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The Protein Tyrosine Phosphatase SHP-1 Regulates Phagolysosome Biogenesis

Carolina P. Gómez,*† Marina Tiemi Shio,†‡§ Pascale Duplay,* Martin Olivier,†‡§ and Albert Descoteaux*†

The process of phagocytosis and phagosome maturation involves the recruitment of effector proteins that participate in phagosome formation and in the acidification and/or fusion with various endocytic vesicles. In the current study, we investigated the role of the Src homology region 2 domain-containing phosphatase 1 (SHP-1) in phagolysosome biogenesis. To this end, we used immortalized bone marrow macrophages derived from SHP-1–deficient motheaten mice and their wild-type littermates. We found that SHP-1 is recruited early and remains present on phagosomes for up to 4 h postphagocytosis. Using confocal immunofluorescence microscopy and Western blot analyses on purified phagosome extracts, we observed an impaired recruitment of lysosomal-associated membrane protein 1 in SHP-1–deficient macrophages. Moreover, Western blot analyses revealed that whereas the 51-kDa procathepsin D is recruited to phagosomes, it is not processed into the 46-kDa cathepsin D in the absence of SHP-1, suggesting a defect in acidification. Using the lysosomotropic agent LysoTracker as an indicator of phagosomal pH, we obtained evidence that in the absence of SHP-1, phagosome acidification was impaired. Taken together, these results are consistent with a role for SHP-1 in the regulation of signaling or membrane fusion events involved in phagolysosome biogenesis. The Journal of Immunology, 2012, 189: 2203–2210.

Phagocytosis is the process by which cells internalize large particulate materials from their milieu. In contrast to endocytosis, phagocytosis involves clathrin-independent mechanisms and requires actin polymerization. Internalization is triggered by the interaction of ligands on the target particles with surface receptors of phagocytic cells. The nascent phagosome is formed to a large extent by invagination of the plasma membrane (1). The particles are surrounded by pseudopods and, following internalization, they end up in a vacuole, the phagosome. The newly formed phagosome rapidly matures into a phagolysosome where the internalized material is degraded. Phagolysosome biogenesis is a dynamic process, requiring the coordinated action of multiple signaling molecules that regulate the interactions between the phagosome and the cytoskeleton, and the endosomal/lysosomal compartments (2–5). These interactions are transient and are characterized by the formation of small fusion pores between the two organelles, allowing the exchange of membrane and contents (6). Through these interactions, phagosomes acidify and sequentially acquire an array of hydrolases culminating in the generation of a highly hydrolytic environment (7). This process takes place rapidly, as within a few hours newly formed phagosomes acquire phagolysosomal functionality (8).

Tyrosine (Tyr) phosphorylation is a critical mechanism for the control of numerous physiological processes in eukaryotes (9), including cytoskeletal function, signal transduction, intracellular traffic, and immune defense (9, 10). Phosphotyrosine signaling is regulated by the dynamic interplay of three distinct functional modules, as follows: protein tyrosine kinases, protein tyrosine phosphatases (PTP), and Src homology 2 domains (11). In metazoans, the multiple possible combinations of these modules illustrate the complexity of phosphotyrosine signaling, which can regulate cellular functions either positively or negatively. A recent large-scale quantitative proteomic and phosphoproteomic analysis revealed that Tyr phosphorylation sites are overrepresented in phagosomes (12), as compared with global phosphoproteome (13). This finding suggested that Tyr phosphorylation plays an important role in phagosome signaling. Given their role in controlling Tyr phosphorylation levels (10, 11), PTPs are expected to participate in the regulation of phagosome function. Interestingly, characterization of the nonreceptor PTP MEG2 in neutrophils revealed that it is present on the cytoplasmic face of secretory vesicles, and is recruited to nascent phagosomes (14). A physiological substrate for PTP-MEG2 is the vesicle fusion protein N-ethylmaleimide–sensitive factor (NSF), the dephosphorylation of which stimulates the homotypic fusion of secretory vesicles (15). Additional PTP-MEG2–interacting proteins are associated with vesicle traffic, small GTPases, and lipid interaction (16). However, a role for PTP-MEG2 or any other PTPs in the regulation of phagosome maturation remains to be demonstrated.

