SREBP-1a–stimulated lipid synthesis is required for macrophage phagocytosis downstream of TLR4-directed mTORC1

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There is a growing appreciation for a fundamental connection between lipid metabolism and the immune response. Macrophage phagocytosis is a signature innate immune response to pathogen exposure, and cytoplasmic membrane expansion is required to engulf the phagocytic target. The sterol regulatory element binding proteins (SREBPs) are key transcriptional regulatory proteins that sense the intracellular lipid environment and modulate expression of key genes of fatty acid and cholesterol metabolism to maintain lipid homeostasis. In this study, we show that TLR4-dependent stimulation of macrophage phagocytosis requires mTORC1-directed SREBP-1a–dependent lipid synthesis. We also show that the phagocytic defect in macrophages from SREBP-1a–deficient mice results from decreased interaction between membrane lipid rafts and the actin cytoskeleton, presumably due to reduced accumulation of newly synthesized fatty acyl chains within major membrane phospholipids. We show that mTORC1-deficient macrophages also have a phagocytic block downstream from TLR4 signaling, and, interestingly, the reduced level of phagocytosis in both SREBP-1a– and mTORC1-deficient macrophages can be restored by ectopic SREBP-1a expression. Taken together, these observations indicate SREBP-1a is a major downstream effector of TLR4–mTORC1 directed interactions between membrane lipid rafts and the actin cytoskeleton that are required for pathogen-stimulated phagocytosis in macrophages.

macrophages | lipid synthesis | mTORC1 | TLR4 | SREBPs

Macrophages play an essential role in the mammalian innate immune response (1). They are part of a first line of defense to challenge from external biological threats and have evolved specialized mechanisms to detect and destroy pathogenic microbes. In response to an invading pathogen, macrophages need to increase membrane lipid levels significantly to repair cell surface damage caused by membrane-disrupting microbial toxins and to envelop phagocytic cargo before engulfment and uptake through phagocytosis (2). Evidence suggests that membrane expansion emanates from the endoplasmic reticulum (3), and previous studies have demonstrated that the lipid regulated sterol regulatory element binding proteins (SREBPs) are up-regulated to respond to microbial pore-forming toxins and for phagocytosis, at least in fibroblasts. The increase in SREBPs is presumably to increase the cellular lipid synthetic capacity (4, 5), but the underlying mechanism has remained elusive.

SREBPs are key transcriptional regulators of genes that couple lipid metabolism to cell dynamics, and proper regulation of their activity is important for cellular homeostasis (6). Mammals express three different SREBP isoforms: SREBP-1a and SREB-1c are encoded by the *Srebf-1* gene and differ through alternative

transcription initiation and splicing of SREBP-1 mRNAs, whereas SREBP-2 is encoded by an unlinked distinct gene (7). The expression patterns for the three SREBPs vary dramatically across different tissues, and, in liver, the SREBP-1c isoform is 10-fold more abundant than SREBP-1a. However, SREBP-1a is expressed more abundantly in macrophages, and we showed it activates expression of the proinflammatory Nlrp1a gene (8). In a follow-up study, we demonstrated that macrophages from a mutant mouse line with a selective deficiency in expression of SREBP-1a (SREBP-1aDF) develop normally, but they are more susceptible to apoptosis after exposure to bacterial pore-forming toxins (9).

These studies, along with recent publications from others (10), demonstrate that SREBP-1a plays a crucial role in the inflammatory response in macrophages. Phagocytosis is also a key innate immune function for macrophages that requires ongoing lipid synthesis (3), and, as mentioned above, earlier work showed

Significance

There is a growing appreciation for a fundamental connection between lipid metabolism and the innate immune response. Phagocytosis is a key macrophage innate immune response to pathogen exposure, and cytoplasmic membrane expansion is required to surround and capture the target pathogen prior to internalization. Sterol regulatory element binding proteins (SREBPs) are gene regulatory factors that sense the intracellular lipid environment and modulate key genes that drive fatty acid and cholesterol synthesis to maintain lipid homeostasis. In this study, we show that, in mutant cells that lack a key SREBP isoform, phagocytosis is impaired, and we track the defect to altered lipid composition of membrane phospholipids that results in decreased interaction between membrane lipid rafts and the actin cytoskeletal network.

that SREBP protein maturation is stimulated during phagocytosis of latex beads in fibroblasts (4). Another study showed that siRNA-mediated knockdown of SREBP-1 during macrophage differentiation reduced phagocytosis in mature macrophages (11). These two studies provide evidence that SREBP-1 plays a pivotal role in phagocytosis, but the mechanism for its activation and how it was connected to pathogen engagement was not investigated. To address these outstanding issues, we examined the role of SREBP-1a in macrophage phagocytosis with a mouse model containing a gene trap insertion in the first intron of the *Srebf1* gene that results in a deficiency in SREBP-1a, with minimal effects on expression of SREBP-1c from its downstream promoter (12). In the current studies, we show that phagocytosis is reduced in these SREBP-1aDF/B6 mice in vivo and in cultured bone marrow-derived macrophages (BMDM).

