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Phagosome-lysosome fusion hijack - An art of intracellular pathogens

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# Equal contribution

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Running Head: Role of phagosome-lysosome fusion in bacterial pathogenesis

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Abstract

Phagosome-lysosome fusion is an important innate-effector immune response of host macrophages. After entering the macrophages through phagocytosis, intracellular bacteria and parasites reside inside the phagosomes. In many cases, these pathogens prevent maturation of phagosomes and its fusion with lysosomes. Several signaling cascades are shown to be associated with blocking of phagosome maturation process. Understanding the mechanism of phagosome-lysosome fusion and factors regulating this process, as well as the strategies adopted by the intracellular pathogens to prevent phagosome-lysosome fusion might provide insights for the development of new drugs and more effective treatment options to combat infectious diseases.

Introduction

Infectious diseases caused by intracellular pathogens are major threats to the human health worldwide. Intense efforts have been directed towards understanding the mechanisms by which intracellular pathogens could compromise protective responses of host such as, protection from circulating antibodies (Evering and Weiss, 2006), free access to nutrients and establishment of their replicative niches to cytosol or specialized compartments (Hackstadt, 2000; Mitchell *et al*., 2016). According to their certain lifestyles and need, many bacterial pathogens such as *Listeria monocytogenes* (Brzoza *et al.*, 2004; Sanger and Sanger, 2012), *Shigella flexneri* (Jehl *et al*., 2012; Campbell-Valois *et al*., 2015) and *Trypanosoma cruzi* (Camandaroba *et al*., 2006) reproduce in cytoplasm (Thi *et al*., 2012), while others like *Mycobacterium tuberculosis* (*M. tb*) target specific vesicles called pathogen containing vacuoles for their lodging and multiplication. This class of parasitism is utilized by *M. tb* (Clemens *et al*., 2002), *M. leprae* (Frehel and Rastogi, 1987), *Coxiella burnetii* (Ghigo *et al*., 2012), *Toxoplasma gondii* (Sibley, 2013). In response to pathogen assault, phagocytic cells employ multiple mechanisms to ensure elimination of intracellular pathogens or restrict them under stringent control. Pathogens enter

into the phagocytes via cell-surface receptors and are trapped within the phagosomes (phagocytosis), which then fuse with lysosomes to form phagolysosomes, where the pathogens are degraded due to respiratory or oxidative burst, low pH, action of lysosomal acid hydrolases and secretion of microbicidal substances, such as elastase (Flannagan *et al*., 2009; Hussain Bhat and Mukhopadhyay, 2015). Thus, phagocytosis is an important process of the innate immune response aimed towards the removal of pathogens. Invading pathogens hijack phagosome maturation process to escape from getting wiped out, which is controlled by numerous factors and signaling cascades. Various pathogens directly or indirectly modulate this process to generate a favorable intracellular niche. In this review, we have discussed signaling cascades which influence phagosome maturation process and the strategies adopted by various intracellular pathogens, especially *M. tb* to arrest phagosome maturation. This information is likely to be helpful to define new therapeutic targets against various intracellular pathogens.

Phagosome maturation

Phagocytic cells viz., monocytes/macrophages, dendritic cells, neutrophils and other antigen (Ag) presenting cells in heamatopoietic lineage not only process the extracellular larger particles but also present them onto cell surface (Ag presentation). They comprise the largest number and complex cell population to primarily counter attack the pathogens, hence instituted as part of so called "first line of defense" (Aderem and Underhill, 1999; Greenberg and Grinstein, 2002). Macrophages are one of the important body's first line of defense cells that are involved in recognition and uptake of the pathogen into cells, and destroy the pathogens by utilizing various processes called macrophage effector functions (Garin *et al.*, 2001; Greenberg and Grinstein, 2002; Murray and Wynn, 2011). Based on functional plasticity of macrophages, they can be classified as M1 or M2 macrophages (Mills, 2012; Martinez and Gordon, 2014). Granulocyte-macrophage colony-stimulating factor (GM-CSF) and inflammatory stimuli like interferon-gamma (IFN-γ) and lipopolysaccharide (LPS) induce M1 macrophages whereas stimuli of macrophage colony-stimulating factor (M-CSF) and IL-4 give rise to M2 macrophages (Mia *et al.*, 2014). Phagocytosis or receptor-mediated endocytosis is an active process whereby phagocytic cells engulf pathogens and antigens into membrane bound vacuoles called phagosomes. Phagosome itself neither can destroy the pathogens nor inhibits the replication of pathogens, because the lumen of nascent vacuole is similar to the fluid phase outside macrophages, except that it is surrounded by a membrane. Thus, phagosome acquires the machinery needed to destroy the pathogens. Acquisition and removal of many proteins by fission and fusion events, a severe drop in lumen pH and procurement of many hydrolases and peptidases, significantly alter the biochemical nature of phagosome lumen that ultimately degrade pathogens. This entire process is termed as phagosome maturation (Desjardins *et al.*, 1994; Garin *et al.*, 2001; Vieira *et al.*, 2002; Smith *et al.*, 2007). Phagosomal maturation is controlled by highly complex and regulated signaling events, which engages many factors and take part in host-pathogen interactions, autophagy and apoptosis (Mariño *et al.*, 2014).

