysosomal membrane but not only in the cytoplasm, similar to mTORC1 activation (Zhang et al. 2014). Some authors proposed a model of reciprocal regulation between AMPK and mTORC1, which are activated under opposite circumstances, scarcity versus availability of nutrients, respectively (Bar-Peled and Sabatini 2014; Zhang et al. 2014).

To restore the unbalanced energetic status, AMPK upregulates mitochondrial pathways for the recovery of ATP levels (Foretz and Viollet 2011). Among its downstream targets, AMPK activates by phosphorylation of the peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α), a transcription coactivator that induces mitochondria biogenesis (Jäger et al. 2007). In parallel, the AMPK pathway has profound effects on the regulation of lipid metabolism. It induces the phosphorylation and translocation to the membrane of FAT/CD36 that is responsible, a least in part, for the uptake of fatty acids, and controls the activity of acetyl coA carboxylase (ACC) enzyme by phosphorylation. The latter mechanism is involved in the internalization and oxidation of fatty acids into the mitochondria. AMPK also affects glycolysis controlling the transcriptional levels of GLUT4 and HKII enzymes and enhancing the glucose uptake by the modulation of signalling events as Rab-GTPase-activating proteins (TBC1D1 and TBC1D4) (Hardie 2011). Finally, AMPK can also reestablish the nutrient and energetic pool of the cell activating ULK1, a highly conserved autophagy kinase, promoting mitophagy (Egan et al. 2011; Kim et al. 2011). Oppositely, AMPK diminishes biosynthetic pathways to decrease ATP consumption. Fatty acid synthesis is regulated negatively by ACC phosphorylation through the suppression of malonyl-coA formation. Other biosynthetic pathways such as cholesterol synthesis and transcription of genes involved in lipid biosynthesis are also suppressed by AMPK protein (Foretz and Viollet 2011).

#### 12.2.1.3 mTOR–AMPK Interplay

In scarce nutritional conditions or in response to hypoxia, AMPK acts as a metabolic checkpoint, blocking the cellular growth, through mTORC1 inhibition. Such mechanism is achieved by the direct phosphorylation of TSC2, inactivating its GAP activity, and by the phosphorylation of the Raptor component, leading to the allosteric inhibition of mTORC1 (Gwinn et al. 2008; Kalender et al. 2010). The antagonistic interaction between AMPK and mTORC1 is clearly observed in the regulation of autophagy mainly by ULK1 phosphorylation. AMPK can trigger autophagy in a double-prolonged mechanism by directly activating ULK1 and by inhibiting simultaneously the suppressive effect of mTORC1 on ULK1. In fact, some authors were able to find distinct sites in ULK1 target by AMPK and also a direct mTOR phosphorylation site (Shang et al. 2011; Wang et al. 2001). Another convergence point between AMPK and mTOR signalling can be observed in the context of DNA damage. This major event signals through mTORC1 axis, inducing the expression of TSC2 and phosphatase and tensin homologue deleted on chromosome 10 (Pten), causing a downregulation of the entire PI3K-mTORC1 signalling (Stambolic et al. 2001). AMPK is then able to be activated through a mechanism that depends on the induction of sestrin1/2 proteins (Budanov and Karin 2008).

### *12.2.2 Subversion of Host Nutrient Sensing Pathways*

The modulation of mTOR and AMPK sensing pathways constitutes an important strategy observed during the infection with apicomplexa parasites, such as *Plasmodium spp.* and *Toxoplasma gondii*, as well as with trypanosomatida parasites, like *Leishmania spp.* and *Trypanosoma cruzi*. The strategies used by these parasites as well as their downstream metabolic effects will be discussed extensively as follows and are synthesized in Box 12.1.

**Box 12.1 Why Parasites Hijack Host Metabolic Checkpoints During Infection?**

- The modulation of mTOR and AMPK metabolic checkpoints helps the parasites to modulate host metabolic pathways in order to scavenge nutrients/metabolites from the host.
- The nutritional virulence imposed by the parasite may divert those nutrients/metabolites from the host, altering consequently the host immune response against the parasites.
- The modulation of mTOR/AMPK sensing pathways reflects distinct functional/nutritional requirements of the parasites, which are continuously challenged within host–parasite coupling.

(continued)

**Box 12.1** (continued)

- AMPK and mTOR metabolic subversion facilitates the adaptation of the parasites, during their life cycle to distinct hosts, which in turn exert different nutritional and immunological pressure.
- The hijacking of host metabolic checkpoints constitutes a successful strategy favouring parasites invasion, survival and eventually proliferation.

#### 12.2.2.1 *Plasmodium spp.*

A possible alteration of mTOR signalling was initially observed during the *P. yoelii* liver stage infection. An increase of p-Akt and p-mTOR and a decrease of p53 expression were detected after performing protein microarray in infected hepatocytes (HepG2 expressing CD81) (Kaushansky et al. 2013). Although the mTOR activation was suggested to play an important role for the survival of *Plasmodium*, other studies generated controversy on the real impact of mTOR during a *Plasmodium* infection. Indeed, the siRNA knockdown of mTORC1 components (mTOR and raptor) in HepG2 cells failed to modify the percentage of infected cells or *P. berghei* development (Hanson et al. 2013). A significant impact of mTOR signalling during *Plasmodium* infection was more recently described. Using an experimental cerebral malaria (ECM) model, mice subjected to brief periods of dietary restriction (10–50%) displayed reduced ECM symptoms associated with a decrease of leptin levels and a reduction of mTORC1 activation in $CD4^+$ and $CD8^+$ T cells. A higher number of active T cells were found in the spleen with less infiltration into the brain tissues, supporting the decreased levels of parasitaemia found in the brain and in the spleen. As a proof of concept, the inhibition of leptin or mTOR signalling in this experimental model of infection can reduce both ECM symptoms and mortality. The authors observed an upregulation of S6 phosphorylation and CXCR3, which is commonly linked to mTORC1 activation, in $CD4^+$ and $CD8^+$ T cells after leptin treatment. Overall, these results establish mTORC1 as a downstream target of leptin in $CD4^+$ and $CD8^+$ T cells (Mejia et al. 2015). The potential for mTOR inhibition in the development of ECM has been further examined. Rapamycin treatment induces a reduction of $CD4^+$ and $CD8^+$ T cells' flux associated with a decreased accumulation of parasitized RBCs in the brain tissues. Concurrently, a decrease of ECM symptoms, with the reduction of blood–brain barrier breakdown and brain haemorrhage and an increase of mice survival, was established. In the light of these results and regarding the recent knowledge concerning the activation of metabolic pathways in T cells, the hypothesis of a possible impact of additional metabolic pathways in the control of this disease, with AMPK protein being one of the most obvious candidates, was raised (Gordon et al. 2015).

The potential impact of AMPK was initially addressed during *P. berghei* infection. A decrease of host p-AMPK and its downstream target p-ACC (S79) protein level was depicted in Huh7-infected cells, until 30 h post-infection. The pharmacological activation of AMPK in vitro and in vivo led to a reduction of *Plasmodium*

liver stage infection (Ruivo et al. 2016). The reduction of AMPK activity in this context of infection comes in line with the work from Kashansky and colleagues, showing an increase of p-mTOR, a negative regulator of AMPK signalling, as well as the decrease of p53, which is in fact an AMPK downstream target. In such context, *Plasmodium*-infected cells might have a higher biosynthetic capacity, upregulating the fermentative pathway, the cholesterol and fatty acid synthesis, which is crucial to sustain the higher rate of *Plasmodium* growth (Ruivo et al. 2016). Several authors have previously reported the massive dependency of *Plasmodium* blood and liver stages on host glycolysis to provide the glucose used as the main energy source (Itani et al. 2014; Kirk et al. 1996; Pfaller et al. 1982; Slavic et al. 2011). Recently, Meireles and colleagues described how *Plasmodium* parasites modulate host machinery to acquire host glucose suggesting a possible role for AMPK in such context (Meireles et al. 2017). During the liver stage infection, *P. berghei* parasites lead to a reduction of host glucose intracellular levels associated with a reduction of intracellular ATP and consequently affecting the host energetic pool. The consequent binding of ADP/AMP to the GLUT1 glucose transporters induced a higher translocation of GLUT1 transporters to the surface of infected cells, ultimately leading to a further enhancement of glucose uptake, in a process that was suggested to be AMPK mediated. The relevance of glucose uptake via the GLUT1 transporter for the infection outcome was demonstrated by the chemical inhibition of GLUT1 transporter that impaired *P. berghei* hepatic infection (Meireles et al. 2017). Combining these studies, a restoration/increase of AMPK phosphorylation during *Plasmodium* liver stage infection might be found at later time points of infection, particularly after 30 h post-infection (p.i.). The strategies used by *Plasmodium* to subvert host nutrient sensing pathways are depicted in Fig. 12.1.