Conversion of the endoplasmic reticulum membrane-bound SREBP1 precursor protein to its nuclear-targeted form is regulated by cellular lipid status and requires transport to the Golgi apparatus where two proteases sequentially cleave and release the mature nuclear-targeted SREBP-1. The proteolytic activation is regulated not only by cellular lipid status but also by TORC1 signaling (13–15), and LPS treatment is known to stimulate macrophage phagocytosis through a TLR4 signaling cascade that requires activation of mTORC1 (16). Therefore, we investigated whether SREBP-1 might connect TLR4-mediated TORC1 signaling to phagocytosis. Our studies show that LPS/mTORC1 activation of phagocytosis is reduced in SREBP-1aDF/B6 macrophages and that expression of a constitutively nuclear-targeted form of SREBP-1a overcomes both genetic and pharmacologic blockade of mTORC1-stimulated phagocytosis. We also show that SREBP-1a–deficient macrophages have reduced incorporation of de novo synthesized fatty acyl chains into major membrane phospholipids, and this is associated with reduced association of the actin cytoskeletal proteins moesin and cofilin with plasma membrane lipid rafts. Because early events in phagocytosis require lipid raft–actin cytoskeletal interactions (17, 18), our results suggest that a loss of SREBP-1a leads to decreased phagocytosis, because SREBP-1a–dependent lipid species are required to facilitate optimal association between membrane lipid rafts and the actin cytoskeleton.

Results

Decreased Phagocytosis in SREBP-1aDF/B6 Macrophages. The phagocytic clearance of pathogens is a major role for macrophages as part of the innate immune response (2), and it requires new membrane synthesis that is likely derived from expansion of the endoplasmic reticulum (3). Based on its role in de novo lipogenesis, we hypothesized that SREBP-1a might be required for phagocytosis to provide the fatty acyl chains for phospholipid biosynthesis required to drive membrane expansion. To test this hypothesis, we measured phagocytic activity in BMDM from WT and SREBP-1aDF/B6 mice using IgG-opsonized sheep red blood cells as the target. The results showed that BMDMs from SREBP-1aDF/B6 displayed significantly reduced phagocytic activity as measured by both the percentage of macrophages ingesting targets (% phagocytosis) and the number of ingested opsonized targets per phagocytic cell (phagocytic index) compared with WT controls (*SI Appendix*, Fig. S1), suggesting that SREBP-1a is required for efficient levels of basal phagocytosis. Phagocytosis of a fluorescently labeled invasion defective strain of *Salmonella typhimurium* was also reduced in SREBP-1aDF/B6 macrophages (*SI Appendix*, Fig. S2).

To determine whether SREBP-1a is also essential for phagocytosis in vivo, we evaluated phagocytosis by following the fate of iron-containing phytate particles (IC-IP$_6$) after injection in live mice. Previous studies have shown IC-IP$_6$ are phagocytic targets of macrophages in vivo (19) and their biodistribution provides a measure of phagocytosis in whole animals. There were higher levels of plasma IC-IP$_6$ in SREBP-1aDF/B6 mice relative to WT mice, whereas uptake into liver and spleen was reduced (Fig. 1

A–C, E, and *F*). There was also lower IC-IP$_6$ uptake by peritoneal macrophages isolated from SREBP-1aDF/B6 mice (Fig. 1*D*). Phagocytosis of apoptotic jurkat T cells was also reduced (*SI Appendix*, Fig. S3). Taken together, these results demonstrate SREBP-1a is required for efficient macrophage phagocytosis.

LPS activation of phagocytosis is defective in TLR4 knockout BMDM (*SI Appendix*, Fig. S4), and TLR4 stimulates both mTORC1 signaling (16) and SREBP protein maturation (13–15). Thus, we hypothesized that SREBP-1a might be responsible for the stimulation of phagocytosis downstream of LPS-dependent TLR4 signaling and mTORC1. To test this hypothesis, we compared LPS stimulation of phagocytosis in BMDM from WT and SREBP-1aDF/B6 mice treated with vehicle control or rapamycin to inhibit mTORC1 signaling. The results in Fig. 2 *A* and *B* demonstrate that rapamycin blunts the LPS-dependent stimulation of phagocytosis in WT BMDM. LPS did not further induce the low level of phagocytosis observed in the SREBP-1aDF/B6 macrophages (Fig. 2*C*). Rapamycin had the expected decrease in mTORC1 signaling as measured by phosphorylation of ribosomal S6 and S6 kinase. Interestingly, LPS also increased the accumulation of SREBP-1 in the nucleus, which was reduced by rapamycin treatment and is consistent with prior studies that show mTORC1 signaling in SREBP-1 maturation in other cell response settings (13–15).
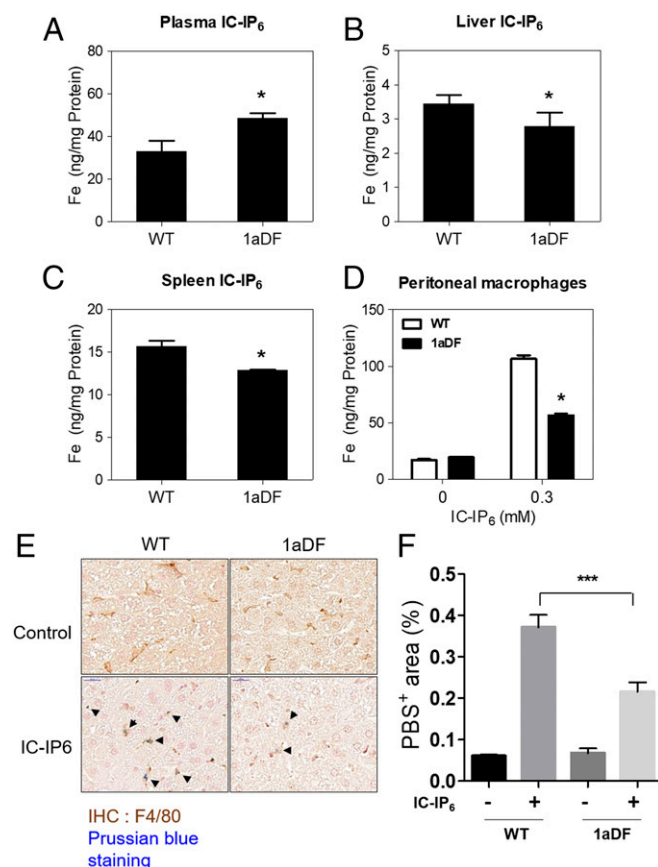
Fig. 1. Reduced phagocytosis in SREBP-1aDF/B6 mice in vivo. (*A*) Plasma IC-IP$_6$ levels in WT and SREBP-1aDF/B6 mice. Quantification of Fe^{3+} level in the plasma of mice i.v. injected with 10 μM IC-IP$_6$ for 4 h. Fe concentrations in the livers (*B*) and spleens (*C*) of WT and SREBP-1aDF/B6 mice. (*D*) Uptake level of Fe^{3+} in peritoneal macrophages of WT and SREBP-1aDF/B6 mice. Data are represented as mean ± SEM; *P < 0.05. (*E*) Costaining of Prussian blue and F4/80 for assessment of IC-IP$_6$ uptake by liver macrophages isolated from WT and SREBP-1aDF/B6 mice. (*F*) Quantification of data from *E*. ***P < 0.001.