The nonreceptor PTP Src homology region 2 domain-containing phosphatase 1 (SHP-1) is expressed at highest levels in hemopoietic cells. In macrophages, SHP-1 participates in the regulation of various processes, including proinflammatory and innate immune...
responses (17–20). SHP-1 has previously been shown to associate with phagosomes (21, 22), thereby suggesting a role in the biogenesis of phagolysosomes. In this study, we present evidence that SHP-1 plays a role in phagosome maturation and that it is required for acidification of this organelle.

Materials and Methods

Cell culture

All animals were handled in strict accordance with good animal practice as defined by the Canadian Council on Animal Care, and all animal work was approved by the Comité Institutionnel de Protection des Animaux de l’Institut National de la Recherche Scientifique–Institut Armand-Frappier. Bone marrow-derived macrophages (BMM) were obtained by extracting the femur and tibia from 8- to 10-wk-old female BALB/c mice (Charles River Laboratories, QC, Canada) at 37°C in 5% CO2 for 7 d in DMEM with t-glutamine (Life Technologies) supplemented with 10% heat-inactivated FBS (HyClone, Logan, UT), 10 mM HEPES (pH 7.4), and antibiotics (complete medium) in the presence of 15% (v/v) L929 cell-conditioned medium as a source of CSF-1. SHP-1-deficient (C57Bl6C3 me/me, me-3, and me-5) and wild-type (WT) immortalized BMM were previously described (23) and were grown in complete medium at 37°C in 5% CO2.

Phagosome preparation and isolation

Adherent macrophages (2 × 10^7 per 150 × 20-mm tissue culture dishes) were incubated with magnetic beads (3 μm average diameter, 2.5% w/v suspension; Spherotech) diluted in 1:50 in 10 ml complete medium at 37°C for 1, 2, or 4 h. Cells were then washed with cold PBS at 4°C and resuspended in lysis buffer (50 mM Tris [pH 7.5], 0.1 mM EDTA, 0.1 mM EGTA, 0.1% 2-ME, 1% Nonidet P-40, 1 mM Na3VO4, and protease inhibitors). Samples were loaded on a gel containing many) and phosphatase inhibitors (Na 3VO4 and NaF). Phagosomal and total cell lysate proteins were separated by SDS-PAGE and transferred onto Hybond-ECL membranes (Amersham Biosciences, QC, Canada), and immunodetection was achieved by chemiluminescence (Amersham Bio- sciences) encompassing aa 252–482 of murine Dok-1 (a gift of J. Nunes, Institut Paoli-Calmettes, Marseille, France). Anti–Dok-1 Abs were affinity purified from ECM Biosciences. Abs directed against the C-terminal region of murine Dok-1 were produced in rabbits using a GST fusion protein (McGill University, QC, Canada). The rabbit Ab against phospho–Dok-1 (Y362) were developed by J. August (1D4B) was obtained through the Developmental Studies Hybridoma Bank at the University of Iowa and the National Institute of Child Health and Human Development. The rabbit Ab against flotillin-2 of Child Health and Human Development. The rabbit Ab against lysosomal-associated membrane protein 1 (LAMP-1) was from BD Laboratories, QC, Canada) at 37°C in 5% CO2 for 7 d in DMEM with 15% (v/v) L929 cell-conditioned medium and the SHP-1 band was observed by in-gel PTP assay, as previously described (25).

Phagocytosis assays

Zymosan (Sigma-Aldrich) and latex beads (3 μm; Polyscience) were opsonized with mouse serum. For synchronized phagocytosis assays, cells were incubated with particles at a particle-to-cell ratio of 5:1 for 10 min at 4°C. Excess particles were removed, and phagocytosis was triggered by transferring the cells to 37°C for the indicated time points prior to proceeding for microscopy.