#### 12.2.2.2 *Toxoplasma gondii*

*T. gondii* parasites induce a sustained activation of mTOR nutrient sensing pathway, which is essential for the host cell entering S phase of the growth cycle, although not induced through its classical upstream modulators PI3K-Akt and ERK pathways. Interestingly, only an increase of phosphorylation of ribosomal protein S6 was observed without any modulation of growth-associated mTOR substrates such as 4E-BP1 and S6K1. A striking interaction was noticed between mTOR-bearing vesicles and the parasitophorous vacuole (PV) in infected cells, which is considered the intracellular niche of *T. gondii* parasites, highlighting a possible functional interaction. Overall, these data lead to the suggestion of a potential impact of mTOR signalling in host translational regulation of mRNA, encoding for example mediators of host defence (Wang et al. 2009a). Additionally, due to the known role of autophagy, as a supportive mechanism for *T. gondii* growth during amino acid deprivation, the authors raised a hypothesis based on host–parasite metabolic adaptation (Wang et al. 2009b). It was suggested that *Toxoplasma* might obtain nutritional benefits, guiding host amino acids through a "futile cycle" of protein synthesis (mTOR activation) and degradation (autophagy) (Orlofsky 2009), increasing the

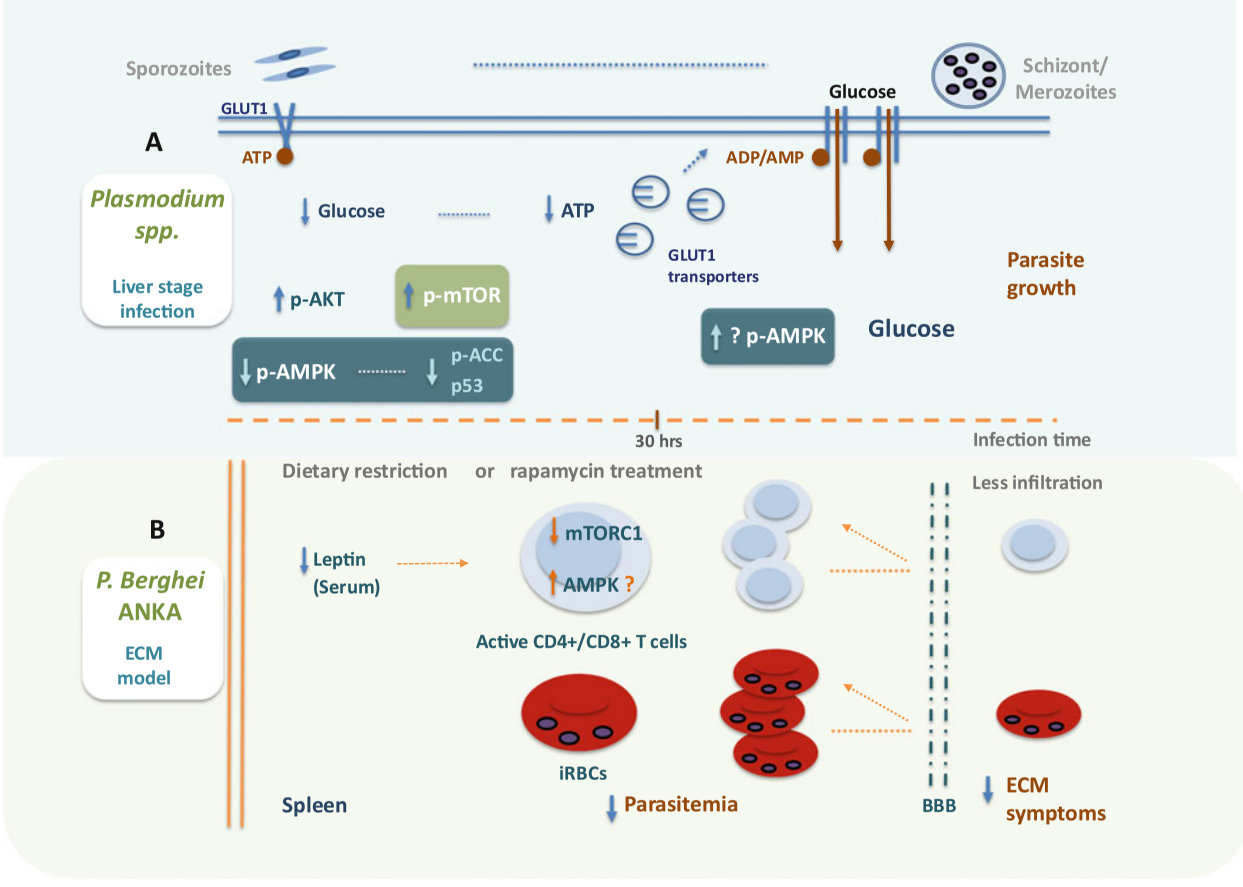


**Fig. 12.1** Major strategies used by *Plasmodium* parasites to modulate host mTOR and AMPK sensing pathways. (**a**) Immediately after entry of the host liver cells, sporozoites alter host sensing pathways to accomplish their differentiation into schizont/merozoites forms. At the early interaction phase, early than 30 h p.i., the

amino acid flux through host lysosomes that can be internalized by the PV, eventually becoming available to the parasites (Coppens et al. 2006).

Wang and colleagues defined an interesting phenomenon in another work where a crucial role for mTORC2-AKT-GSK3 pathway in the migration and reorganization of *T. gondii*-infected cell structure was found. A migratory suppression of infected cells in a wounded monolayer model, explained by the inability of centrosomes to reorient in the direction of migration, seems to be governed by mTORC2 pathway (Wang et al. 2010). Moreover, the localization of centrosome and Golgi apparatus to the vacuole and the recruitment of mitochondria, microtubules and endolysosomes, which are homogenously distributed within the vacuole perimeter, constitutes vital mechanisms hijacked by *T. gondii* parasites, and modulated ultimately by mTORC2. The mechanisms used by *T. gondii* to modulate host nutrient sensing pathways are represented in Fig. 12.2. These data suggest that mitochondria and lysosomes can associate with traffic via microtubules with parasites inside the PV (Wang et al. 2010). PV is surrounded by a membrane that is freely permeable to many host metabolites (Schwab et al. 1994), which may support the relevance of homogenous organelle distribution referred earlier, particularly during nutrient starvation, to secure an efficient delivery of host resources to the PV (Wang et al. 2010).

#### 12.2.2.3 *Leishmania spp.*

A global blockage of host translation was described initially on *L. major*-infected B10R macrophages, a BMM cell line, upon 6 h of infection through a mechanism dependent on the action of the glycoprotein GP63. The direct cleavage of mTOR protein by GP63 consequently led to mTORC1 inhibition and the activation of a well-known translational repressor named 4E-BP1 by dephosphorylation. Such mechanism is likely to be important for *Leishmania* survival since the activation

**Fig. 12.1** (continued) infected cells exhibit a lower uptake of glucose through GLUT1 transporter. Within this time frame, a significant alteration of host pathways is achieved with the increased expression of p-AKT and p-mTOR and simultaneously a decrease of p-AMPK and its downstream targets, p-ACC and p53. Thus, an energetic deficit is established in such environment, turning possible the interaction between ADP/AMP with GLUT1 transporter (after 30 h p.i.), increasing their translocation to the membranes and subsequently the glucose uptake. In this event, AMPK may play an important role in restoring the energetic levels and increasing the expression of GLUT1 that is important to increase intracellular glucose levels. These early metabolic alterations guide the infected cells to establish a more permissive niche to *Plasmodium* during liver stage infection. (**b**) The modulation of host metabolism plays a crucial role dictating the infection outcome of *P. berghei ANKA* infection, particularly in a model of experimental cerebral malaria (ECM). After dietary restriction or rapamycin treatment, a reduction of leptin levels in the serum of infected mice is obtained, which leads subsequently to the downregulation of mTORC1 and possibly to an increase of AMPK pathway in splenic T cells. T cells become active establishing an environment less permissive to *Plasmodium* in the spleen. A lower infiltration of active T cells and infected RBCs through the blood–brain barrier (BBB) is also acquired, which leads to the reduction of ECM symptoms and to decreased levels of parasite load in the brain