Fig. 2. LPS-stimulated phagocytosis requires mTOR signaling and SREBP-1a. (A) Percentage of phagocytosis after LPS and rapamycin treatment in WT and SREBP-1aDF/B6 BMDMs. LPS (100 ng/mL) and rapamycin (25 μM) were treated respectively or together in BMDMs from WT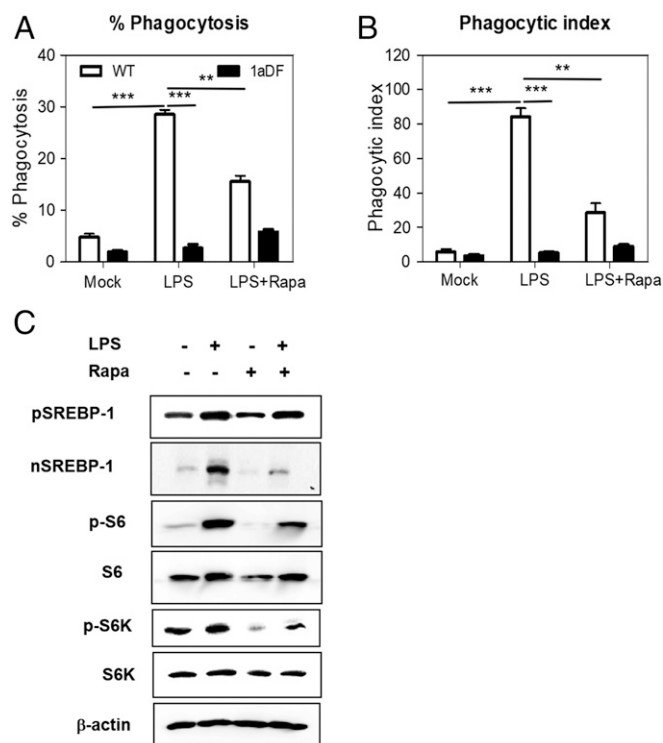 and SREBP-1aDF/B6 mice for 16 h; BMDMs were incubated with sheep red blood cells for 30 min at 37 °C, and targets taken into 200 cells were counted. The percent of cells that displayed active phagocytosis was calculated. (B) Phagocytic index levels in WT and SREBP-1aDF/B6 BMDMs. The phagocytic index indicates the average number of targets per 200 cells. (C) Protein induction level for SREBP-1 and mTOR signaling by LPS and Rapamycin challenge in BMDMs. Values are expressed as mean ± SEM; **$P < 0.01$, ***$P < 0.001$.

Reintroduction of SREBP-1a Reverses the Phagocytic Defect in SREBP-1aDF/B6 Macrophages and in Response to Rapamycin and Loss of Raptor. When we ectopically expressed a soluble version of SREBP-1a that lacks its membrane-anchoring domain and is constitutively targeted to the nucleus, phagocytosis efficiency was restored to WT levels in SREBP-1aDF/B6 BMDM (Fig. 3 A and B). Thus, the impaired phagocytosis in the absence of SREBP-1a is not an indirect effect due to its chronic loss or to a general defect in TLR4 signaling. Importantly, nuclear-targeted SREBP-1a also reversed the inhibitory effect of rapamycin on LPS-stimulated phagocytosis in WT BMDM (Fig. 3 C and D), providing further evidence that nuclear SREBP-1 is essential for mTORC1-stimulated macrophage phagocytosis.

Next, to further address the importance of mTORC1-mediated SREBP-1 maturation, we analyzed phagocytosis in BMDMs from mice with a macrophage-specific knockout of raptor (raptor mKO), which is a key unique component of mTORC1 (20). We found that LPS-stimulated phagocytosis was reduced in raptor mKO BMDMs (Fig. 4 A and B), providing genetic support for the pharmacologic effect of rapamycin. Once again, the impaired phagocytosis in macrophages lacking raptor was also reversed by ectopic expression of nuclear-targeted SREBP-1a (Fig. 4 C and D). Taken together, these results are consistent with a model placing SREBP-1a as a downstream effector of LPS/TORC1-stimulated phagocytosis.

Ongoing Lipid Synthesis Is Required for Phagocytosis. To address the molecular mechanism of impaired phagocytosis in SREBP-1aDF/

B6 BMDMs, we first evaluated our previous differential gene expression profiles for BMDMs from WT and SREBP-1aDF (8). However, this analysis did not show significant changes in expression of genes associated with phagocytosis between the two genotypes. In this same report, we showed that de novo synthesis of fatty acids was reduced in the SREBP-1aDF BMDM. Thus, we hypothesize that SREBP-1a–dependent de novo fatty acid biosynthesis might be directly required for phagocytosis.