Important signaling cascades regulating phagosome-lysosome fusion and its manipulation by intracellular pathogens

Intracellular pathogens operate by confronting key signaling pathways in their hosts. These pathogens usually target more than one signaling cascade and often interact at several points to

seize them fully. Although different intracellular pathogens tend to exploit these machineries in the host, the ways in which they commandeer host cells usually differ. Modulation of phagosomal maturation by intracellular pathogens is initiated at very early stage of pathogen recognition, either by avoiding the fusion of the pathogen containing phagosomes with lysosomes or by escaping from phagosomes to replicate in cytosol. Recognition of pathogens by various receptors on cell surface many times determine the fate of endocytosed/phagocytosed pathogens. Here, we have discussed about the pathogen recognition by important surface receptors of macrophages and its effect in host-pathogen interaction in the context of phagosome-lysosome fusion.

Receptor-triggered signaling events

i. Complement receptors (CR)

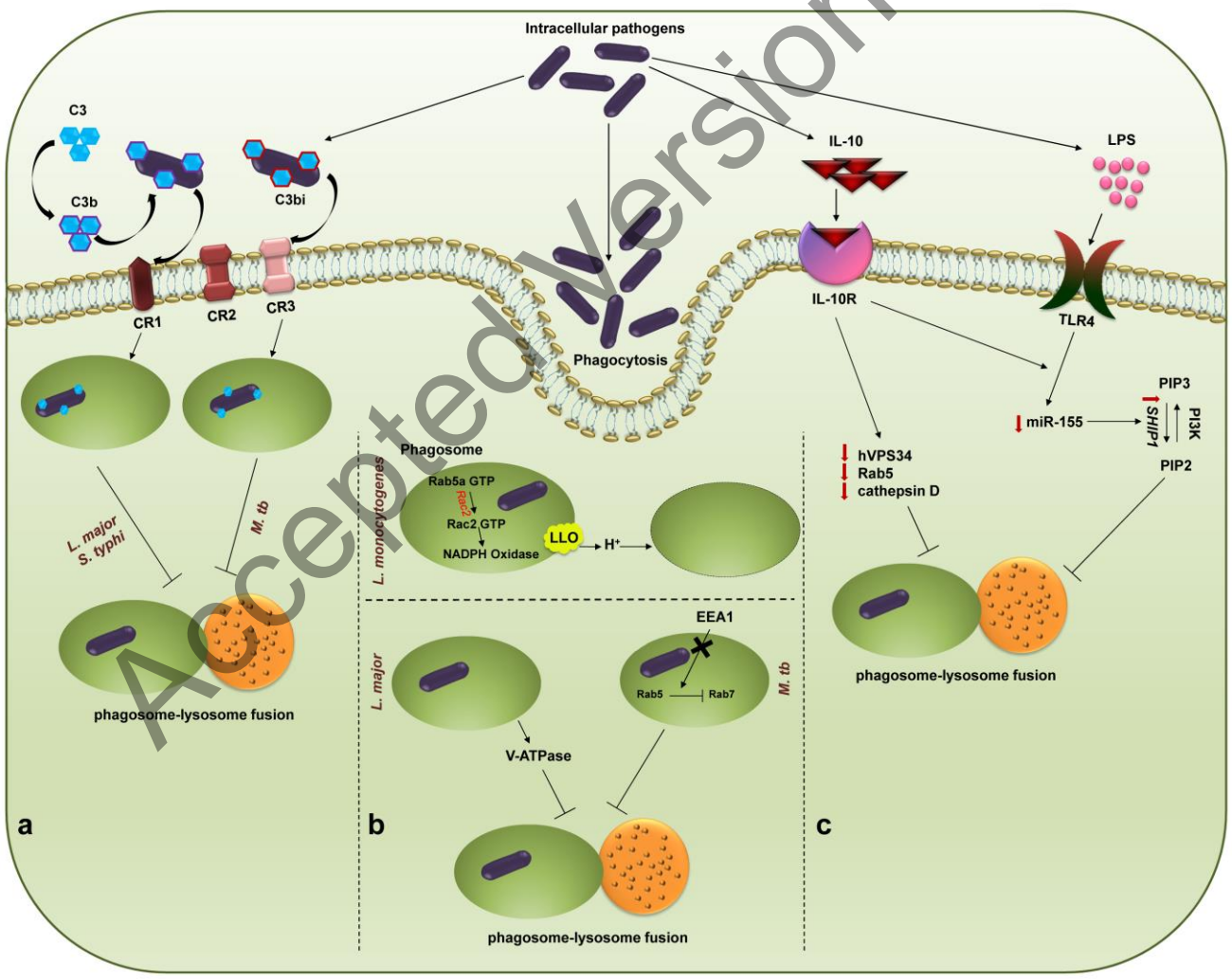

Figure 1

Figure 1. Schematic illustration deciphering exploitation of signaling mechanisms by various intracellular pathogens to inhibit phagosome-lysosome fusion in macrophage. (a) Opsonized

mycobacteria are internalized through CR3 receptor, while *L. major* and *S. typhi* binds to CR1 and inhibit phagosome-lysosome fusion. (b) After internalization of *L. monocytogenes* into phagosome, secretion of listeriolysin O (LLO) which facilitates escape of pathogen due to pore formation. *M. tb* inhibits recruitment of EEA1 to phagosome which stops further downstream signaling for maturation whereas *L. major* cause exclusion of V-ATPase from phagosome and inhibits phagosome-lysosome fusion. (c) The anti-inflammatory cytokine IL-10 binds to IL-10 receptor (IL-10R), down-regulates hVPS34, Rab5, cathepsin D expression important for phagosome-lysosome function. IL-10 also suppresses LPS-induced miR-155 expression causing up-regulation of miR-155-targeted gene *SHIP1*, thus promoting conversion of PIP3 to its inactive PIP2 state and arresting of phagosome-lysosome fusion.