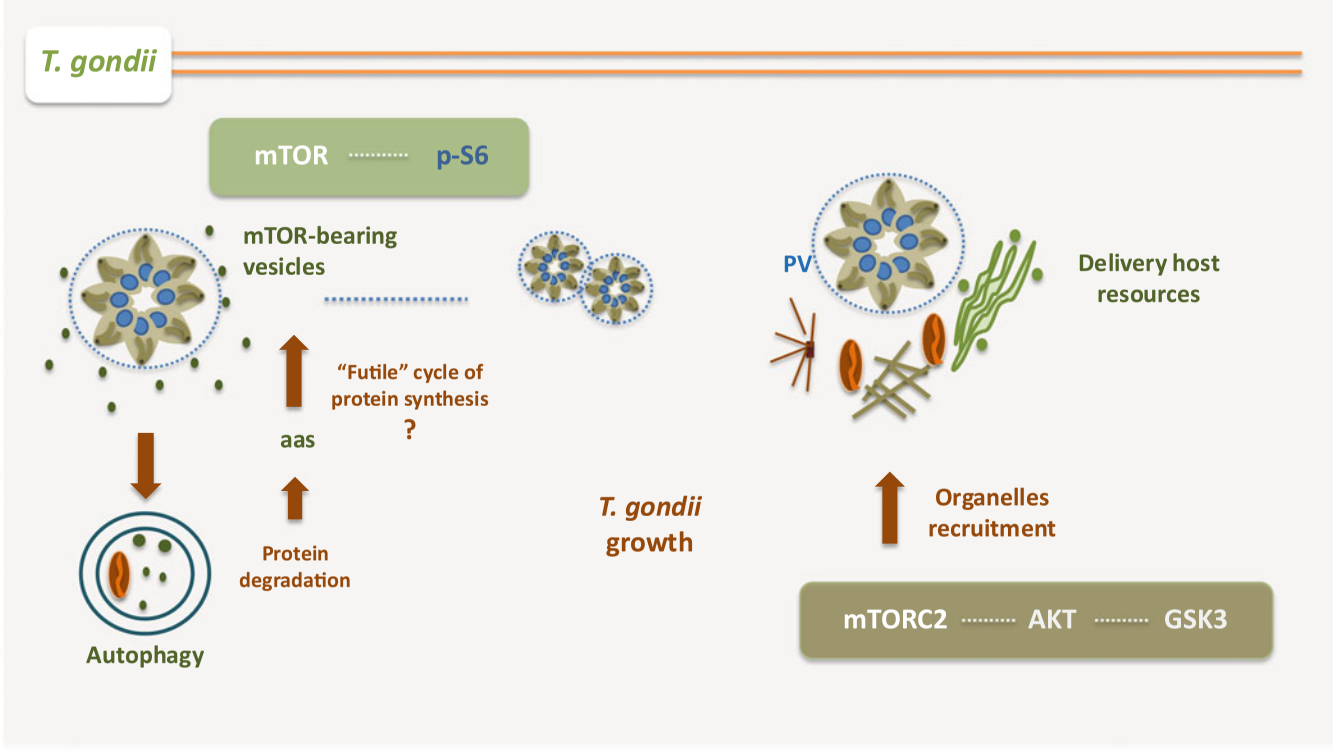


**Fig. 12.2** Modulation of host mTOR sensing pathways in the context of *T. gondii* infection. *T. gondii* parasites after interacting with their host cells trigger mTOR pathway leading subsequently to the activation of S6 mTOR substrate and also to the deposition of mTOR-bearing vesicles around the PV structure. In this context was proposed a model, where host proteins degraded by autophagy, a mechanism that is known to be activated during *T. gondii* infection, can be afterwards recycled by mTOR pathway in a continuous cycle defined as futile cycle of protein synthesis. Additionally, *T. gondii* parasites can also hijack mTORC2 nutrient sensing pathway that results in the further activation of AKT and GSK3 signalling components. The activation of this pathway network led to the recruitment of mitochondria, microtubules, Golgi apparatus and centrosomes, among others, to the vacuole perimeter, which is then critical for the delivery of host resources to the parasite. Importantly, all these mechanisms are crucially implicated in the growth of *T. gondii* parasites

of 4E-BP1 through rapamycin treatment increases the parasite load in WT and not in 4EB-P1/2 double knockout (DKO) macrophages. Importantly, the genetic deletion of 4E-BP1/2 reduces parasite load in macrophages ex vivo and decreases the susceptibility to cutaneous leishmaniasis in vivo (Jaramillo et al. 2011). Yet, a contradictory report demonstrated an early hyperphosphorylation of mTOR upon infection with *L. major,* which was interpreted as a counteracting mechanism to prevent autophagic digestion of parasites in the early infection phase, ensuring promastigote differentiation into amastigotes (Frank et al. 2015). Although these data suggested a dynamic process of mTOR activation/deactivation during the initial phase of *L. major* infection, downregulation of mTOR expression by siRNA before infection decreased the infection rate compared to *L. major*-infected BMDM. Thus, mTOR phosphorylation may develop a protective role for the parasite during the early stages of infection. At 24 h p.i., mTOR hyperphosphorylation is already repressed, and an increase of glycolysis, inflammation- and autophagy-related mRNAs and miRNAs was observed, as well as a reduction of *Prkaa2* gene generating a harsh environment to eliminate intracellular amastigotes by an autophagy-dependent mechanism (Frank et al. 2015).

In the same context of infection, Rabhi and colleagues were able to detect an upregulation of a glycolytic and lipid transcriptional signature (Rabhi et al. 2012), which seems to correlate with the reduction of *prkaa2* transcript found earlier, suggesting a decrease of AMPK activity (Frank et al. 2015). Among the glycolytic transcripts, increased levels of mRNA were found for glucose transporters and glycolytic enzymes such as hexokinases (*Hk*), pyruvate kinase M2 (*Pkm2*) and lactate dehydrogenase a (*Ldha*). This phenotype was simultaneously associated with a downregulation of tricarboxylic acid (TCA) and oxidative phosphorylation (OXPHOS)-related genes. This work suggested that upon establishment, *L. major*-infected macrophages rely mainly on increased glycolytic flow for energy production. *L. major* also disturbs cholesterol and triglyceride homeostasis leading to their accumulation through the expression of scavenger receptors involved in the uptake of low-density lipoprotein (LDL), inhibiting cholesterol efflux and increasing the synthesis of triacylglycerides (TAG) (Rabhi et al. 2012).

While exploring the metabolic interactions between macrophages and the visceral *L. infantum*, we identified the activation of host AMPK as a key mechanism favouring parasite survival (Moreira et al. 2015b). We observed that following *L. infantum* infection, macrophages switch from an early glycolytic to an oxidative metabolism, in a process requiring SIRT1 and LKB1/AMPK. In the absence of SIRT1 or LKB1, infected macrophages are not able to induce AMPK activation leading to an impairment of the metabolic switch. In that sense, the AICAR-induced AMPK activation contributes to parasite survival while inhibition of AMPK using compound C resulted in lower parasite numbers in vitro. Interestingly, we demonstrated that AMPK inactivation specifically in the myeloid population led to a reduced *L. infantum* burden in vivo (Moreira et al. 2015b). The mechanisms used to hijack host nutrient sensing during *Leishmania* infection are presented in Fig. 12.3. All these examples highlight the strategies used by *Leishmania* to explore host resources, shedding light on cellular metabolic subversion mechanisms induced by a pathogen that resides strictly within the phagolysosome compartment of the host cell.

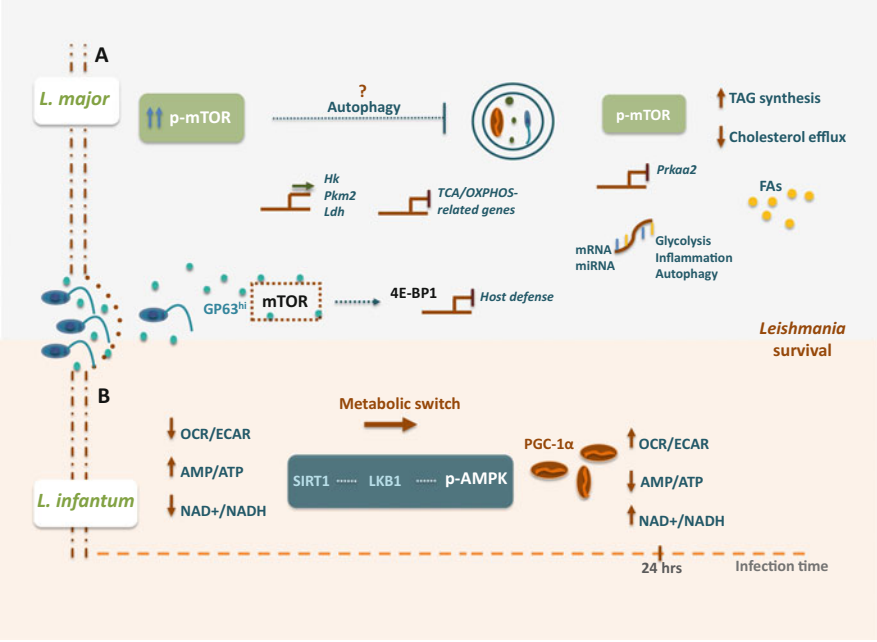


**Fig. 12.3** Strategies employed by different species of *Leishmania* to subvert mTOR and AMPK host sensing pathways. (**a**) At early phase of infection (until 24 h p.i.), *L. major* parasites lead to the hyperphosphorylation of mTOR, which has been suggested to inhibit the autophagic machinery. Simultaneously, *Leishmania*-infected cells exhibit an upregulation of glycolytic enzymes and a downregulation of TCA/OXPHOS components at transcriptional levels. At 24 h p.i., a significant reduction of mTOR hyperphosphorylation state is observed with the concomitant reduction of *Prkaa2* gene and the mRNA and miRNA of glycolysis, inflammation and autophagy programs. Finally, a metabolic reprogramming towards the enhancement of TAG synthesis and the reduction of cholesterol efflux then become established. Still in the context of *L. major* infection, a striking cleavage of mTOR can be eventually acquired, being such mechanism regulated by the levels of GP63 secreted by *Leishmania*. The resultant activation of 4E-BP1 protein dampens the transcription of important genes related to host defence. (**b**) At early phase of *L. infantum* infection, a decrease of OCR/ECAR ratio is acquired along with the establishment of an energetic deficit and a reduction of $NAD^+$/NADH ratio. These metabolic alterations support the induction of a metabolic switch through the activation of SIRT1–LKB1–AMPK axis. A mitochondria-dependent niche is acquired through the increased expression of PGC-1α, a master regulator of mitochondria biogenesis, leading ultimately to the increase of OXPHOS pathway and to the restoration of the energetic and redox levels. Altogether the strategies used are crucial for *Leishmania spp.* survival

#### 12.2.2.4 *Trypanosoma cruzi*

The mammalian invasion strategy used by *T. cruzi* parasite involves the host autophagic machinery, which is crucial for the lysosomal-dependent entry of tissue culture-derived trypomastigotes (TCT). As such, induction of autophagy by starvation or rapamycin-induced mTOR inhibition leads to a significant increase in the number of infected cells, while the opposite holds true as well (Romano et al. 2009).