To directly analyze whether a defect in lipid synthesis may alter the lipid species of cellular membrane phospholipids, we performed shotgun lipidomics on BMDMs from WT and SREBP-1aDF/B6 mice. The lipidomics showed that loss of SREBP-1a specifically resulted in a reduction of major membrane phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) species that contain fatty acyl chains preferentially derived from de novo fatty acid biosynthesis (Fig. 5 A and B). Similarly, BMDMs from mice lacking the SREBP escort protein SCAP, which attenuates nuclear accumulation of all SREBP species, also dramatically and selectively reduced this same class of membrane PC and PE species (Fig. 5 C and D). There were no other significant changes in any other PC or PE species in these samples. To determine whether the phagocytosis defect might be directly linked to fatty acid deficiency, we analyzed phagocytosis in BMDM supplemented with exogenous oleic acid. The results in Fig. 5 E and F demonstrate that oleic acid supplementation rescued the phagocytosis defect in the SREBP-1aDF/B6 macrophages.

In addition to reduced de novo synthesis of fatty acids, Wei et al. (21) showed that deletion of the fatty acid synthase gene (*Fasn*) in macrophages resulted in impaired inflammatory responses, which the authors attributed to a severe defect in lipid raft protein composition and function. Because lipid rafts are involved in early steps in phagosome assembly (22), we partially profiled lipid raft protein content in WT vs. SREBP-1aDF/B6 BMDM using differential centrifugation of membrane preparations
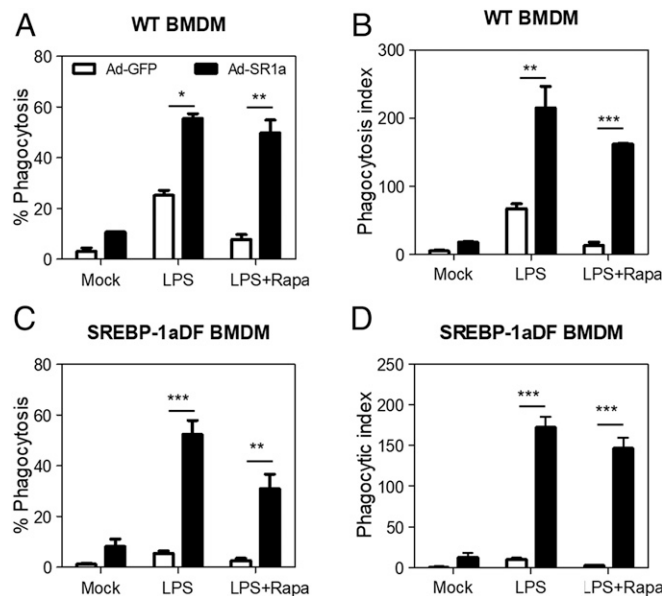
Fig. 3. SREBP-1a reverses phagocytosis defect in SREBP-1aDF/B6 BMDMs. Phagocytosis was repaired by SREBP-1a overexpression in SREBP-1aDF/B6 macrophages. A control (GFP) or SREBP-1a adenovirus was infected into the BMDM of SREBP-1aDF/B6 mice. After incubating adenoviruses for 48 h, BMDMs were treated with 100 ng/mL of LPS and 20 μM rapamycin for 16 h. Percentage of phagocytosis (A) and phagocytic index levels (B) were measured in WT BMDMs. Percentage of phagocytosis (C) and phagocytic index level (D) were measured in BMDMs from SREBP-1aDF/B6 mice. Data are represented as mean ± SEM; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, vs. Ad-GFP.

Fig. 4. SREBP-1a restores phagocytosis defect in raptor mKO BMDMs. (*A*) Percentage of phagocytosis is increased by LPS in WT BMDMs, and phagocytosis is reduced in raptor mKO BMDMs. (*B*) Phagocytic index level by LPS in raptor^fl/fl and raptor mKO BMDMs. Adenovirus of GFP or SREBP-1a (10 MOI) was infected in SREBP-1aDF/B6 BMDMs for 48 h, and BMDMs were treated with 100 ng/mL of LPS for 16 h. (*C*) Percentage of phagocytosis and (*D*) phagocytic index were measured. Data are represented as mean ± SEM; *P < 0.05; **P < 0.01, ***P < 0.001.
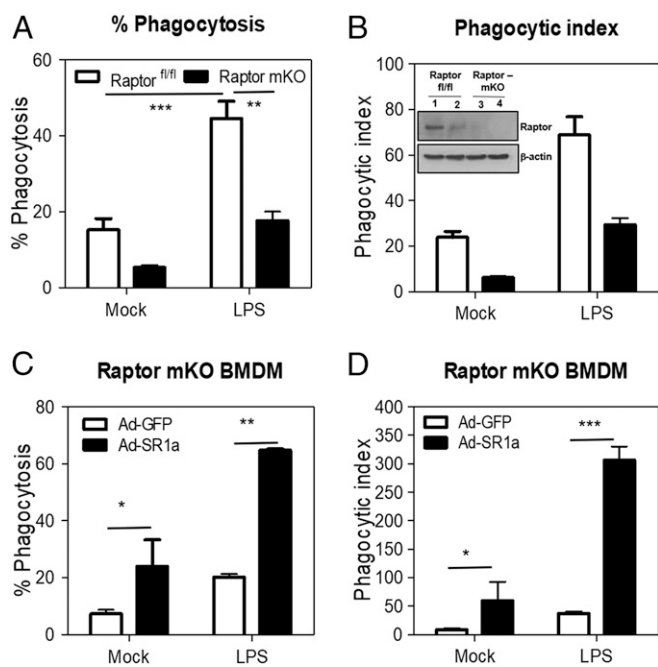
followed by immunoblotting (Fig. 6 *A* and *B*). The results show that, unlike in *Fasn*-deficient macrophages, lipid raft proteins were present at equivalent levels in the corresponding fractions from WT and SREBP-1aDF/B6 samples. However, the presence of moesin and cofilin, two proteins that are known to connect the actin cytoskeletal network to membrane lipid rafts (17, 18), was selectively depleted from the SREBP-1aDF/B6 lipid raft fractions. These results suggest that the reduced level of de novo lipid synthesis in SREBP-1aDF/B6 does not significantly alter lipid raft integrity but does impair interactions between the membrane domains and the actin cytoskeletal network. When oleic acid was supplemented to the culture medium of the SREBP-1aDF/B6 BMDM, the presence of moesin and cofilin in lipid raft fractions was restored (Fig. 6 *C* and *D*). We also analyzed moesin and cofilin association with lipid rafts by immunofluorescence colocalization with antibodies detecting ganglioside GM1. In this experiment (*SI Appendix*, Fig. S5), LPS stimulated colocalization in WT cells, but this was significantly reduced in SREBP-1aDF. Consistent with the results from the ultracentrifugation method, oleic acid supplementation increased lipid raft association for both moesin and cofilin in SREBP-1aDF cells.