Microscopy and immunofluorescence

Macrophages were fixed and permeabilized using 0.1% Triton X-100, and nonspecific surface FcγR binding was blocked using 1% BSA, 2% goat serum, 6% milk, and 50% PBS (26). Particle internalization was quantified by immunofluorescence microscopy. Quantification of phagosomal recruitment was performed as follows. Slides were prepared with three coverslips per time point for each experiment, and 100 phagosomes were counted per coverslip for a total of 300 phagosomes per experiment. Experiments were repeated a minimum of three times. For distribution and colocalization experiments, anti-mouse AlexaFluor 488, anti-rabbit AlexaFluor 488, anti-rat AlexaFluor 488, or anti-rat AlexaFluor 568 (Molecular Probes) was used. DRAQ5 (Biostatus, Leicestershire, U.K.) was used to visualize cells’ nuclei. All coverslips were mounted on slides with Fluoromount-G (Southern Biotechnology Associates). Detailed analysis of protein presence and localization on the phagosome was performed using an oil immersion Nikon Plan Apo 100 (N.A. 1.4) objective mounted on a Nikon Eclipse E800 microscope equipped with a Bio-Rad Radiance 2000 confocal imaging system (Bio-Rad, Zeiss). Images were obtained using appropriate filters with a Kalman filter of at least 3. To quantify the intensity of SHP-1 recruitment to phagosomes, a line spanning representative phagosomes and the surrounding cytoplasm was manually traced with a one-pixel width, and fluorescence intensity profile was represented in a graph for each phagocytosis time point. Intensity of the signal was measured using the MetaMorph software package.

Phagosome acidification

Cells were preloaded with the lysosomotropic agent LysoTracker (LT) Red (Molecular Probes) diluted in DMEM (1:1000) for 2 h at 37°C. Cells were washed and synchronized phagocytosis was performed for indicated time points at 37°C, as described above. Cells were then rinsed, fixed with 2% paraformaldehyde for 10 min, washed, and directly mounted for confocal analysis.

Statistical analyses

Statistical analyses were performed using the statistical tools provided in GraphPad prism software. SDs were calculated from three independent experiments.

Results

SHP-1 is recruited to nascent phagosomes and remains throughout the maturation process

SHP-1 has previously been shown to associate with phagosomes, raising the possibility that it participates in the biogenesis of phagolysosomes (21, 22). To investigate a potential role for SHP-1 in this process, we first performed confocal immunofluorescence experiments to visualize SHP-1 redistribution in murine BMM upon phagocytosis of serum-opsonized zymosan particles (27–29). As shown in Fig. 1A, SHP-1 was detectable on nascent phagosomes (15 min) and remained associated to the phagosome throughout the maturation process (up to 4 h after the initiation of phagocytosis). Quantification of the kinetics of SHP-1 association (Fig. 1B) revealed that, by 30 min, 50% of phagosomes were positive for SHP-1. From 1 h postphagocytosis, SHP-1 was present on $>80\%$ of phagosomes (Fig. 1B). These results confirmed the early recruitment and presence of SHP-1 throughout the maturation process. To further analyze the phagosomal association/recruitment of SHP-1, we purified phagosome extracts using magnetic beads at different time points after the initiation of phagocytosis (26, 30). To this end, we used immortalized bone marrow macrophages derived from SHP-1–deficient motheaten mice (me-3) and their control WT littermates (25). Purified phagosome extracts of various ages (15 min, 1 h, and 2 h)
were isolated from WT macrophage cells and were analyzed by Western blot to detect the presence of SHP-1. In agreement with our confocal immunofluorescence data, we detected a progressive increase in SHP-1 levels in phagosome extracts from 15 min to 2 h after the initiation of phagocytosis (Fig. 2A). As expected, no SHP-1 was detected in SHP-1–deficient me-3 macrophage extracts (Fig. 2A), confirming the specificity of the Ab against SHP-1.