To clarify the contribution of mTOR signalling during *T. cruzi* host invasion, a comparative analysis was performed of the molecular mechanisms of epithelial cell invasion upon metacyclic trypomastigote (MT) and TCT parasites' entry. MT parasites begin early to explore the host cell through the activation of mTOR and/or PI3K/PKC by the parasite molecule gp82. Cytoskeleton disorganization and lysosomes mobilization to the periphery of infected cells support the induction of parasites' lysosome exocytosis (Martins et al. 2011). The reversion of these effects was established through the inhibition of mTOR, PI3K and PKC pathways, which led to a consequent decrease of MT invasion. Interestingly, TCT parasites also rearrange in a similar manner the host cell structures referred previously, although through a mTOR-independent pathway. As a consequence, rapamycin treatment was responsible for enhancing TCT invasion (Martins et al. 2011). Overall, MT and TCT engage in different sets of pathways interacting differently with the host cell structures.

Recently, the application of a genome-wide RNA interference screen using as a target host gene HeLa cells allowed the identification of cellular processes that fuel *T. cruzi* growth (Caradonna et al. 2013). In this experimental setup, AKT-mTORC1 pathway stands out as a possible regulator of *T. cruzi* growth. The maintenance of intracellular ATP/ADP ratios at higher levels provided a distinct advantage for the parasite, which therefore kept AMPK activity in check. In addition, acute silencing of AMPK catalytic (PRKAA1) or the regulatory subunit (PRKAB1) provides a more favourable growth environment for intracellular *T. cruzi* (Caradonna et al. 2013).

*T. cruzi* can modulate the type of environment established during infection, leading to a significant inflammatory reaction which is considered one of the major alterations regarding Chagas disease. Strikingly, Nagajyothi and colleagues found that adipocytes constitute an important site of inflammation during the progression of infection. Infected adipocytes exhibited an increase in the transcription of inflammatory cytokines and chemokines, such as IL-1β, IFN-γ, TNF-α, CCL2, CCL5 and CXCL10. A higher expression of PI3K and the consequent activation of AKT, along with the concomitant reduction of adiponectin levels, are key events for the establishment of such inflammatory niche (Nagajyothi et al. 2008). Importantly, the authors suggested an indirect impairment of host AMPK in infected cells, since it constitutes one of the targets of the adiponectin pathway. Adiponectin AMPK interplay may constitute one of the mechanisms behind the anti-inflammatory role of adiponectin (Nagajyothi et al. 2008). The major strategies used by *T. cruzi* to modulate host nutrient sensing pathways are represented in Fig. 12.4. An additional work developed by Vilar-Pereira and colleagues demonstrated that AMPK activity could impair reactive oxygen species (ROS) production, an important inflammatory mediator, improving the cardiac function in established Chagas heart disease. The chronic Chagas cardiomyopathy (CCC) can be developed years after *T. cruzi* infection. Using a mice model that mimics the human CCC, treatment with resveratrol after the onset of CCC led to an activation of the AMPK pathway associated with a decrease of ROS production and heart parasite burden. Moreover, resveratrol could also ameliorate the heart function even when treatment is performed late after infection. The use of metformin, an AMPK activator, or temple

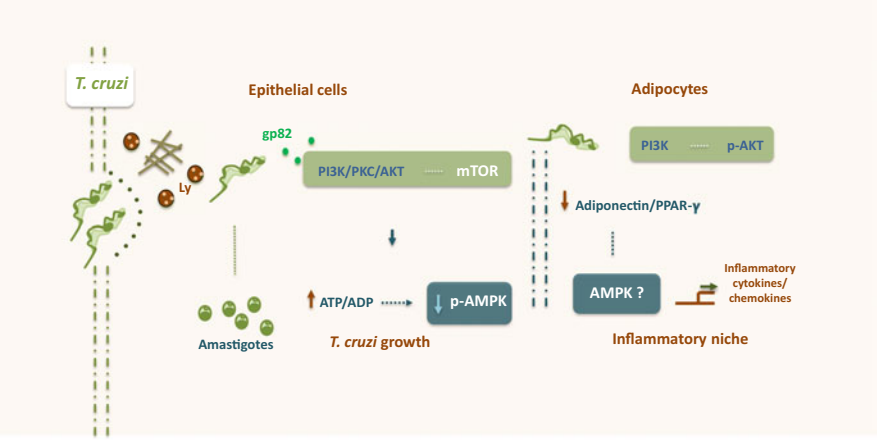


**Fig. 12.4** *T. cruzi* virulence mechanisms used to manipulate AMPK/mTOR host sensing pathways. After *T cruzi* internalization, gp82, a secreted virulence factor, triggers the PI3K/PKC/AKT signalling pathways which consequently supports the activation of mTOR protein in *T. cruzi*-infected cells. Importantly, at this early contact with the host, *T. cruzi* parasites are able to alter cytoskeleton organization, recruiting it, as well as lysosomes, to the membrane site of host interaction. This mechanism may facilitate lysosome exocytosis and further *T. cruzi* invasion. The infected cells in such complex niche exhibit a higher energetic level that seems to be crucial to sustain lower levels of AMPK phosphorylation, promoting later on *T. cruzi* growth. In a context of adipocytes infection, *T. cruzi* impose an activation of PI3K-AKT pathway and a simultaneous decrease of adiponectin/PPARγ levels in those cells. All these signalling networks contribute to the establishment of an inflammatory environment with the upregulation of inflammatory cytokines and chemokine transcripts, being such process regulated possibly by AMPK sensing pathway. This local response may support the overall inflammation generated commonly during *T. cruzi* infection

(SOD mimetic) led to similar effects as resveratrol, in terms of heart function improvement and lipid peroxidation reduction, although no modifications were observed on heart parasite burden (Vilar-Pereira et al. 2016).

## 12.3 Redefining Host Nutritional Content During Parasitic Infections

Apart from the ability to explore host sensing pathways to remodel host metabolic fluxes, parasites can hijack important host reserves, which is considered one of the most crucial parasite nutritional strategies. Despite the variability observed among the distinct parasites regarding their specific niches, nutritional properties and nutritional pressure, a certain degree of conservation is maintained that might account for the large similarities found among them. The specific nutritional requirements of each parasite press them to hijack host nutrient pools with the modulation of host amino acids synthesis, particularly arginine and tryptophan. They can also

take advantage of the host iron pool. Apart from these striking strategies that redefine host nutritional content, parasites are also able to alter host fatty acid metabolism, e.g. scavenging fatty acids (FA) such as cholesterol and/or trigger of the flux of lipid droplets (LDs) to the parasite's vicinity. The focus of the next section will be reserved to depict the strategies developed by the parasite to subvert host lipid content that is considered the most broader effect among parasitic infections.

### *12.3.1 Lipids: A Control Node of Host–Parasite Metabolic Coupling*

Lipids constitute the most scavenged class of host nutrients with a relevant impact to the parasite energetic pool. Lipids can be incorporated as fatty acid intermediates in parasite fatty acid metabolic pathways and ultimately define the landscape of host immune response. Despite the fact that the majority of the parasites can perform de novo fatty acid synthesis, these organisms depend on the lipids scavenged from the host to compensate for their rapid replication and their urgent environment adaptation (Ginger 2006). The remodelling of host cholesterol and fatty acid pool and the modulation of lipid droplet biogenesis are strategies used by *Plasmodium spp*., *Toxoplasma gondii*, *Leishmania spp.*, *Trypanosoma cruzi* and *Trypanosoma brucei* parasites.

#### 12.3.1.1 Cholesterol and Fatty Acids

During the blood stage of *Plasmodium* infection, infected erythrocytes display a significant increase in the phospholipid content alongside a concomitant decrease of polyunsaturated phospholipids. These fatty acids' balance appears to be crucial for the remodelling of parasite membranes during parasite growth and division (Beaumelle and Vial 1988; Simões et al. 1992; Vial et al. 2003). The modulation of lipid metabolism in the host cell is not restricted to blood stage of infection given that a remarkable alteration in the transcription program of host lipid profile was similarly detected in *Plasmodium*-infected hepatocytes (Albuquerque et al. 2009). Such alteration seems to be time dependent, being the metabolism of fatty acids particularly enriched at 12 h post-infection. More recently, an enhancement of the host lipids repertoire, namely in neutral lipids and in phosphatidylcholine (PC), was detected in infected hepatocytes. PC is internalized by the parasite, supporting the correct localization of parasite proteins within the PV membrane (PVM), which was shown to be essential for the parasite survival (Itoe et al. 2014).