Discussion

Membrane lipid composition has a profound effect on many innate immune responses in macrophages (23). In fact, in the 1970s, changes in membrane fatty acid composition were first shown to influence phagocytosis (24). In this early report and several more-recent follow-up studies, supplementation of cultured macrophages with unsaturated fatty acids increased, while saturated fatty acids decreased phagocytosis activity (25). Based on these observations, altered membrane fluidity was proposed as an explanation, but compelling evidence in support of this

hypothesis and, more importantly, an underlying mechanism has not been addressed. In fact, we now know that exogenous fatty acid supplementation has a major effect on cellular metabolism, and recent studies show that elimination of de novo fatty acid biosynthesis by knocking out the *Fasn* gene in macrophages has a major impact on inflammatory responses (21). This is mediated, at least in part, due to reduced TLR4 signaling. Because TLR4 signaling also activates phagocytosis, we explored the relationship between TLR4 signaling, fatty acid biosynthesis, and macrophage phagocytosis.

The *Fasn* gene is a direct target of SREBP-1, and we showed *Fasn* gene expression and endogenous fatty acid synthesis were reduced in SREBP-1aDF/B6 macrophages (8). Previous studies showed SREBP-1 was activated during phagocytosis in fibroblasts (4) and during phagocytic differentiation of human monocytes (11), but the mechanism and the SREBP-1 isoform(s) involved was not addressed. In this study, we examined the mechanism and further characterized the role of SREBP-1a and de novo lipogenesis in macrophage phagocytosis. First, we showed that SREBP-1aDF/B6 mice had a system-wide defect in phagocytosis of IC-IP$_6$ in whole animals in vivo. Then we showed that phagocytosis of opsonized red blood cells and apoptotic cells was reduced in isolated BMDM from SREBP-1aDF/B6 mice.

TLR4 signaling stimulates mTORC1 signaling, phagocytosis, and proteolytic activation of SREBP-1, so we investigated their interrelationship. In Fig. 2, we showed that TLR4-dependent activation of phagocytosis was reduced in SREBP-1aDF/B6 macrophages and that reintroduction of SREBP-1a through viral vector delivery restored phagocytic capacity to WT levels. In addition, the TLR4-dependent activation of phagocytosis was inhibited by pretreatment with rapamycin or in raptor-deficient BMDMs, providing pharmacologic and complementary genetic evidence that TLR4-dependent activation of phagocytosis requires signaling through TORC1. Importantly, in both cases, exogenously provided SREBP-1a restored the phagocytic defect, which strongly suggests that TLR4 signaling through mTORC1 requires SREBP-1a to stimulate phagocytosis. It is possible that mTORC1 signaling also stimulates phagocytosis through more-rapid effects on membrane structure or function; however, because exogenous SREBP-1a can reverse the pharmacologic and genetic block due to rapamycin or raptor knockout, the SREBP effect is clearly more important.

When we compared gene expression profiles in WT versus SREBP-1aDF/B6 macrophages, we did not observe reduced expression of any key phagocytic genes (8). Based on this analysis and the prior literature suggesting fatty acid composition plays a significant role in macrophage phagocytosis, we proposed that the reduced phagocytosis in SREBP-1aDF/B6 macrophages was from a defect in de novo fatty acid synthesis.

We showed previously that de novo fatty acid synthesis was reduced in SREBP-1a–deficient BMDM (8). To extend this observation, we compared the fatty acyl chains contained in major phospholipid species from WT and SREBP-1aDF/B6 BMDMs using shotgun lipidomics (26). This comparison demonstrated that the reduction in newly synthesized lipids in SREBP-1aDF/B6 cells resulted in reduced amounts of the major PC and PE species containing fatty acyl chains such as palmitate and oleate, which are derived from de novo synthesized lipids (26). Similar results were obtained in BMDM isolated from SCAP-deficient macrophages where all SREBP maturation is prohibited.