Complements are heat labile serum proteins that act together to destroy invading pathogens. Complements are normally present in inactive forms. These proteins are activated by proteolysis through series of steps of three major pathways, i.e. the classical pathway, the alternate pathway and the lectin pathway, all of which lead to the production of C3b (crucial component of the complement system) which is essential to activate latter steps of complement activation (Ahearn and Fearon, 1989; Carroll, 1998). Pathogens coated with serum-derived ligands bind to complement receptors CR1, CR3, and CR4 accordingly and are subsequently phagocytosed in membrane-bound phagosomes (Schlesinger, 1993; Hirsch *et al*., 1994). *M. tb,* like other pathogens can activate the alternative pathway of complement system that leads to opsonization with C3b and C3bi and binding to the complement receptors (Schlesinger *et al*., 1990). Pathogenic mycobacterial cell wall component distinctively recruit the complement fragment C2a to form C3 convertase and produce opsonically active C3b even in the absence of early activation components of the alternative or classical pathways. This mechanism leads to binding of C3b opsonized mycobacteria, especially to CR1 rather than to CR3 or CR4 (Schorey *et al*., 1997; Hu *et al*. 2000). Endogenous capsular polysaccharides of non-opsonized *M. tb* interact with the β-glucan binding site near the C-terminus of CD11b, while opsonized *M. tb* binds C3bi binding domain of CR3, suggesting binding of *M. tb* at two distinct sites on the CR3 receptor. CR3 is relatively predominant among the CRs. Human monocytes and macrophages showed about 70 to 80% reduced ability in phagocytosis of *M. tb* in absence of CR3 (Schlesinger *et al*., 1990; Schlesinger, 1993). Moreover, interaction of *M. tb* to CR3 causes phagosomal arrest due to interruption of respiratory burst (Wright and Silverstein, 1983). Thus, the internalization of *M. tb* through binding with CRs is distinctly beneficial route for *M. tb* to aid in survival and pathogenesis (Hirsch *et al*., 1994) (Figure 1). Certain pathogens ensure their survival by activating alternate pathways of complement activation. For example, *Leishmania major* activates the alternative complement pathway to recruit C3b on its surface. When opsonized metacyclic promastigotes (infective form) bind to CR1, they survive and replicate intracellularly, while promastigotes (non-infective forms) are killed when enter macrophages through the lectin-like domain of CR3 (Mosser and Edelson, 1987; Polando *et al*., 2013). *Salmonella typhi* enters murine macrophages through CR3, phagocytosed in a vesicle that fuses with lysosomes, while entry via CR1 allows *S. typhi* to survive in a phagosome that does not acquire lysosomal markers (Ishibashi and Arai, 1990) (Figure 1). Thus, internalization of other intracellular pathogens through CR decide the fate of phagosome-lysosome fusion.

ii. Mannose receptors (MR)

Mannose receptors (MR) are transmembrane C-type (calcium dependent) lectins that specifically bind mannose sugars present on the surface of pathogens. Mannose receptors are not expressed on monocytes, whereas the differentiated monocytes (macrophages) have been reported for MR expression on their cell surface (Ezekowitz *et al.*, 1990; Schreiber *et al.*, 1993). Phagocytosis of the *M. tb* is known to be mediated via MR, mainly when it is bound to the terminal mannose residues of lipoarabinomannan (LAM) expressed on cell surface of pathogens and influence survival of bacilli within macrophages (Schlesinger, 1993; Schlesinger *et al*., 1994, 1996). Phagocytosis via MR could result in reduced production of reactive oxygen intermediates (ROI) and pro-inflammatory cytokines like IL-12, tumor necrosis factor-alpha (TNF-α) but increased production of anti-inflammatory cytokines, IL-4 and IL-13 by macrophages, suggesting a role of MR in host-pathogen interaction and modulation of macrophage immune responses in tuberculosis (Ezekowitz *et al.*, 1990; Zhang *et al.*, 2005; Gazi and Martinez-Pomares, 2009). Interestingly, interaction of *M. tb* ManLAM with MR promotes inhibition or delay in phagosome-lysosome fusion (Vergne *et al*., 2003; Fratti *et al*., 2003; Kang *et al.*, 2005). Virulent Erdman and H37Rv strains of *M. tb* mainly use MR in addition to CR for endocytosis and inhibition of phagosome-lysosome fusion to enhance its survival (Schlesinger *et al.*, 1996). Shimada *et al.* (2006), found that treatment of THP-1 cells with *Staphylococcus aures* glycopeptidolipid (GPL) led to binding of GPL to MR causing arresting of phagosome-lysosome fusion. Moreover, treatment with competitive inhibitors against MR or use of anti-MR monoclonal antibody (mAb) rescued GPL-induced inhibition of phagosome-lysosome fusion indicating that the inhibition of phagosome-lysosome fusion by GPL is mediated through MRs but not through CRs. GPL of *Mycobacterium* sp. is also reported to inhibit phagosome-lysosome fusion in MR-dependent manner (Sweet *et al*., 2010). Furthermore, few species of phosphatidyl-*myo*-inositol mannosides (PIMs) present on surface of *M. tb* could bind to the MR and beads coated with these PIMs also caused an MR-dependent delay in phagosome-lysosome fusion (Torrelles *et al*., 2006). Another study has shown that mycobacterial cell wall component ManLAM upon binding to MRs, upregulate IRAK-M, a negative regulator of TLR signaling (Kobayashi *et al.*, 2002; Pathak *et al*., 2005). ManLAM is also shown to activate ERK and PI3K pathways in TLR-dependent manner leading to production of IL-10 (Caparros *et al.*, 2006), which has been shown to directly inhibit phagosome-lysosome fusion (O'Leary *et al.*, 2011). Thus, ManLAM might negatively affect phagosome-lysosome fusion. However, recent work by Aplemelk and colleagues has indicated that mannose capping of ManLAM may not influence phagosome maturation. They used isogenic cap-less mutants where mannose cap of ManLAM were removed from *M. marinum* and *M. bovis* BCG, and these bacteria did not show any alteration in phagosome-lysosome fusion and cytokine profile in macrophages (Appelmelk *et al.*, 2008). Burgdorf *et al.* (2007) have indicated that MR-internalized Ag (the model Ag ovalbumin, OVA) is targeted into early endosome where it gets co-localized with early endosomal markers Rab5 and EEA1, but not with late endosomes or lysosomal markers Rab7 and LAMP-1. In this study it was shown that while pinocytosed Ag (Lucifer yellow) and scavenger receptor (SR)-internalized OVA co-localized specifically with lysosomal MHC class-II, MR endocytosed OVA co-localized with MHC class-I. This explains that, MR associated endocytosis inhibits the phagosomal maturation process and directs OVA into the stable early endosome compartment for cross-presentation (Burgdorf *et al.*, 2007).