During *Plasmodium* infection, a striking interaction was uncovered between the *Plasmodium* protein UIS3, located at the PVM, and the host liver-fatty acid binding protein (L-FABP), which is responsible for the delivery of fatty acids to cytoplasmic compartments (Mikolajczak et al. 2007). This process might support the host fatty acid supply to the parasites, since *Plasmodium* PVM, as well as during *Toxoplasma*

infection, is highly permeable to small metabolites forming long tubular extensions that cross the host cytoplasm to facilitate the metabolites interchange (Bano et al. 2007; Mueller et al. 2005). Upon *Plasmodium* invasion, PVM closely associates with the endoplasmic reticulum (ER), which approximates the host lipid biosynthesis machinery to the PV, corroborating the host–parasite fatty acid flow (Bano et al. 2007; Jayabalasingham et al. 2010; Labaied et al. 2011). Besides fatty acids, *Plasmodium* infection also impacts cholesterol metabolism. The expression of the scavenger receptor BI (SR-BI), a major cholesterol provider, is altered in the host membrane during infection. The increased surface expression of the SR-BI receptor supports a higher permissiveness concerning the entry and the development of *Plasmodium* parasite in the host cells (Rodrigues et al. 2008; Yalaoui et al. 2008). In the liver, *Plasmodium* can scavenge continuously cholesterol from hepatocytes until release of merozoites, via host LDL interaction (Labaied et al. 2011). Thus, these authors argue that malaria parasites are moderately dependent on host sterols for optimal development in the liver. Interestingly, they also noticed the plasticity of these parasites to survive in the liver, adapting easily to a cholesterol-deprived environment. *Plasmodium* is able to explore other sources of sterols in order to maintain their proper infectivity (Labaied et al. 2011).

Similarly, *Plasmodium T. gondii* parasites can scavenge host lipids compartmentalized in parasite endomembrane or in the proximity of PV membrane. The ability to use host lipids, such as long-chain fatty acids and cholesterol, may contribute to the biogenesis of parasite membranes (Charron and Sibley 2002). *T. gondii*-infected cells alter host cholesterol metabolism, with increased LDL endocytosis using the LDL receptor (LDLr), inducing as well a higher activity of hydroxymethylglutaryl-CoA (HMG-CoA) reductase (Blader et al. 2001; Coppens et al. 2000, 2006). Both sources are responsible for the lower levels of cholesterol and TAG in serum of infected mice. This reduction could be partially associated with the parasite acquisition of cholesterol and TAG from the host. Yet, under hypercholesterolemia conditions *T. gondii* developed a strategy to uptake lipids via an alternative receptor(s), which maintains intracellular cholesterol at optimal levels, contributing to parasite survival and replication in LDLr-deficient mice (Portugal et al. 2008). The host cell membrane cholesterol constitutes the main source of cholesterol within the PV membrane where *T. gondii* resides. Interestingly, such mechanism is considered a major strategy to support the discharge of organelles of *T. gondii*-infected cells, which is crucial for *Toxoplasma* invasion (Coppens and Joiner 2003).

The remodelling of PV membrane with the intravacuolar network (IVN), which is an extensive collection of elongated nanotubules in the vacuolar space participating in the delivery of nutrients from the PVM to the parasite, is also dependent on lipids. A continuous flow of lipids supports the growth and maturation of PV and IVN, which reflects the dynamic nature of host–parasite lipidome (Caffaro and Boothroyd 2011; Sibley et al. 1995). Additionally, *Toxoplasma* is able to explore host organelles through the recruitment of mitochondria to the cytosolic face of the PV, allowing the scavenging of lipoic acid from the host to fulfil its nutritional requirements (Crawford et al. 2006; Sinai et al. 1997). Finally, *T. gondii* induce the fragmentation of the Golgi apparatus into ministack structures, around the PV, triggering host Golgi-derived vesicles, marked with Rab14, Rab30 or Rab43, within

the PV. These mechanisms allow *T. gondii* to scavenge sphingolipids from the host Golgi contributing to the parasite replication (Romano and Coppens 2013).

Several reports have been demonstrating that *Leishmania* parasites are also capable of modulating fatty acid metabolism, particularly regarding the role of cholesterol uptake and metabolism. *L. major* infection induces a transcriptional increase of key enzymes related with cholesterol synthesis, namely HMG-CoA reductase and squalene epoxidase. *L. major* interfere as well with the efflux of cholesterol and phospholipids through the downregulation of ATP-binding cassette transporter A1 (ABCA1). The diminished expression of sterol 27-hydroxylase (CYP27) is also crucial to block the prevention of cholesterol accumulation in the membranes (Rabhi et al. 2012). The disruption of cholesterol metabolism in *L. major*-infected cells was attributed to the cleavage of the pre-miRNA processor Dicer enzyme mediated by GP63. The downregulation of miR-122 is coupled with low levels of serum cholesterol in VL mice, leading to disease progression (Ghosh et al. 2013). Interestingly, VL patients present a reduced serum cholesterol that could be correlated with the splenic parasite load (Ghosh et al. 2011; Lal et al. 2010). The depletion of cholesterol from the cytosol or treatment with cholesterol-lowering drugs such as statins reduced the parasite burden demonstrating the impact of cholesterol for the progression of *Leishmania* infection (Dinesh et al. 2014; Kumar et al. 2016; Tewary et al. 2006).

Semini and colleagues recently tried to clarify more recently the complex interaction between host cholesterol and parasite survival. Cholesteryl esters are sequestered by the infected cells forming a halo around the parasites within the PV structure. A reduction of Niemann-Pick Disease type C1 (NPC1) protein, which is responsible for cholesterol efflux from endocytic compartments, supports the intracellular retention of cholesteryl esters as well as the concomitant increase of mRNA-encoding proteins, which are important modulators of cholesterol biosynthesis (Semini et al. 2017). Importantly, during *Leishmania* infection the reduction of cholesterol from the membranes alters the membrane fluidity, impairing the antigen-presenting capacity of the innate immune cells (Chakraborty et al. 2005). In agreement, the replenishment of cholesterol to the membrane increases the resistance in *L. donovani*-infected hamsters (Banerjee et al. 2009).

Some controversy exists among the different authors concerning the content levels of cholesterol following *Leishmania* infection. Mukherjee and colleagues found that at early phase of infection, between 0.5 and 4 h p.i., *L. donovani* lead to an increase of host cell membrane cholesterol through the upregulation and nuclear stabilization of SREBP2. This transcription factor upregulates mitochondrial uncoupling protein 2 (*UCP2*) and *HMGCR* transcripts that are crucial for the cholesterol biosynthesis-mediated intact membrane raft formation (Mukherjee et al. 2014). Such mechanism facilitates the parasite entry and suppresses mitochondrial-ROS production supporting parasite survival.

Within the context of cholesterol trafficking, Rabhi et al. found an enhancement of CD36 receptor, LDLr and low-density lipoprotein receptor-related protein 12 precursors (Lrp12), which are crucial for the uptake of LDL, during *L. major* infection (Rabhi et al. 2012). Additionally, an increase of lipoprotein lipase precursor (Lpl)

was detected that has been associated with a hydrolysis of triglycerides. Increased levels of LDL uptake were also noticed during *L. amazonensis* infection, where cholesterol from LDL is esterified and localized in compartments along the parasite body. Interestingly, such mechanism seems to rely on parasite membrane lipid microdomains (De Cicco et al. 2012). Finally, *L. amazonensis* can also alter the lipid metabolism of dendritic cells (DCs), an additional host target for *Leishmania* parasite, with the upregulation of cholesterol and long-chain fatty acids (LVFAs) uptake (Lecoeur et al. 2013). Despite the existence of some antagonism and in the light of serum cholesterol content found in VL patients, *Leishmania* has an impact on the modulation of influx/efflux cholesterol mechanisms, which become accumulated and potentially available to the parasite.

*T. cruzi* parasites infect a variety of host cells, including adipocytes, and are also dependent on host cholesterol metabolism, which was demonstrated by the impact of LDLr for the parasite invasion and for the fusion of the PV with host-cell lysosomes (Nagajyothi et al. 2011). In fact, the early interaction between the parasite and the host led to an accumulation of LDLr in coated pits, triggering the signalling cascades for lysosome recruitment (Nagajyothi et al. 2011; Rodriguez et al. 1996; Wilkowsky et al. 2001). Strikingly, an increased expression of LDLr was noticed in the heart of *T. cruzi*-infected CD1 mice (Nagajyothi et al. 2011). Recently, an increase in LDL levels and cholesterol was described in host tissues from acute and chronic murine Chagas disease, via LDLr–*T. cruzi* interaction (Johndrow et al. 2014). Interestingly, a direct association between LDL and *T. cruzi* trypomastigotes, but not amastigotes, was also observed, with the LDL covering the parasite cellular surface in close proximity of trans-sialidase localization. Trans-sialidase/glycoprotein 85 (gp85) is a neuraminidase that transfers sialic acid onto parasitic receptors, being involved in trypomastigote cell adhesion and invasion. Prioli and colleagues suggested that LDL inhibition of *T. cruzi* trans-sialidase may facilitate LDL endocytosis favouring *T. cruzi* infection (Prioli and Rosenberg 1990). LDL was also found in *T. cruzi* reservosomes, which are sites to accumulate endocytosed proteins and lipids, supporting a role for LDL in parasite metacyclogenesis from epimastigotes to trypomastigotes (Pereira et al. 2011; Soares and De Souza 1988; Soares et al. 1989). Overall, LDL and LDLr expression has been associated with the pathology and progression of atherosclerosis (Sunnemark et al. 2000). As a proof of concept, *T. cruzi* infection in animals fed with a high cholesterol diet led to early symptoms of atherosclerosis in mice (Nievelstein-Post et al. 1994). Therefore, the interaction between *T. cruzi* and LDL or LDLr may enhance host susceptibility for the development of atherosclerosis disease (Miao and Ndao 2014).