Next, we performed an in-gel PTP activity assay (31) to monitor the presence of PTPs in magnetic bead–phagosome extracts isolated from WT and SHP-1–deficient me-3 macrophages at 1, 2, and 4 h after the initiation of phagocytosis (Fig. 2B). Active SHP-1 was present in phagosome extracts from WT cells at all time points tested and, as expected, was not detected in phagosome extracts from SHP-1–deficient me-3 macrophages (upper band).
Interestingly, our in-gel PTP activity assay revealed the presence of other PTPs in phagosome extracts that, based on their apparent molecular masses, corresponded to TC-PTP (Fig. 2B, lower band) and PTP-1B (Fig. 2B, middle band). The presence of both TC-PTP and PTP-1B was confirmed by Western blot analysis (Fig. 2C). Of note, we consistently observed a reduction in the activity of TC-PTP and PTP-1B in phagosome extracts from SHP-1–deficient macrophages, despite that the levels of both phosphatases were comparable in phagosome extracts from WT and SHP-1–deficient cells. Comparison of the patterns of tyrosine-phosphorylated proteins present in phagosome extracts from WT and SHP-1–deficient me-3 macrophages revealed an increased tyrosine phosphorylation in the absence of SHP-1 (Fig. 2D). To determine whether known SHP-1 substrates were differentially phosphorylated and recruited to phagosomes in the absence of SHP-1, we focused on Dok-1 (p62Dok), a major SHP-1 substrate (32) present in the phagosome proteome (22). Western blot analyses revealed that Dok-1 levels in phagosome lysates increased progressively over time, independently of the presence of SHP-1 (Fig. 2E). Consistent with previous observations (32), we found a significant increase in the phosphorylation of Dok-1 (Tyr $^{66}$) in total lysates from SHP-1–deficient macrophages with respect to lysates from WT macrophages (Fig. 2E). Surprisingly, phosphorylation of Dok-1 (Tyr $^{66}$) was below detectable levels in phagosome lysates purified from both WT and SHP-1–deficient macrophages (Fig. 2E). Together, these results indicate that SHP-1 is recruited early and remains associated to phagosomes, and suggest that it may participate in phagolysosome biogenesis by modulating phosphotyrosine signaling in phagosomes.

**Impaired recruitment of lysosomal markers in the absence of SHP-1**

As they mature, phagosomes interact with various endosomal populations to acquire lysosomal features (2). To evaluate the potential impact of SHP-1 on the maturation process, we fed WT and SHP-1–deficient me-3 macrophages with serum-opsonized zymosan, and at the indicated time points we assessed the phagosomal association of endosomal markers by confocal immunofluorescence microscopy. In the case of the early endosomal marker EEA1, phagosomal recruitment occurred with similar kinetics in WT and SHP-1–deficient me-3 macrophages (Fig. 3A). In the case of the late endosomal marker Rab7 (33, 34), we observed no major difference in its recruitment at early time points postphagocytosis (Fig. 3B). However, at 1 h postphagocytosis, whereas ~60% of phagosomes in WT cells were positive for Rab7, ~40% of phagosomes were positive in SHP-1–deficient macrophages. For LAMP-1, ~90% of phagosomes were positive at 2 h after the initiation of phagocytosis by WT cells, which is very similar to the kinetics of LAMP-1 phagosomal recruitment observed in BMM (Fig. 4A, 4B). In contrast, the phagosomal recruitment of LAMP-1 was reduced and delayed in SHP-1–deficient macrophages, with <60% of phagosomes being positive at the same time point. At 4 h after the start of phagocytosis, we observed that ~80% of phagosomes were positive for LAMP-1 in SHP-1–deficient macrophages (Fig. 4A, 4B). We next assessed by Western blot the presence of LAMP-1 in purified magnetic bead–phagosome extracts isolated at various time points from WT and SHP-1–deficient macrophages (Fig. 4C, upper panel). Consistent with the results obtained by confocal immunofluorescence microscopy, we found reduced levels of LAMP-1 in phagosome extracts from SHP-1–deficient at all time points tested. Flotillin is recruited to phagosomes from compartments distinct from those bearing LAMP-1 (35). As shown in Fig. 4C (middle panel), flotillin-2 levels were slightly reduced in phagosome extracts isolated from SHP-1–deficient macrophages with respect to WT macrophages.