iii. Toll like receptors (TLRs)

TLRs are expressed on host macrophages as pattern recognition receptors and recognize the pathogens by their common patterns (Medzhitov *et al.*, 1997). Though triggering of TLR-signaling is shown to be important to activate various macrophage effector responses like cytokine production, oxidative and nitrosative responses and antigen presentation (Nair *et al*., 2014), its role in regulating phagosome-lysosome fusion is controversial. Blander and Medzhitov in their experiments with mice lacking TLR2, TLR4, and TLR signaling adaptor molecule MyD88 explored the fate of phagosomes containing gram-negative (*E. coli*) or gram-positive bacteria (*S. aureus*). They reported that in the absence of TLR signaling, acquisition of lysosomal markers LAMP-2 and fluorescent lysotracker dye were significantly impaired (Blander and Medzhitov, 2004). In their study they observed that phagosomes containing *E. coli* or *S. aureus* could fuse with lysosomes in macrophages from wild-type mice, whereas failed to fuse with lysosomes in macrophages from $MyD88^{-/-}$ or $TLR2\times4^{-/-}$ mice, suggesting that phagosome maturation might be controlled by TLR-dependent signaling cascades. However, Yates and Russell explored extremely opposite views in their report, where they found that TLR signaling had no role in phagosomal maturation process (Yates and Russell, 2005). To validate this, mannose- or IgG-coupled silica particles that were free of any TLR-stimulating activity were used. TLR stimulation was induced by coating the beads with LPS (a TLR4 agonist) or PAM_3CSK_4 (a TLR2 agonist), which hinted no alteration in phagosomal acidification, even by internalization of mannose- or IgG-coupled silica particles or with inclusion of TLR agonists. They demonstrated that phagosome-lysosome fusion happened at the same rate whether TLRs were activated or not. However, Yates and Russell indicated the role of MyD88 in phagosomal maturation process in agreement with Blander and Medzhitov (Blander and Medzhitov, 2004; Yates and Russell, 2005). In a study, McCoy *et al.* (2010), demonstrated that IL-10 suppressed miR-155 expression in response to LPS (a potent TLR4 ligand), leading to an increase in the expression of miR-155 targeted gene *SHIP1* (Src homology 2 domain-containing inositol-5-phosphatase 1), an inositol phosphatase that converts phosphatidyl inositol 3 phosphate (PIP3) to PIP2 (McCoy *et al.*, 2010). PIP2 to PIP3 conversion by phosphoinositide 3-kinase (PI3K) is one of the critical steps in phagosomal maturation process and TLR4 signaling can promote PI3K activation. Thus, inhibition of TLR4-dependent signaling by IL-10 may influence phagosomal maturation process (An *et al*., 2005; McCoy *et al.*, 2010) (Figure 1). Also, O'Leary *et al*. (2011), reported that IL-10 could inhibit phagosome-lysosome fusion and impeding IL-10 activity could then rescue the inhibited phagosome-lysosome fusion in case of *M. tb* infection. IL-10 production in *M. tb* infection is strongly related with TLR signaling (Redford *et al.*, 2011). These studies are important with respect to correlating many of the signaling cascades influencing phagosome-lysosome fusion, which may be regulated by TLR signaling but do not establish a direct connection and further investigations are required to draw a strong conclusion.