*T. cruzi* parasites are also able to interact with high-density lipoprotein (HDL), which has major functions such as removing excess cholesterol from the tissues, inhibiting LDL oxidation, inducing endothelial inflammation and promoting endothelial nitric oxide production (Miao and Ndao 2014). HDL inhibits *T. cruzi* trans-sialidase enhancing parasite infection in vitro (Prioli and Rosenberg 1990). Furthermore, it was suggested that such inhibition led to a decreased rate of trypomastigotes escaping from the PV and possibly delaying the process of trypomastigote transformation (Rubin-de-celis et al. 2006). This latter process of trypomastigotes transformation seems to be oppositely regulated by LDL-trans-sialidase inhibition. Also, HDL has

been suggested to play a role as a nutritional supply in *T. cruzi* epimastigote form (Prioli and Rosenberg 1990). Finally, the potential modulation of HDL during *T. cruzi* infection was highlighted by the presence of truncated fragments of apolipoprotein A-I (Apo A-I), the major structural component of HDL, in sera of *T. cruzi*-infected patients (Ndao et al. 2010).

#### 12.3.1.2 Lipid Droplets

Lipid droplets (LDs or lipid bodies) are present in almost all cell types, being able to store and supply fatty acids in all eukaryote and some prokaryote cells. Their main role is to secure the balance of lipid availability with metabolic and energetic demand (Pol et al. 2014). These organelles emerge from the ER lipid bilayer or from a subset of ER membranes. In eukaryotes, LDs can form de novo by progressive accumulation of neutral lipids in ER and also through fission processes that have been suggested due to the presence of such mechanism in yeasts (Long et al. 2012). They are composed of a core of neutral lipids, mainly TAG and sterol esters surrounded by a phospholipid hemimembrane associated with proteins (Murphy 2001; Tauchi-Sato et al. 2002). LDs also comprise different types of proteins such as perilipin family proteins perilipin/PLIN1, adipose differentiation-related proteins (ADRP/adipophilin/PLIN2) and tail-interacting protein of 47 kDa (TIP47/PLIN3), which are constitutively associated with the borders of LDs regulating lipid metabolism (Brasaemle et al. 2004; Wolins et al. 2001). In addition, the presence of enzymes of the lipid metabolism and membrane trafficking proteins (GTPases of the Rab family) that are important for vesicular traffic and organelle interaction can also be detected (Wan et al. 2007). Importantly, LDs also contain specific protein markers of other organelles, such as ER luminal chaperones and components of mitochondrial oxidative phosphorylation (Gao and Goodman 2015). During host/pathogen interactions, different pathogens modulate host fatty acid metabolism inducing an enrichment of LDs in proximity or in the PV, where eventually LDs may contact/internalize with the parasites, establishing a protein/nutritional flow.

Two reports found an enhancement of diacylglycerol (DAG) and TAG in *P. falciparum*-infected erythrocytes that accumulated closely to an acidic food vacuole (FV) of the parasite (Jackson et al. 2004; Palacpac et al. 2004). Jackson and colleagues suggested that an organelle storage with lipid intermediates (neutral lipids) might be generated during the digestion of phospholipids in the FV (Jackson et al. 2004), which has also an important role in the digestion of host haemoglobin. The neutral pool accumulated close to the PV might be involved in haem detoxification avoiding the toxic effects resulting from its accumulation such as oxidative damage, membrane disruption and inhibition of enzymatic reactions (Campanale et al. 2003; Loria et al. 1999) (Jackson et al. 2004; Rosenthal and Meshnick 1996). Finally, an enrichment of LDs was also observed in the liver and in the kidney of *Plasmodium*-infected mice, supporting the crucial impact of LDs during *Plasmodium* infection (Dumont et al. 1988; Pulido-Mendez et al. 2006; Rodriguez-Acosta et al. 1998).

The mobilization of host lipid resources for the intracellular *Toxoplasma* parasite was defined as one of the major strategies to sustain the remodelling of parasite membranes (Charron and Sibley 2002). LDs within the parasite may function as a storage of host lipid scavenged from the host, also being a source of lipids for parasite membrane biogenesis and eventually a site of lipid metabolism (Charron and Sibley 2002). In fact, LDs were described as a nutritional source for *T. gondii* parasites with the host lipid content and the lipolytic activity crucial for the parasite's development (Nolan et al. 2017). Upon *T. gondii* infection, there is an association between an upregulation of host LD-associated transcripts adipose differentiation-related protein (*ADRP*), diacylglycerol O-acyltransferase 2 (*DGAT2*) and LD accumulation being the PV become surrounded by LDs. Host LDs were also found to be upregulated in human cells, in a FABP-dependent process (Hu et al. 2017). The LDs enrichment is regulated upstream by two host signalling pathways, namely, c-Jun kinase (JNK) and mTOR. Such mechanism is apparently evolutionarily conserved, being distributed across different *Toxoplasma* strains and in the related parasite *Neospora caninum,* also a member of the apicomplexa phylum (Hu et al. 2017). Importantly, the impairment of LDs or the LD-associated biogenesis mechanisms reduce significantly *Toxoplasma* replication in the host cell. Moreover, *Toxoplasma* has access to host LDs storage that will be used for their own membrane remodelling and LD biogenesis, in a process regulated by Rab 7 protein and IVN structure (Nolan et al. 2017).

An increased transcription of key enzymes involved in the lipid metabolism and modulation of LDs biogenesis was also observed during *L. major* infection. As an example, *L. major* infection enhanced stearoyl-CoA desaturase 2 (*SCD2*) that regulates the ratio of monounsaturated to saturated fatty acids (Rabhi et al. 2016). Infected macrophages shift the transcriptional program towards the synthesis of TAG that is associated with the formation of lipid droplets. As a result, *L. major*-infected macrophages accumulate LDs that have been localized in a close association or even co-localized with the parasites within the PV. Live time lapse imaging allows the observation of a direct cumulative recruitment of LDs into the parasites. This suggests that LDs have a dynamic profile interacting continuously with PV and parasites (Rabhi et al. 2016). Importantly, LD accumulation is independent of parasite viability being detected in uninfected cells, although to a lower extent possibly through paracrine stimulation (Rabhi et al. 2012). LD enrichment is not exclusive of macrophages given that the same phenotype is observed in DCs infected with *L. amazonensis* amastigotes (Lecoeur et al. 2013). Some discrepancies were found concerning LD recruitment during *L. infantum chagasi* infection. Although no significant accumulation of LDs was observed throughout infection, all LDs were detected in infected cells and co-localizing specifically with the parasite in the PV. These LDs could be the result of de novo LDs synthesis performed by the parasite or could be eventually recruited from the host cells (Araújo-Santos et al. 2014).

The work developed by Melo et al. described for the first time an increased number of lipid droplets in infected macrophages during acute *T. cruzi* infection (Melo 1999). The modulation of LDs using C75, an inhibitor of fatty acid synthase, reduced

critically the LD formation as well as the parasite division within macrophages highlighting the impact of LDs for a successful infection (D'Avila et al. 2011). LD biogenesis during *T. cruzi* infection is regulated upstream by the activation of toll-like receptor 2 (TLR-2). Yet, the enhanced uptake of apoptotic cells by macrophages also constitutes an important mechanism to trigger LD synthesis. Uptake of apoptotic cells by macrophages together with TLR-2 triggering supports the interaction with $\alpha v \beta 3$ integrin. The activation of TGF-β-dependent lipid droplet formation and $PGE_2$ synthesis is then established. Overall, this mechanism increases the permissiveness of macrophages to *T. cruzi* infection (D'Avila et al. 2011; Freire-de-lima et al. 2011; Ming et al. 1995; Silva et al. 1991). Most importantly, there is a direct correlation between lipid droplet formation during *T. cruzi* infection and increased parasite load in vivo (D'Avila et al. 2011). Similarly to *Leishmania* infection, LDs accumulate in the vicinity of *T. cruzi*-infected macrophage phagolysosome or eventually within these structures. This suggests again a possible interaction between LDs and the phagolysosome throughout *T. cruzi* infection, which may serve as a platform for lipid exchange (Melo et al. 2003). On the other hand, such interaction might be important to mount a host response. The lipids recruited during LD biogenesis, such as arachidonic acid (AA) an upstream regulator of prostaglandins, can activate actin assembly, phagosome–lysosome fusion, phagosome maturation and ultimately the dinucleotide phosphate (NADPH-) oxidase. The latter structure is implicated in the elimination of intracellular pathogens, contributing to the macrophage microbicidal properties (Anes et al. 2003; D'Avila et al. 2012; Suh et al. 2006). Fig. 12.5 represents the major strategies used by the parasites to modulate host LD biogenesis with severe implications for parasite survival.