Oishi et al. (10) recently reported that, after an initial shutdown of lipid synthesis following TLR4 stimulation, SREBP-1–mediated lipid synthesis rises to provide antiinflammatory lipid mediators to assist in resolution of the initial proinflammatory response. This is consistent with the results reported here, as we performed all of our studies following 16 h to 24 h of
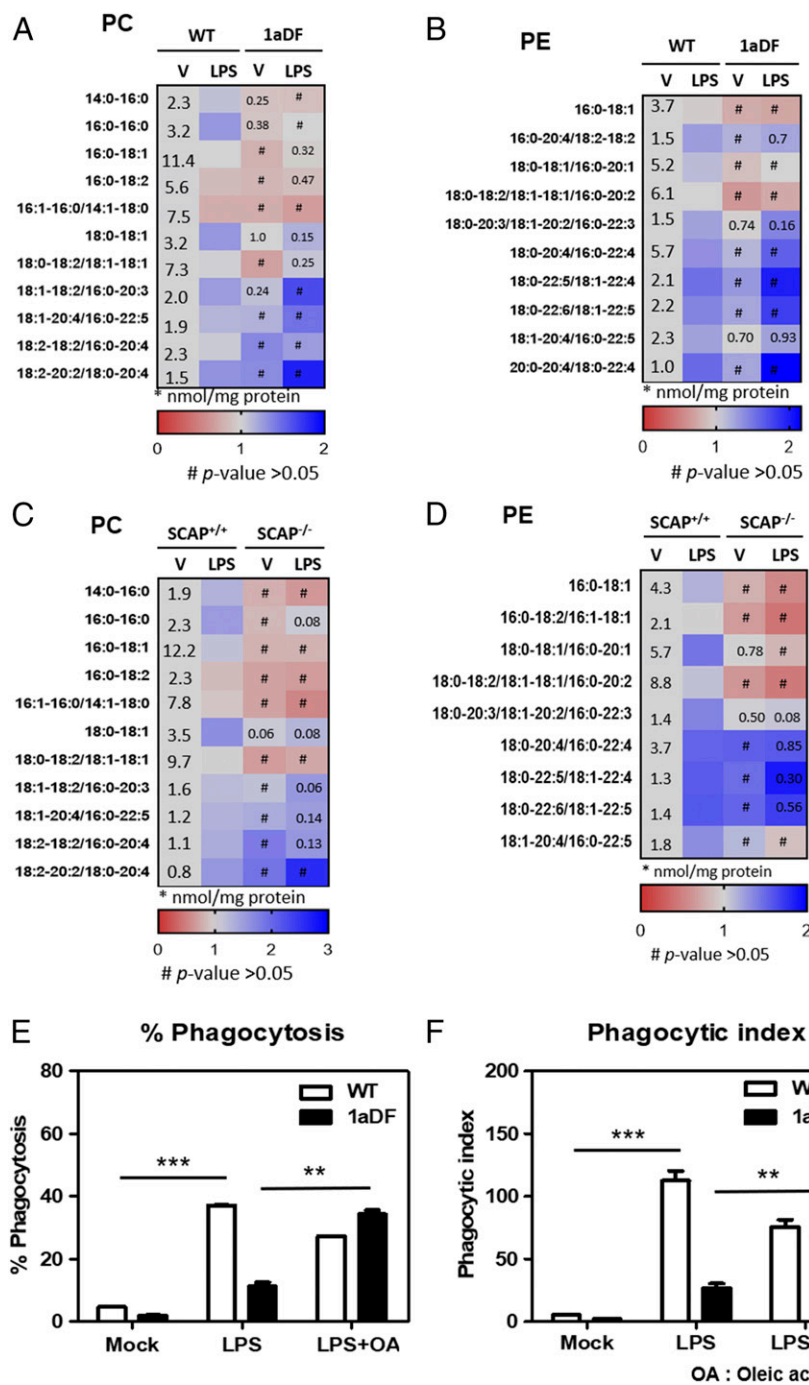
A — PC

	WT		1aDF	
	V	LPS	V	LPS
14:0-16:0	2.3		0.25	#
16:0-16:0	3.2		0.38	#
16:0-18:1	11.4		#	0.32
16:0-18:2	5.6		#	0.47
16:1-16:0/14:1-18:0	7.5		#	#
18:0-18:1	3.2		1.0	0.15
18:0-18:2/18:1-18:1	7.3		#	0.25
18:1-18:2/16:0-20:3	2.0		0.24	#
18:1-20:4/16:0-22:5	1.9		#	#
18:2-18:2/16:0-20:4	2.3		#	#
18:2-20:2/18:0-20:4	1.5		#	#

* nmol/mg protein

0   1   2

\# p-value >0.05

B — PE

	WT		1aDF	
	V	LPS	V	LPS
16:0-18:1	3.7		#	#
16:0-20:4/18:2-18:2	1.5		#	0.7
18:0-18:1/16:0-20:1	5.2		#	#
18:0-18:2/18:1-18:1/16:0-20:2	6.1		#	#
18:0-20:3/18:1-20:2/16:0-22:3	1.5		0.74	0.16
18:0-20:4/16:0-22:4	5.7		#	#
18:0-22:5/18:1-22:4	2.1		#	#
18:0-22:6/18:1-22:5	2.2		#	#
18:1-20:4/16:0-22:5	2.3		0.70	0.93
20:0-20:4/18:0-22:4	1.0		#	#

* nmol/mg protein

0   1   2

\# p-value >0.05

C — PC

	SCAP+/+		SCAP-/-	
	V	LPS	V	LPS
14:0-16:0	1.9		#	#
16:0-16:0	2.3		#	0.08
16:0-18:1	12.2		#	#
16:0-18:2	2.3		#	#
16:1-16:0/14:1-18:0	7.8		#	#
18:0-18:1	3.5		0.06	0.08
18:0-18:2/18:1-18:1	9.7		#	#
18:1-18:2/16:0-20:3	1.6		#	0.06
18:1-20:4/16:0-22:5	1.2		#	0.14
18:2-18:2/16:0-20:4	1.1		#	0.13
18:2-20:2/18:0-20:4	0.8		#	#