Cytokine signaling

Cytokines secreted during innate and adaptive phase of immune responses can influence the maturation of phagosomes into phagolysosomes. The pro-inflammatory cytokines such as IFN-γ and TNF-α, and the anti-inflammatory cytokines like IL-10 has been shown to affect the endocytic pathways and thus can modify phagosome biogenesis during bacterial infections (Via *et al.*, 1998). Cytokines change the key endocytic regulators which are involved in membrane trafficking, endosome conversion and phagosome conversion. Jouanguy *et al*. (1996),

demonstrated that individuals defective in IFN-γ or IFN-γ receptor gene had severe BCG infection in vaccinated children or to atypical mycobacterial infection in unvaccinated persons. Macrophage stimulated with IFN-γ and LPS resulted in increased co-localization of mycobacteria with lysosomal markers such as LAMP-1 and cathepsin D, indicating that IFN-γ enhanced fusion of phagosomes with lysosomes (Via *et al.*, 1997). Schaible and group found that IFN-γ activated maturation and acidification of *M. avium*-containing phagosomes that enhanced killing of the bacilli in macrophages. Further biochemical analysis of mycobacterial phagosomes confirmed that the low intra-phagosomal pH was correlated with the increased accumulation of vacuolar H^+-ATPase (V-ATPase) (Schaible *et al.*, 1998), suggesting a direct role of IFN-γ induced signaling in phagosome maturation and their acidification. However, treatment of macrophages with IFN-γ prior to *C. burnetii* infection induced alkalization of *C. burnetii* vacuoles independent of V-ATPase exclusion (Ghigo *et al.*, 2002) which is in contrast with the findings that IFN-γ could reduce the pH of mycobacterium-containing phagosomes by accumulating V-ATPase (Schaible *et al.*, 1998). IFN-γ also inhibited remodeling of *Legionella pneumophila* containing phagosomes into ER-derived vesicles through their conversion into LAMP-2 and cathepsin D-expressing phagolysosomes (Santic *et al.*, 2005). Further, IFN-γ is also shown to be involved in induction of Rab5a expression and phagosome conversion in *L. monocytogenes* infection (Prada-Delgado *et al.*, 2001). IFN-γ induced Rab5 causes remodeling of the phagosomal environment, assisting the translocation of Rac2 to phagosomes harboring *Listeria* sp. and regulating the Rac2 GTPase activity. After recruitment to phagosome, Rac2 directs phagocyte NADPH oxidase activity and the subsequent production of oxidative free radicals (Prada-Delgado *et al.*, 2001). These events facilitate the transition of early phagosomes to late phagosomes and the subsequent killing of *L. monocytogenes*. Even though role of IFN-γ in induction of phagosome-lysosome fusion is well established, a study by Trost *et al*. (2009), using quantitative proteomics and bioinformatics approach has indicated that latex beads containing phagosome, induced by IFN-γ, delay their maturation despite the abundance of proteins mainly involved in phagosome-lysosome fusion like VAMP8, Syntexin-binding proteins (1, 2 and 3), Syntexin (4, 8 and 11), LAMP-1 and many Rab GTPases. They found that IFN-γ delayed acquiring lysosomal hydrolases and peptidases that resulted in the gain of MHC class-I antigen presentation. By network analysis they have proposed that enhanced antigen presentation is dependent on phagosomal networks of the actin cytoskeleton and vesicle-trafficking proteins. Further, they found that IFN-γ-activated macrophages delayed disassembly of actin filaments during phagosome-lysosome fusion (Yam and Theriot, 2004; Trost *et al.*, 2009). Even though this report deviates from the early finding that IFN-γ induces phagosome-lysosome fusion, however it does not completely cast-off the involvement of IFN-γ in inducing phagosomal maturation. Moreover, IFN-γ might have delayed phagosome-lysosome fusion, quantitative estimation of phagosomal lysosomal markers support an enhanced phagosome-lysosome fusion by IFN-γ treatment. Other pro-inflammatory cytokines, such as IL-6, IL-12 and IL-22 have also been shown to modulate the conversion of phagosomes into phagolysosomes. IL-22 producing NK cells inhibit intracellular growth of *M. tb* by enhancing phagosome-lysosome fusion (Dhiman *et al.*, 2009). When cells are treated with IL-12, the salmonella-containing vacuole is targeted to lysosomes, while the transport of *Salmonella* sp. to lysosomes is inhibited in the presence of IL-6 (Bhattacharya *et al.*, 2006).

As indicated in the earlier section, the anti-inflammatory cytokine such as IL-10 has been shown to affect phagosome conversion in opposite fashion. It was observed that IL-10 strongly

decreases the expression of Rab5 and VPS34 transcripts, and thus slows down endosome and phagosome conversion (Barry *et al*., 2011) (Figure 1). As suggested by Barry *et al*. (2011), it is also possible that IL-10 inhibits Rab-prenylation or GDI activity to inhibit phagosome maturation. IL-10 along with IL-4 and IL-13 was found to reduce the expression of cathepsin D in monocytes from patients with inflammatory bowel disease (Lugering *et al.*, 1998) and affects fluid-phase and mannose receptor-mediated endocytosis in human primary macrophages (Montaner *et al.*, 1999). Consequently, the delivery of cathepsin D to lysosomes is decreased (Figure 1). In macrophages derived from the bone marrow of IL-10 knockout mice, the co-localization of mycobacteria with lysosomal markers was shown to be enhanced relative to macrophages from control mice, suggesting an increase in the acidification of mycobacterial phagosomes in these mice (Via *et al.*, 1998). IL-10 has been reported to be a highly produced cytokine during chronic Q fever (Capo *et al.*, 1996; Honstettre *et al.*, 2003) and monocytes from patients with chronic Q fever are not able to kill *C. burnetii* and exhibit defective phagosome conversion (Ghigo *et al*., 2004).