Overall, the formation/recruitment of LDs to the vicinity of PV, where the parasites reside, could be an important strategy to obtain a high-energy substrate source, protecting the parasites against toxic intermediates and/or modulating the host immune response.

#### 12.3.1.3 Lipid Droplets: An Immune Signalling Hub

The composition of LDs, as a reflection of their dynamic nature, could vary depending on the cell type and physiological state (Melo and Dvorak 2012). As an example, in immune cells, LDs have the entire enzymatic machinery to produce eicosanoids that are mainly inflammatory lipid mediators (Dvorak et al. 1993; Triggiani et al. 1995). Macrophages, eosinophils and neutrophils can store arachidonic acid that can be associated with phospholipids and/or neutral lipids. The LDs also possess the major enzymes for eicosanoid synthesis, cyclooxygenases (COX), lipoxygenases (LO) and leukotriene C4 (LTC4)-synthase as well as other enzymes such as phospholipase $A_2$ ($cPLA_2$) and MAPK proteins that are crucial to release arachidonic acid (Bozza et al. 1997, 1998).

LDs also play a crucial role within the immunologic synapse in the process of antigen presentation. LD organelles can regulate the cross-presentation of exogenous antigens to $CD8^+$ T cells in association with MHC class I, given that this process is

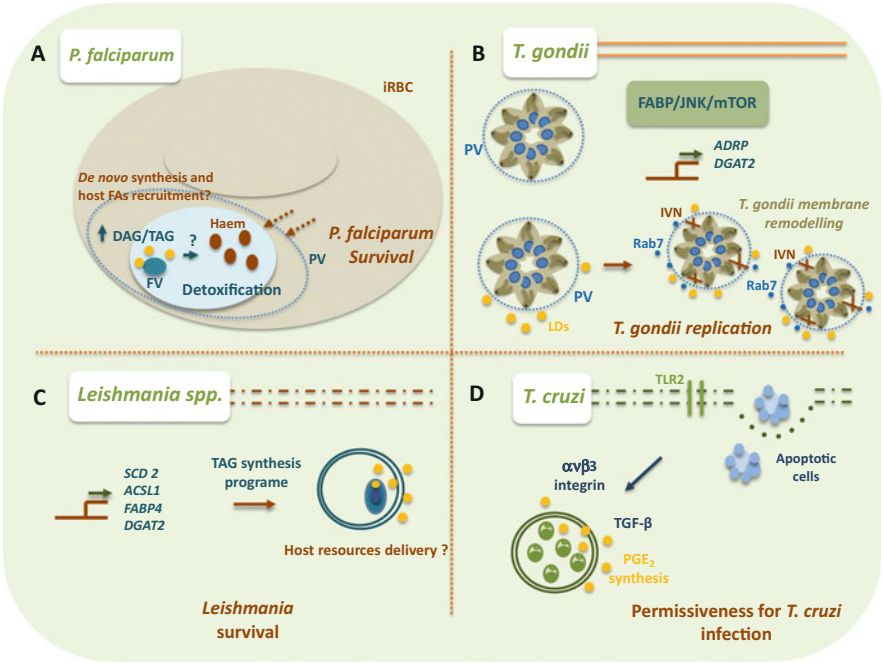


**Fig. 12.5** Different mechanisms used by the parasites to hijack host LDs. (**a**) *P. falciparum*-infected red blood cells (iRBCs) exhibit an increase of DAG and TAG synthesis, which are then accumulated closely to the parasite food vacuole (FV) in the form of LDs. LDs may play a critical role in the regulation of parasite oxidative damage via haem detoxification, favouring *Plasmodium* survival. (**b**) During *T. gondii* infection, the upregulation of FABP/JNK/mTOR network together with the increase of *ADRP* and *DGAT2* transcripts guides the infected cells to synthesize LDs. These LDs become closely located to the PV. The presence of Rab7 and the formation of an intravacuolar network (IVN) structure drive the lipids stored in LDs for the remodelling of *T. gondii* membrane. (**c**) *Leishmania spp.* upregulate the transcriptional program of TAG synthesis, potentiating the LD biogenesis in the host cells. These organelles are then found in the vicinity of parasites PV and eventually some of them co-localize with the parasites. Such mechanism may favour host resource delivery and ultimately *Leishmania* survival. (**d**) *T. cruzi* internalization leads to the activation of TLR2 receptor at the surface of host membrane. This mechanism along with the uptake of apoptotic cells by infected macrophages potentiates the program of LD synthesis via $\alpha v\beta 3$ integrin interaction and TGF-β production. All these events and the subsequent production of $PGE_2$ create a permissive environment for *T. cruzi* survival

critically reduced when in the presence of defects in LD formation (Bougnères et al. 2009). Recently, LDs have been defined as an important regulator of the activity of saponin-based adjuvants (SBAs) used in animal and human vaccines. The enhancement of antigen cross-presentation induced by SBAs is related to their capacity to induce LDs in the $CD11b^+$ DC in vitro and in vivo. LDs induction results in a saponin-dependent increase in cross-presentation and T cell activation (den Brok et al. 2016).

Several authors have begun to describe a potential role of LDs as crucial intermediates of host immune and microbicidal response. Two reports in the context of *T. gondii* infection described an increase in the number of LDs in the host cytoplasm of macrophages and skeletal muscle cells that fine-tune the capacity to reduce host microbicidal properties (Gomes et al. 2014; Mota et al. 2014). Additionally, a higher production of prostaglandin E2 ($PGE_2$), an inhibitor of Th1 immune response, eicosanoids, and an impairment of nitric oxide production were found, which benefits ultimately the parasite persistence in the host cell (Gomes et al. 2014; Mota et al. 2014). The correlation between LD formation and enhanced generation of $PGE_2$ by host cells has been also observed during *T. cruzi* and *L. amazonensis* infection (Melo et al. 2003; Pinheiro et al. 2009). Interestingly, within *T. cruzi*-infected macrophages, COX-2 and PGE2 immunolocalized in LDs indicates that these organelles could be the source of PGE2 in macrophages (D'Avila et al. 2011). Moreover, treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) or COX inhibitors decreases LDs and $PGE_2$ production, resulting in the reduction of parasite growth (D'Avila et al. 2011; Freire-de-Lima et al. 2000). As such, LDs enrichment may modulate macrophage immune response with the production of $PGE_2$, becoming the intracellular niche more suitable for parasite survival (Toledo et al. 2016).

#### 12.3.1.4 Is the Adipose Tissue a Safe Niche to the Parasite?

Different sets of parasites can exert a pressure over host lipid metabolism, leading eventually to the accumulation of important lipid reserves in the host cells. Yet, what is the potential role of adipocytes, which are lipid-enriched sites for the success of infection? Can the parasites use this niche to gain access to different sort of nutrients, or to evade the immune response constituting critical reservoirs for chronic infections? So far, parasites such as *T. cruzi*, *T. brucei* and *P. berghei* were observed to survive and persist in the adipose tissue (AT) of infected mice. During *P. berghei* infection, Frank-Fayerd et al. found that sequestration of malaria parasite-infected erythrocytes, also defined as the adherence of *Plasmodium*-infected red blood cells (iRBCs) to host tissue, occurs specifically in AT and lungs in a CD36-dependent mechanism (Franke-Fayard et al. 2005). Postmortem analysis of tissues from individuals who died due to malaria infection confirms that *P. falciparum*-iRBCs are sequestered not only in the lung and spleen but also in AT (Miller 1969; Wilairatana et al. 2000).

*T. cruzi* was also found in both white (WAT) and brown adipose tissues (BAT) (Shoemaker et al. 1970) as well as adipocytes of infected mice (Andrade and Silva 1995). In fact, both adipose tissues may represent important reservoirs for chronic infections in mice and humans (Combs et al. 2005; Ferreira et al. 2011). Although *T. brucei* are mainly extracellular parasites, residing in the blood and in interstitial spaces of many different tissues of the host, a recent report described the presence of *T. brucei* in mice adipose tissue, more specifically in the interstitial space between the adipocytes (Trindade et al. 2016). Based on scanning electron microscopy, the parasites were shown to interact with subcutaneous adipocytes, with entanglement

by reticular fibres and embedment between collagen bundles. However, it is still unknown yet if the parasites can eventually contact directly with the adipocytes (Caljon et al. 2016; Capewell et al. 2016; Trindade et al. 2016). Trindade et al. also found a significant increase in the levels of parasite DNA in adipose tissue compared with the levels in blood, during the chronic stage of infection. The parasites close to the AT modify their genetic profile with the upregulation of genes that encode for fatty acid oxidation enzymes in an adaptive behaviour to its microenvironment allowing the parasitesurvival and replication in close association with that niche. Ultimately, the authors suggested a flow of nutrients between the parasite and AT to explain parasite genetic alteration (Trindade et al. 2016).