The aspartic protease cathepsin D is delivered to phagosomes as a 51-kDa procathepsin D and is processed into an active 46-kDa form (36, 37). As shown in Fig. 4C, lower panel, the 51-kDa procathepsin D was present in purified phagosome lysates isolated at 1, 2, and 4 h from both WT and SHP-1–deficient macrophages. In contrast, the 46-kDa mature form of cathepsin D, which gradually appeared in purified phagosome lysates from WT macrophages, was absent from SHP-1–deficient phagosome lysates. We ensured that the effect of SHP-1 deficiency on phagosome maturation was not the consequence of a defect in particle internalization. As shown in Fig. 5, WT and SHP-1–deficient macrophtages internalized zymosan and latex beads to the same extent. Collectively, these results indicate that phagosomal acquisition and processing of late endosomal/lysosomal markers are impaired in cells lacking SHP-1, suggesting that this PTP participates in the regulation of signaling or membrane fusion events involved in phagolysosome biogenesis. Impaired processing of procathepsin D also raised the possibility that phagosomal acidification may be defective in the absence of SHP-1 (37).
SHP-1 regulates phagosomal acidification

Phagosomal acidification is essential for the acquisition of microbicidal properties, as most lysosomal hydrolases are optimally active at acidic pH (38, 39). To assess the impact of SHP-1 deficiency on phagosomal acidification, we used the lysosomotropic agent LT Red as an indicator of phagosome acidity. WT macrophages and two SHP-1–deficient clones (me-3 and me-5) were preloaded with LT prior to being fed with either latex beads or zymosan for various time points (Fig. 6A). Quantification of the number of phagosomes positive for LT showed that, in WT macrophages, ∼80% of phagosomes containing latex beads were acidified after 1 h. In the absence of SHP-1, there was a significant reduction in the percentage of phagosomes positive for LT (Fig. 6A–C). Hence, in SHP-1–deficient me-3 macrophages, 35% of phagosomes containing latex beads were positive for LT even 4 h after the initiation of phagocytosis. In the case of SHP-1–deficient me-5 macrophages, 20% of phagosomes were positive for LT. We obtained similar results when phagocytosis was performed with zymosan (Fig. 6C). The absence of SHP-1 did not affect the overall staining and distribution of LT (Fig. 6A), indicating that the defective accumulation of LT to phagosomes is not the consequence of a generalized lysosomal acidification defect in SHP-1–deficient macrophages. To further investigate the potential role of SHP-1 in phagosomal acidification, we performed confocal immunofluorescence experiments to visualize the distribution of the c subunit of the v-ATPase in WT and SHP-1–deficient me-3 macrophages following the phagocytosis of latex beads. As shown in Fig. 6D, the c subunit of the v-ATPase was present on a majority of phagosomes in WT macrophages. In contrast, barely 20% of phagosomes were positive for the c subunit of the v-ATPase in me-3 macrophages. These data suggest that SHP-1 is involved in the phagosome acidification process.

Discussion

The present study was aimed at investigating the role of the PTP SHP-1 in the regulation of phagolysosome biogenesis. We report that SHP-1 is recruited early to phagosomes and that it is required for the acquisition of lysosomal features and acidification. Previous studies established that particle internalization is accompanied by the tyrosine phosphorylation of numerous proteins, through the action of Syk and Src family kinases (40–44). Investigation by Strzelecka-Kiliszek et al. (21) showed that binding of IgG-coated particles to the surface of macrophages induces rapid and transient tyrosine phosphorylation of several proteins. In contrast, when particle internalization starts, most proteins are dephosphorylated. The observation that tyrosine dephosphorylation coincides with the phagosomal association of SHP-1 is consistent with this PTP
being an important modulator of tyrosine phosphorylation-dependent signaling during FcγR-mediated phagocytosis (20, 45).