Rab GTPases and Calcium signaling

Rab GTPase are the largest group of monomeric GTPases within Ras superfamily. Over 70 Rab GTPases have been reported so far and shown to regulate vesicular transport and phagosomal maturation process (Roberts *et al.*, 2006; Markgraf *et al*., 2007; Schwartz *et al.*, 2007). The endocytic compartment, termed as early endosome obtain an important Rab GTPase protein Rab5 due to activity of GEF (Guanine nucleotide exchange factor) molecules, Rabex-5 and Rabaptin-5 (Stenmark *et al.*, 1995; Horiuchi *et al.*, 1997). The procurement of Rab5 is responsible for early endosomes to undergo homotypic fusion and initiates successive binding of other effector molecules like EEA1 and hVPS34 (PI3K3) (Gorvel *et al.*, 1991; Simonsen *et al.*, 1998; Callaghan *et al.*, 1999; Fratti *et al*., 2001). Interaction of EEA1-FYVE domain with phosphatidyl inositol 3 phosphate (PI3P) leads to hetero-oligomerisation with other Rab5 effectors like Rabaptin5, Rabex-5 and NSF to cluster into a macromolecular complex on endosomal membrane. Surprisingly, Rab5 is not a part of this macromolecular complex even though Rab5 directly interacts with EEA1 and Rabex5-Rabaptin5 complex (Callaghan *et al.*, 1999; McBride *et al.*, 1999). Recruitment of EEA1 to early endosomal membrane and its molecular assembly with other Rab5 effector proteins is critical for phagosomal maturation process as EEA1 recruitment to early endosomes makes the phagosomal maturation process directional from early to late endosome (Simonsen *et al.*, 1998; Rubino *et al*., 2000). Along with EEA1, Rab5 effector hVPS34 (PI3K3) is also recruited to early endosome, which is responsible for synthesis of PI3P (Fratti *et al.*, 2001; Futter *et al.*, 2001; Vieira *et al.*, 2002). Because of the homotypic fusion and accumulation of cargo, size of these vacuoles increases as a result PI3P level increases. Interestingly, the sizes of these vacuoles or level of PI3P determines the early to late endosomal transition. At a particular size, Mon1a/b, a molecular switch acts upon early endosomes to remove Rab5 and recruit Rab7, giving rise to late endosomes (Poteryaev *et al.*, 2010). The HOPS (homotypic fusion and vacuole protein sorting) complex is the GEF for Rab7 (Poteryaev *et al.*, 2010). Mon1a and Mon1b interact with the core component of HOPS complex and play an important role in fusion of late endosomes to lysosomes (Poteryaev *et al.*, 2010). Also, other Rab GTPases have been shown to play important roles during endocytic process and phagosomal maturation indicating a cumulative action of Rab GTPases (Desjardins *et al.*, 1994). Rab33 and Rab24 are illustrated for their role in formation and maturation of autophagosomes

respectively (Munafo and Colombo, 2002; Itoh *et al.*, 2008). Rab8 is shown to mediate constitutive biosynthetic trafficking from the *trans*-Golgi network (TGN) to the plasma membrane and participation in GLUT4 vesicle translocation in association with Rab10 and Rab14 and ciliogenesis process through its cooperation with Rab17 and Rab23 (Miinea *et al.*, 2005; Yoshimura *et al.*, 2007; Sano *et al.*, 2008). Rab32 and Rab38 are involved in the biogenesis of melanosomes (Wasmeier *et al.*, 2006) and Rab32 also controls mitochondrial fission (Bui *et al.*, 2010). Recently Rab32 has also been reported to influence phagosomal maturation process in association with other Rab GTPase proteins (Li *et al.*, 2016). Rab22a mediates trafficking between early/recycling endosome to TGN (retrograde) (Mesa *et al*., 2005) and Rab22b anterograde trafficking from TGN to endosome/cell surface (Ng *et al*., 2007). Rab5 mediates endocytosis and endosome fusion of clathrin-coated vesicles (CCVs) (Robinson *et al.*, 1996), macro-pinocytosis with Rab34 (Coyne *et al.*, 2007) and maturation of early phagosomes with Rab14 and Rab22 (Kyei *et al.*, 2006; Ng *et al.*, 2007). Rab21 mediates integrin endocytosis. Further, Rab11 and Rab35 are shown to involved in endocytic recycling through recycling endosomes, whereas Rab4 facilitates fast endocytic recycling directly from early endosomes (Riggs *et al.*, 2003; Kouranti *et al.*, 2006). The late endosome-associated Rab7 is shown to play a crucial role in maturation of late endosomes and phagosomes and their fusion with lysosomes (Via *et al.*, 1997; Bucci *et al.*, 2000). Another late endosomal GTPase, Rab9 is shown to involved in trafficking from late endosomes to the TGN (Barbero *et al.*, 2002) (Table 1).