AT could represent an advantage reservoir, especially during the chronic infections given the adipocyte large half-life (Spalding et al. 2008; Wang et al. 2013). A striking mechanism can exemplify the major importance of this tissue for chronic infections. *T. cruzi*-infected mice fed with a high-fat diet present low parasitaemia levels with a reduced myocardial pathology, although, despite the parasitaemia reduction, a significant increase of parasites was detected in WAT. Within this tissue, parasites can survive and be maintained without major impact for the host (Nagajyothi et al. 2014). Overall, adipose tissue may constitute an important reservoir, being nutritionally advantageous to the parasites that need to adapt successfully to this niche to overcome the inflammatory pressure exerted within.

## 12.4 Concluding Remarks

The immunometabolic network established during a host–parasite interaction is one of the most relevant mechanisms dictating the outcome of infection. It can be regulated through the action of distinct parasite factors, which affect different host pathways, and/or even by the nutritional pressure exerted by the parasite and the host nutritional requirements. The parasites' exquisite metabolic demand, to support their growth, occupying either extracellular or intracellular niches, exerts a critical nutritional pressure, which ultimately helps to define host metabolic landscape. In fact, this host–parasite metabolic coupling is dynamically regulated, reflecting different nutritional requirements in each stage of parasite differentiation, a process that is dependent on the host (mammalian/insect vector) to be fully accomplished. In that sense, we may define parasites as the master renovators of their host, becoming eventually impossible to dissociate from their host counterpart, constituting host–parasite interaction a unique entity with their own metabolic requirements.

The modulation of nutrient sensing pathways constitutes one of the major strategies employed by the parasites, especially during the early interaction phase. The redirection of metabolite flux and the dampening of a proper immune response against the parasites serve as critical platforms to support parasite adaptation, survival and proliferation. Interestingly, parasites that live restricted to the PV or to the cytosol, as *Plasmodium*, *Toxoplasma* and *T. cruzi* appear to be dependent on mTOR activation to support their survival. Instead, *Leishmania* parasites that reside

inside phagolysosome, despite the early detection of mTOR hyperphosphorylation, become highly dependent on AMPK activation to induce an increase of mitochondria pathways, crucial for *Leishmania* survival. This disparity among the modulation of host sensing pathways might be explained possibly by the nutritional content of phagolysosome intracellular niche. *Leishmania* complex auxotrophic requirement is indeed a tropism for this type of intracellular niche (Naderer and McConville 2011). Other pathogens with similar nutrient auxotrophies, such as the bacteria *Coxiella burnetii,* also display a phagolysosome tropism (Omsland et al. 2013).

The activation of host mTOR or AMPK sensing pathways could be a strategy of the parasites to cope their metabolic phenotype with the metabolic profile of the host cell. *Leishmania* amastigotes inside phagolysosome acquire a stringent metabolic state, with a lower metabolic and growth rate but still dependent on fatty acid oxidation to survive inside that niche (Saunders et al. 2014). The activation of catabolic pathways by AMPK might ensure a flow of tricarboxylic acid (TCA) cycle and fatty acid oxidation (FAO), intermediates and cofactors into the phagolysosome that could be consequently used by the amastigotes to support their own FAO pathway. Oppositely, *T. gondii* and *Plasmodium* parasites use glucose as a carbon source for ATP generation and various anabolic reactions, which might explain the possible modulation of host mTOR sensing pathway (Blume et al. 2009; Itani et al. 2014; Kirk et al. 1996; MacRae et al. 2012; Pfaller et al. 1982; Slavic et al. 2011). The modulation of host sensing pathways during infection might be crucial to define the baseline of the host–parasite metabolic phenotype, tracing the path, for the subsequent activation of further metabolic intermediates/pathways, creating ultimately a permissive niche to the parasites.

The remodelling of host nutrient reserves, particularly through the modulation of cholesterol and fatty acids metabolism, appears to constitute a general marker of a parasite infection. These specific mechanisms may enhance the parasite fatty acids pool to be used in the membrane remodelling, as a source of energy, or to maintain a continuous flow of lipids to specialized vacuoles where parasites reside (Lingelbach and Joiner 1998; Naderer and McConville 2011). Lipids may also be an important pathogenic factor to overcome immune response and manipulate host pathways during parasitic infections. The specialized class of lipids defined as glycosylphosphatidylinositol (GPI) lipids are widely distributed among protozoan parasites (Ramakrishnan et al. 2013). In *Leishmania*, the GPI-anchored lipophosphoglycans constitute an important virulence factor counteracting the host response (Dobson et al. 2010; Sacks 2001). Meanwhile in African trypanosomes, GPI-anchored variant surface glycoproteins are considered the key process to sustain antigenic variation mechanism used to manipulate host immune system. *Plasmodium* and *Toxoplasma* can induce the production of toxins and immune modulators that seems to be dependent on precursor GPI lipids (Debierre-Grockiego and Schwarz 2010; Nebl et al. 2005; Schofield et al. 2002). Even though many of these specialized lipids could be synthesized by the parasites, the host lipids might play a role in this pathogenic process supporting the importance of host lipid manipulation during infection.

The salvage of host lipids through the manipulation of host lipid metabolism indicates the degree of dependence of the parasites on this class of nutrients. It also may explain their tropism for the adipose tissue, a site that constitutes the most important source of lipids. Apart from the nutritional point of view, adipose tissue has also been recently addressed as one of the factors that could account for the fewer efficacies and subsequently for the relapse episodes of some antiparasitic drugs. The hydrophobic nature of adipose tissue might explain the impairment of the activity of some conventional therapeutic drugs, since most drugs are indeed hydrophilic. Such phenomenon was observed recently with posaconazole for *T. cruzi* treatment. The drug failure was directly linked to its inability to eliminate the parasites present in the mice adipose tissue (Francisco et al. 2015). A striking observation from Trindade et al. shows that *T. brucei* can be released from the adipose tissue and be relocated into the blood. This event suggests that adipose tissue might be an important niche for parasites toescape an immunological response, guiding the infection into the chronicity stage, and simultaneously support some relapse episodes that may occur after treatment (Trindade et al. 2016).

The importance of a lipid-enriched environment for the parasite survival may argue for the existence of a possible link between the success of a parasitic infection with host metabolic dysregulation. In fact, a higher susceptibility *L. infantum chagasi* was observed in an obesity mice model (diet-induced obesity), exhibiting an increased parasite burden compared to the standard-diet animals (Sarnáglia et al. 2016). Interestingly, a correlation was made between individuals with higher body mass index, who were diagnosed previously for cutaneous leishmaniasis, compared to the healthy group (Da Cunha et al. 2009). A positive association was also established between *T. gondii* seropositivity and obesity, where higher levels of *T. gondii* IgG titres were detected in obese individuals compared to the non-obese group (Reeves et al. 2013). During *T. cruzi* infection, Nagajyothi and colleagues observed in infected-high fat diet (HFD) mice, a reduction in the mortality, parasitaemia and cardiac parasite load but at the same time an increase of parasite load in adipocytes. Interestingly, during *P. berghei* infection, obese mice, genetically altered (*ob/ob* mice), were proved to be resistant to cerebral malaria (Robert et al. 2008). Additionally, mice subjected to HFD for 2 or 4 days exhibited a clear reduction of *P. berghei* in the liver, due to ROS production, and consequently a delay in the onset and progression of blood stage infection (Zuzarte-Luis et al. 2017). It is important to notice that these two reports regarding *Plasmodium* infection do not show any measurements of the parasite load in AT. And also if the parasites, in this lipid-enriched environment, become more protected from ROS effect since an antioxidant role was recently defined for LDs in a stem cell niche of the *Drosophila* model (Bailey et al. 2015). Finally, in the context of *T. cruzi* infection, two reports described an interesting relationship between diabetes, a metabolic disease and *T. cruzi* infection. Mice with chemical-induced diabetes or with genetically induced diabetes (*db/db* mice) displayed high parasitaemia levels and mortality rate (Tanowitz et al. 1988). Recently, Cabalén et al. described the ability of *T. cruzi* parasites to potentiate adipose tissue macrophage polarization towards an anti-inflammatory profile, favouring diabetes progression in a diet-obesity model

(Cabalén et al. 2016). *T. cruzi* seems to potentiate a dysregulation of host metabolic status, particularly diabetes, in order to create a glucose-rich environment that may constitute an important nutritional source for the parasite.

Metabolism is a key element of the host–parasite duet, which is able to dictate the success of a pathogen infection. Parasites within the host develop sensing and subversion mechanisms to orchestrate host metabolic pathways. Host–parasite interaction has been modulated through evolution, and this may account for the huge plasticity exhibited by the parasite. In fact, such event is clearly observed by the capacity of the parasites to reside in distinct host niches. Taking advantage of the host nutrient resources, parasites manipulate the fluxes of nutrients in accordance with time, space and demand conditions, giving them tremendous adaptation skills.

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