* nmol/mg protein

0   1   2   3

\# p-value >0.05

D — PE

	SCAP+/+		SCAP-/-	
	V	LPS	V	LPS
16:0-18:1	4.3		#	#
16:0-18:2/16:1-18:1	2.1		#	#
18:0-18:1/16:0-20:1	5.7		0.78	#
18:0-18:2/18:1-18:1/16:0-20:2	8.8		#	#
18:0-20:3/18:1-20:2/16:0-22:3	1.4		0.50	0.08
18:0-20:4/16:0-22:4	3.7		#	0.85
18:0-22:5/18:1-22:4	1.3		#	0.30
18:0-22:6/18:1-22:5	1.4			0.56
18:1-20:4/16:0-22:5	1.8		#	#

* nmol/mg protein

0   1   2

\# p-value >0.05

E — % Phagocytosis

WT, 1aDF; Mock, LPS, LPS+OA; *** , **

F — Phagocytic index

WT, 1aDF; Mock, LPS, LPS+OA; *** , **

OA : Oleic acid

Fig. 5. Phospholipid species with de novo synthesized fatty acyl chains are reduced in SREBP-1aDF/B6 and SCAP mKO BMDMs. (*A*) PC profile after LPS (100 ng/mL) treatment for 24 h, analyzed by liquid chromatography-MS in WT and SREBP-1aDF/B6 BMDMs. (*B*) PE profile after LPS challenge in WT and SREBP-1aDF/B6 BMDMs. (*C*) PC profile after LPS treatment in BMDMs from SCAP^{fl/fl} and SCAP mKO mice. (*D*) PE profile after LPS treatment in BMDMs from SCAP^{fl/fl} and SCAP mKO mice. (*E*) Percentage of phagocytosis with LPS plus oleic acid in WT and SREBP-1aDF/B6 BMDMs. (*F*) Phagocytic index level after LPS treatment plus oleic acid in WT and SREBP-1aDF/B6 BMDMs. Data are represented as mean ± SEM; **P < 0.01, ***P < 0.001.

TLR4 activation. In fact, our results suggest that the activation of SREBP-1 and antiinflammatory lipid production observed by Oishi et al. may be at least partially mediated through the TLR4–mTORC1–SREBP-1a pathway established by our study.

Wei et al. (21) showed that macrophages lacking *Fasn*, which totally eliminates new fatty acid synthesis, have a major defect in lipid raft protein integrity and macrophage inflammatory functions. However, when we profiled lipid raft protein content, we found that classic lipid raft-associated proteins were present in similar levels in lipid raft fractions from WT and SREBP-1aDF/B6 macrophages. This is likely because the loss of SREBP-1a does not lead to a total block in de novo lipid synthesis. Despite apparent normal lipid raft protein content, there were significantly reduced levels of cofilin and moesin in SREBP-1aDF/B6 lipid raft membrane fractions. Oleic acid supplementation rescued the association of moesin and cofilin with lipid rafts, providing direct evidence for a key role in de novo synthesized fatty acyl chains in actin cytoskeletal–lipid raft interactions.

Robust phagocytosis requires a direct interaction between the plasma membrane and actin cytoskeleton (27). In this regard, cofilin promotes actin turnover during cytoskeletal remodeling (18), and moesin is a member of the ezrin radixin moesin family of proteins that connect plasma membranes to the actin cytoskeleton (17). Importantly, both cofilin and moesin also associate with lipid rafts and are required for distortions of the cell membrane that define early stages of the phagocytic process, including membrane ruffling and phagocytic cup formation. Additionally, studies have shown that knockdown of moesin (28)
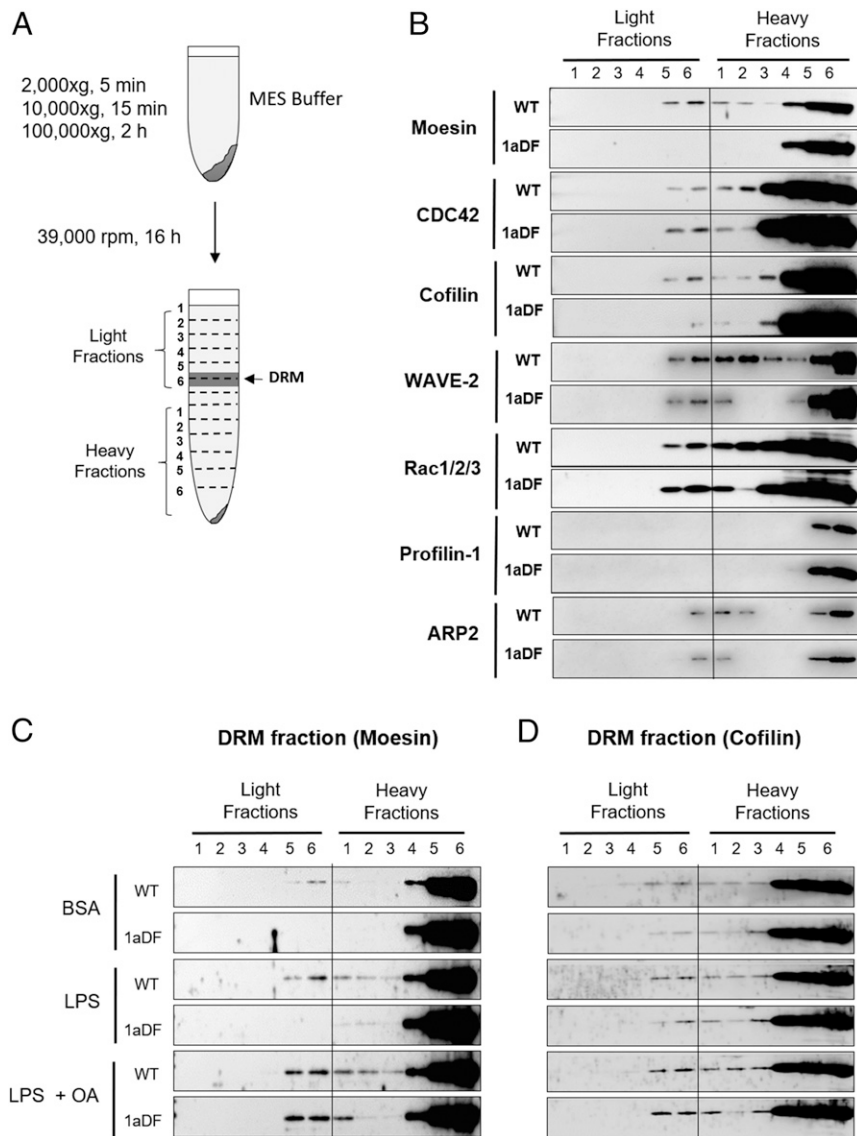
Fig. 6. SREBP-1a is required for actin reorganization proteins in DRM domains. (A) Flowchart illustrating the DRM isolation procedure, as detailed in Material and Methods. Fraction numbers correspond to the lane numbers in B. Each fraction was isolated after spinning down for 17 h, and we collected 1 mL from each fraction. (B) DRM proteins were confirmed by immunoblotting. Extracts isolated from BMDMs of WT and SREBP-1aDF/B6 mice were separated by ultracentrifugation for 16 h at 39,000 rpm in a Beckman SW55Ti swinging bucket rotor. (C) Effects of LPS and oleic acid (OA) on moesin association with DRMs. LPS (100 ng/mL) and OA (500 μM) were treated for 16 h and 24 h, respectively, and isolated DRMs from macrophages. (D) Effects of LPS and OA on cofilin association with DRMs.