Rab GTPases regulate many intracellular functions in macrophages that play physiologically significant role to counter intracellular pathogens like *M. tb*, *Salmonella* sp. and *Listeria* sp. In listeria infection Rab5 is reported to play a role in recruitment of GTPase Rac2 to phagosome which promotes the assembly of NADPH oxidase on phagolysosomal membrane that produces reactive oxygen species (ROS) (Prada-Delgado A *et al*., 2001; Lebreton *et al*., 2015) (Figure 1). The pore-forming toxin listeriolysin O (LLO) protein is shown to be a major virulence factor responsible for escape of *L. monocytogenes* from phagocytic vacuoles. Studies indicated that acidic pH of phagosome lumen triggered LLO activity and vacuolar perforation (Beauregard *et al*., 1997) (Figure 1). Acidification of phagosomes containing leishmania is inhibited due to integration of LPG (lipophosphoglycan) into lipid microdomains (LM). This process leads to exclusion or loss of V-ATPases (Vinet *et al.*, 2009) on the phagosomal membrane, indicating possible role of Rab GTPases, as established in mycobacterial infection (Fratti *et al.*, 2001) (Figure 1). Studies related to mycobacterial evasion of phagosome-lysosome fusion are focused on inhibition of early to late endosomal transition that relies on the conversion of Rab5 to Rab7 (Via *et al.*, 1997; Simonsen *et al.*, 1998; Kelley and Schorey, 2003; Rink *et al.*, 2005; Seto *et al.*, 2009). EEA1 was found to be a critical regulator of Rab5 to Rab7 conversion (Rink *et al.*, 2005) and *M. tb* inhibits recruitment of EEA1 to phagosomes that leads to impediment of Rab conversion eventually inhibiting phagosome-lysosome fusion (Fratti *et al.*, 2001, 2003) (Figure 1). Rab GTPases are thus one of the critical regulators of phagosome-lysosome fusion and are targeted by many intracellular pathogens for their better survival inside the host.

One of the important signaling determinants that escort with phagocytic process is intracellular calcium (Ca^{2+}) level. However, this is not essentially required for the phagocytosis suggesting Ca^{2+} is probably crucial to activate phagocytosis-triggered processes like phagosome-lysosome fusion and antigen presentation. NOX (NADPH oxidase 4) is considered to be one of the sources of ROS within the lumen of phagosome and can also influence phagosomal maturation. Calcium

signaling critically plays a role in NOX-dependent ROS production. Calcium assists endosome-endosome and phagosome-lysosome fusion in part through calmodulin, which interacts with SNAREs and stimulates calmodulin-dependent kinase II (Burgoyne & Clague, 2003; Pryor *et al*., 2000). Actin, in particular F-actin formation on the surface of late endosomes, lysosomes and phagosomes is required for membrane fusion (Jahraus *et al.*, 2001). Protein kinase C alpha (PKCα) also participates in interaction of phagosome with late endosome which is an essential step for phagosomal maturation and evidences suggest a role of calcium to regulate PKCα (Ng Yan Hing *et al.*, 2004).

Phagosome association with lipid bodies (LBs)

Pathogens trigger several changes in the host cell signaling and trafficking mechanisms and one noticeable pathogen-mediated change is the LB biogenesis in the host cell cytoplasm. It was found that *M. tb* and *M. leprae* trigger differentiation of macrophages into foamy macrophages (FMs) during the progression of disease caused in both mice and human. FMs are characterized by a granuloma specific cell population marked with increased accumulation of LBs and play an important role in tuberculosis pathogenesis, both during the initial phases of macrophage infection as well as in granulomas (Cardona *et al*., 2000; Tanigawa *et al*., 2008; Peyron *et al*., 2008; Russell *et al*., 2009; Mattos *et al*., 2010; Daniel *et al*., 2011; Dkhar *et al*., 2014).

Infection with different pathogens demonstrate a clear association of LBs with phagosomes in parallel to LB formation (Melo *et al*., 2003; Mattos *et al*., 2011a; Rank *et al*., 2011) but little is known about the functional meaning of this interaction. The LB-phagosome interaction has been considered as a pathogen strategy for accessing host lipids during infection with *M. tb* (Peyron *et al*., 2008) *M. leprae* (Mattos *et al*., 2011a) and *Chlamydia trachomatis* (Cocchiaro *et al*., 2008). *M. tb* accumulates lipids obtained from the host cell membrane degradation in the form of LBs from which it procure both carbon and energy for its own metabolism (Pandey & Sassetti, 2008). The mycobacteria-phagosome interaction could be important for the pathogen growth and persistence as LBs act as a channel for the transport of potential nutrients, especially neutral lipids, to the phagosome (D'Avila *et al*., 2006; Peyron *et al*., 2008). Further, Luo *et al*. (2005), revealed that mycobactin-metal complex are accumulated in LBs within *M. tb* infected macrophages and these mycobactin-targeted lipid droplets were found in direct contact with phagosomes suggesting that migration of iron-mycobactin complex from LBs to phagosomes would facilitate iron delivery to phagosomal mycobacteria, acting as an iron source for the pathogen to promote their growth.