Our observation that active SHP-1 is present on phagosomes at all time points analyzed suggested that the role of this PTP extends beyond the modulation of tyrosine phosphorylation during the internalization process. Indeed, characterization of the phagosome phosphoproteome and molecular analysis of the evolution of phagosomes highlighted the importance of phosphorylation in the regulation of phagolysosome biogenesis (4, 12, 22). This process is accomplished through transient, highly regulated interactions between the phagosome and the cytoskeleton, as well as the early, late, and recycling endocytic compartments (46–48). Through these interactions, phagosomes acidify and sequentially acquire an array of hydrolases, culminating in the generation of a hydrolytic, microbicidal environment (37, 49, 50). Our results unveiled an important role for SHP-1 in this process, as acquisition of lysosomal features was clearly impaired in the absence of this PTP.

Hence, whereas the absence of SHP-1 had no noticeable effect on the phagosomal association of the early endosomal marker EEA1, it impaired the recruitment of the late endosomal marker Rab7. We also found that phagosomal association of both flotillin and LAMP-1 was reduced in the absence of SHP-1. Most noticeable was the impairment of phagosome acidification in SHP-1–deficient macrophages. Collectively, those results indicated that SHP-1 is involved in the regulation of phagolysosome biogenesis.

Phagosomal acidification is mediated by the vacuolar proton-ATPase (v-ATPase), which is present on various endocytic organelles (51). The v-ATPase is a multimeric complex consisting of the multisubunit cytoplasmic V1 sector that is responsible for ATP hydrolysis, and of the multisubunit transmembrane V0 sector that pumps protons across the bilayer (52). Little is known concerning the mechanism(s) regulating the phagosome recruitment of the v-ATPase as well as the regulation of its activity. Previous studies revealed that phagosomal acidification starts early during phagosome formation, before acquisition of lysosomal features such as LAMP-1 and cathepsins (8, 53, 54), which are recruited to phagosomes through distinct kinetics and mechanisms (7, 35, 55).

Recently, it was shown that early recruitment of the α3 subunit of the v-ATPase to young phagosomes occurs via tubular structures extending from the perinuclear lysosomes along microtubules (56). This finding is consistent with previous studies showing that phagosomes acquire lysosomal features through a microtubule-dependent mechanism (47, 50). The identification of Syt V as an important regulator of the recruitment of the v-ATPase to the phagosome membrane (26) provided additional insights into the mechanism regulating phagosome acidification. How SHP-1 contributes to the phagosomal acidification process remains to be established. The observation that there was no generalized defect in lysosomal acidification in the absence of SHP-1 suggests that the defect in phagosome acidification in SHP-1–deficient cells
is not due to an impaired activity of the v-ATPase. Rather, one may envision that SHP-1 is involved in the phagosomal recruitment of the v-ATPase, by regulating membrane fusion events, a function previously reported for other protein phosphatases. This is the case for the yeast protein phosphate 1, which was shown to control the terminal step of membrane fusion, after the SNARE docking stage and the early priming reaction involving NSF/S-νitrosoacetylpenicillamine (57). This finding led the authors of that study to propose that protein phosphatase 1 may be part of a system other than SNARE complexes and NSF/S-νitrosoacetylpenicillamine, which catalyzes bilayer mixing. Similarly, PTP-MEG2 was shown to dephosphorylate NSF, thereby stimulating the homotypic fusion of secretory vesicles (15). PTPs can also negatively regulate phagosome acidification and recruitment of the v-ATPase by acting on the membrane fusion machinery.