decreases macrophage phagocytosis, and overexpression of a constitutively active form of cofilin increases membrane ruffling, which is an early event in phagocytosis activation (29). Our results suggest that, when SREBP-1a−dependent lipid species are reduced, there is impaired association between the actin cytoskeletal network and the plasma membrane, which leads to reduced phagocytosis.

The resolution of both phagocytosis and autophagy requires phagosome−lysosome fusion to metabolize the encapsulated payload. It is interesting that these two key cellular processes are oppositely regulated by mTORC1 signaling at unique steps before lysosomal fusion that result in either activation or inhibition of phagocytosis vs. autophagy, respectively. This is important, because there is a limited supply of cellular lysosomes, and the converse regulation by mTORC1 ensures that autophagy and phagocytosis do not occur at the same time, to maximize pathogen clearance. In the studies presented here, we show that mTORC1 activates SREBP-1−dependent lipid synthesis to stimulate phagocytosis, whereas prior studies have shown that mTORC1 phosphorylates and inactivates the ULK1 kinase that is essential for early events in formation of the membrane-bound autophagic organelle (30).

Materials and Methods

Phagocytosis Assay. Phagocytosis assays were performed using slight modifications to a previously published procedure (31).

Isolation of BMDMs. BMDMs were isolated from C57B/6J, SREBP-1aDF/B6, SCAP^{fl/fl}, SCAP LysM6-SCAP KO (SCAP mKO), Raptor^{fl/fl}, LysM6-Raptor KO (raptor mKO), or B6(Cg)-Tlr4tm1.2Karp/J (TLR4 KO) mice. Femurs and tibias were isolated and flushed with DMEM supplemented with 2% FBS. After RBC were lysed using ACK lysis buffer (0.15 M NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM Na_2-EDTA, pH 7.2), macrophages were depleted by a preadhesion step for 2 h at 37 °C in 5% CO_2. Following the differentiation of BMDMs for 7 d in culture, BMDMs were subcultured to use for experiments.

Adenovirus Infection in BMDMs. Macrophages were plated in 100-mm dishes (1×10^6 cells per dish). Ad-SREBP-1a [10 multiplicities of infection (MOI)] was added to cells in DMEM containing 20% L929 conditioned medium without FBS at 37 °C with 5% CO_2 for 6 h, and medium with FBS was added. After incubating for an additional 24 h, cells were collected for analysis.

Immunoblotting. Proteins were isolated from BMDM, and immunoblotting was performed following a previous method (9).

Biodistribution and Clearance of IC-IP$_6$ from the Blood. Preparation of IC-IP$_6$, which is phytic acid chelated with Fe^{3+}, was prepared and biodistribution was assessed as described (19).

Shotgun Lipidomics Analysis. Cell pellets were homogenized in 0.5 mL of 10× diluted PBS in 2.0-mL cryogenic vials (Corning Life Sciences) by using a digital sonifier (Branson 450). For shotgun lipidomics, lipid extracts were diluted to a final concentration of ~500 fmol/μL, and the mass spectrometric analysis was performed on a QqQ mass spectrometer (Thermo TSQ Quantiva) equipped with an automated nanospray device (TriVersa NanoMate; Advion Bioscience Ltd.) as previously described (32). Identification and quantification of lipid molecular species were performed using an automated software program (16, 33). Data were normalized to per milligram of protein.

S. Typhimurium Infection of BMDM. *S. typhimurium* (SL1344) ppGpp deficiency strain was cultured overnight and then diluted 100-fold into LB broth and grown for another 4 h at 37 °C. The bacterial inoculums were diluted and added to the BMDM cells at MOI 1:100 for 30 min at 37 °C in 5% CO$_2$/95% air.

Isolation of Detergent Resistance Membrane Fractions. Detergent resistance membrane (DRM) fractions were isolated from BMDMs as described previously (21).

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