As the lipid content of LBs serves as a nutrient source for the pathogen facilitating its survival within the host cell (D'Avila *et al*., 2006; Peyron *et al*., 2008; Cocchiaro *et al*., 2008; Mattos *et al*., 2011a; Mattos *et al*., 2011b), inhibition of LB formation using pharmacological inhibitors within pathogen infected cells causes the reduction of bacterial growth within host cells (Kumar *et al*., 2006; Mattos *et al*., 2011b). Kumar *et al*. (2006), blocked the lipid droplet biogenesis by using triacsin C which specifically inhibits the activity of a subset of long chain acyl-coA synthetases (ACSL), required for triacylglyceride and cholesterol ester biosynthesis. This impediment in lipid droplet biogenesis causes decrease in the size of phagosome and reduction of chlamydial growth within Hep2 cells. Further, use of another inhibitor of lipid metabolism, C75 which inhibits fatty acid synthase (FAS) repressed not only the *M. tb*-induced LB formation but

also the bacterial viability in Schwann cells (Mattos *et al*., 2011b). Taking into account that LBs are the sites for Rab5 and Rab7 GTPases, the association of LBs with phagosomes may comprises a mechanism for Rab transport to and from the phagosome for phagosome maturation (van Manen *et al*., 2005). Also, Igtp (Irgm3), an ER resident 47 kDa GTPase involved in phagosomal maturation and phagocytic cross-presentation is shown to reside on LBs membrane within dendritic cells, where it binds the LB coat component adipose differentiation-related protein (ADRP). This suggests the involvement of LBs in regulation of cross-presentation of phagocytosed antigens in these cells (Bougnères *et al*., 2009). Moreover, association of LBs and phagosomes in dendritic cells may aid a regulatory function of LBs in phagolysosomal progression (Bougnères *et al*., 2009). Therefore, the obscure LB-phagosome interaction cannot be merely considered as a pathogen strategy to perpetuate and support its own survival, but also might be a host approach to impair the survival and multiplication of intracellular pathogens.

Conclusions

In the last few years, attempts were made towards development of innovative therapies due to the limitations of current therapy against many intracellular pathogens. Diseases caused by various intracellular pathogens symbolise a lingering dialogue interplayed between both host and pathogens that leads to extensive signaling manipulation in both organisms. Researchers have targeted the limiting facts of the pathogens which hinder their long time survival and pertaining of the infection by counter-attacking the host molecules. In this process, the major aspect of the discovery has been to target the host process, mainly the phagosome-lysosome fusion which is very crucial for clearance of pathogens. Upon encountering a host, the pathogen is destined to be in phagosome. The signaling cascades controlling fusion of phagosomes with lysosomes are shown to be exploited by various intracellular pathogens and the pathogens inhibit the important processes of phagosome-lysosome fusion at different steps to establish a successful infection. Studies in this review exemplifies that host factors like kinases, surface receptors, LBs and other key molecules in signaling cascades can be targeted to restrict successful infection by pathogen, which are not only important to understand the host-pathogen interaction but also can pave the way for discovery of novel therapeutic targets. Moreover, a detailed understanding of the complicated phagosome-lysosome fusion process seems to afford new insights into the pressure points in the life cycle of pathogen, which, hopefully, will lead to new chemotherapeutic interventions.

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Table 1. Rab GTPases and their functions in phagosome-lysosome fusion

Rab GTPase	Function(s)	References
Rab4	Fast endocytic recycling	van der Sluijs *et al.*,1992; Sheff *et al.*,1999
Rab5	Endocytosis; Homotypic fusion; Maturation of early phagosome; Macro-pinocytosis	Gorvel *et al.*, 1991; Robinson *et al.*, 1996; Simonsen *et al.*, 1998; Callaghan *et al.*, 1999; Fratti *et al.*, 2001; Kyei *et al.*, 2006; Coyne *et al.*, 2007; Ng *et al.*, 2007
Rab7	Late endosome/phagosome fusion with lysosome	Via *et al.*, 1997; Bucci *et al.*, 2000
Rab8	Trafficking from TGN to plasma membrane; GLUT4 vesicle translocation; Ciliogenesis	Miinea *et al.*, 2005; Yoshimura *et al.*, 2007; Sano *et al.*, 2008
Rab9	Trafficking from late endosomes to the TGN	Barbero *et al.*, 2002
Rab 10	Post-Golgi trafficking; Insulin stimulated GLUT4 translocation	Chen *et al.*, 1993; Sano *et al.*, 2008
Rab11	Slow endocytic recycling; Cytokinesis	Riggs *et al.*, 2003; Kouranti *et al.*, 2006
Rab14	Trafficking between TGN and endosomes; GLUT4 vesicle translocation; Phagosome maturation	Junutula *et al.*, 2004; Miinea *et al.*, 2005; Kyei *et al.*, 2006
Rab17	Ciliogenesis	Yoshimura *et al.*, 2007
Rab 21	Integrin endocytosis	Pellinen *et al.*, 2006
Rab22	Trafficking between TGN and early endosomes and *vice versa*	Mesa *et al.*, 2005; Ng *et al.*, 2007
Rab23	Ciliogenesis	Yoshimura *et al.*, 2007
Rab24	Autophagosome maturation	Munafo and Colombo, 2002
Rab32	Biogenesis of melanosomes; Mitochondrial fission	Wasmeier *et al.*, 2006
Rab33	Autophagosome formation	Itoh *et al.*, 2008
Rab34	Macro-pinocytosis	Coyne *et al.*, 2007
Rab35	Endocytic recycling; Cytokinesis	Kouranti *et al.*, 2006
Rab38	Biogenesis of melanosomes	Loftus *et al.*,2002; Wasmeier *et al.*, 2006