Hence, the mycobacterial PTP PtpA was recently shown to dephosphorylate the macrophage protein VPS33B, a key regulator of membrane fusion, leading to the inhibition of phagosome maturation and acidification (58).

Exclusion of the v-ATPase from phagosomes may have an important consequence on the maturation process, because earlier studies revealed that phagosomal acidification is an important element in controlling phagosome maturation (8, 37, 59). Indeed, further studies revealed that the α1 subunit of the V0 sector is a crucial factor in vacuole fusion, acting downstream of trans-SNARE pairing (60, 61). Consistently, the V0 sector was involved in vesicle fusion during exocytosis in neurons (62, 63). More recently, studies with zebrafish microglia established that the α1 subunit mediates fusion independently of its proton pump activity (64). Further studies will be required to determine whether the lack of phagosome acidification observed in SHP-1–deficient macrophages is the consequence of a defective phagosome maturation process, or one of the contributing factors. Similarly, impaired phagosomal recruitment of LAMP-1 may contribute to the inhibition of phagosomal biogenesis in SHP-1–deficient macrophages. Indeed, it has been demonstrated that LAMPS are essential for successful completion of phagosome maturation, specifically for the transition from early to late phagosomes (39). Acidification is also an important characteristic of the phagosome and directly impacts its enzymatic activities. In this regard, Ullrich et al. (37) showed that inhibition of the v-ATPase with bafilomycin A impairs the conversion of the 51-kDa procathepsin D into the 46-kDa mature form. Given the role of SHP-1 in phagosomal acidification, our observation that processing of procathepsin D into the 46-kDa form requires SHP-1 is consistent with the work of Ullrich et al. (37).

To date, several SHP-1–binding proteins and substrates have been identified (65). Many are expressed in macrophages, including PIR-B, SIRPα, Dok-1 (p62Dok), SLP-76, Syk, Vav1, and BLK. Some of those proteins are intermediates in signaling pathways initiated from Fcγ receptors and participate in the regulation of particulate internalization (45). Several of those SHP-1 substrates were identified in recent proteomic analyses of latex bead phagosomes (22), but their potential contribution to phagosome biogenesis/function and the role of SHP-1 in their phagosomal recruitment remain poorly understood. Indeed, we observed that recruitment of Dok-1 to phagosomes is independent of SHP-1. Furthermore, tyrosine phosphorylation of phagosome-associated Dok-1 was below detectable levels in both WT and SHP-1–deficient macrophages. This observation raises the possibility that SHP-1 acts indirectly on the phagosome maturation process. Also, the possibility that SHP-1 acts as an adaptor deserves to be investigated. Clearly, a better understanding of the role(s) of those known phagosomal SHP-1–binding proteins and substrates will be crucial to understand how SHP-1 modulates phagolysosomal biogenesis. Importantly, our results were obtained with immortalized BMM clones, which are very useful to maximize reproducibility. Although those clones bear the characteristics of the macrophage population from which they originate (23), macrophage populations in vivo are heterogeneous. Future studies using primary macrophages from motheaten mice may provide additional information on the impact of SHP-1 deficiency on phagosome maturation in various macrophage populations. In addition to SHP-1, we found that two other PTPs, namely TC-PTP and PTP-1B, were recruited to phagosomes. PTP-1B and TC-PTP are both localized in the endoplasmic reticulum (66, 67), an intracellular compartment known to contribute to the phagosome proteome (68). Interestingly, we observed a reduction in the activity of these two PTPs in phagosome extracts from SHP-1–deficient cells. The relationship between SHP-1 and the activity of these PTPs remains to be established. Previous studies indicated that TC-PTP and PTP-1B play a cooperative but nonredundant role in signaling pathways associated with macrophage development and activation (69, 70). The possibility that these PTPs regulate both phagolysosome biogenesis and/or phagosome function is a scenario that remains to be experimentally addressed.

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Disclosures

The authors have no financial conflicts of interest.

References


