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Inducible Renitence Limits Listeria monocytogenes Escape from Vacuoles in Macrophages

Michael J. Davis,* Brian Gregorka, † Jason E. Gestwicki, ‡ and Joel A. Swanson* †

Membranes of endolysosomal compartments in macrophages are often damaged by physical or chemical effects of particles ingested through phagocytosis or by toxins secreted by intracellular pathogens. This study identified a novel inducible activity in macrophages that increases resistance of phagosomes, late endosomes, and lysosomes to membrane damage. Pretreatment of murine macrophages with LPS, peptidoglycan, TNF-α, or IFN-γ conferred protection against subsequent damage to intracellular membranes caused by photooxidative chemistry or by phagocytosis of ground silica or silica microspheres. Phagolysosome damage was partially dependent on reactive oxygen species but was independent of the phagocyte oxidase. IFN-γ-stimulated macrophages from mice lacking the phagocyte oxidase inhibited escape from vacuoles by the intracellular pathogen Listeria monocytogenes, which suggested a role for this inducible renitence (resistance to pressure) in macrophage resistance to infection by pathogens that damage intracellular membranes. Renitence and inhibition of L. monocytogenes escape were partially attributable to heat shock protein-70. Thus, renitence is a novel, inducible activity of macrophages that maintains or restores the integrity of endolysosomal membranes.

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Abbreviations used in this article: BMM, bone marrow-derived macrophage; DPI, dihydroorotate dehydrogenase; DOPA, dopamine; GSH, glutathione; HSP70, heat shock protein-70; HSP90, heat shock protein-90; IFN-γ, interferon-γ; LAMP, lysosomal-associated membrane protein; LLO, lysteriolysin O; MIP, macrophage inflammatory protein; NADPH, nicotinamide adenine dinucleotide phosphate; NFκB, nuclear factor-κB; NO, nitric oxide; PGN, peptidoglycan; ROS, reactive oxygen species; SFFV, spleen focus forming virus; SMS, silica microspheres; TLR, toll-like receptor; TRDS, Texas red–dextran; TSS, TBS plus 2% sheep serum.

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Abbreviations used in this article: BMM, bone marrow-derived macrophage; DPI, diphosphorylenideonidium; Fdx, fluorescein–dextran; HSP70, heat shock protein-70; LLO, lysteriolysin O; MOL, multiplicity of infection; NAC, N-acetyl-L-cysteine; PGN, peptidoglycan; PLL, poly-L-lysine; ROS, reactive oxygen species; SMS, silica microsphere; TRDS, Texas red–dextran; TSS, TBS plus 2% sheep serum.

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mouse IL-10, recombinant mouse IL-1β, SNARF-1 carboxylic acid acetate succinimidyl ester, Cell Trace CFSE Cell Proliferation Kit, Texas red-dextran (TRdx; average molecular mass 10,000 Da), Texas red-phalloidin, and gentamicin solution were purchased from Invitrogen (Carlsbad, CA).

Recombinant human IFN-β, diphenyleneiodonium (DPI), and N-acetyl-l-cysteine (NAC) were purchased from Sigma Chemical Co. (St. Louis, MO).

LPS no. 225 Salmonella typhimurium was purchased from List Biological Laboratories (Campbell, CA), and 35-mm dishes with attached 14-mm coverslips were purchased from MatTek Corp (Ashland, MA). Peptidoglycan (PGN) from Escherichia coli 0111:B4 was purchased from Invivogen (San Diego, CA). IFN-γ was from R&D Systems (Minneapolis, MN), TNF-α was from eBioscience (San Diego, CA), and murine IL-6 was from Calbiochem (San Diego, CA). MIN-U-SIL-15 ground silica was a gift from U.S. Silica (Berkeley Springs, WV). Oxide silica 3-μm microspheres (SMS); Microspheres-Nanospheres, Cold Spring, NY) were washed overnight in 1 N HCl then rinsed several times in distilled water. For some experiments, SMS were coated with 0.1 M poly-L-lysine (PLL) for 30 min. Reombini

Bone marrow-derived macrophages

C57BL/6j (wild type), B6.129-Cybb<sup>−/−</sup>/J (xox2 deficient), and TLR4<sup>−/−</sup> mice (strain C57BL/6j/10SCN) were purchased from The Jackson Laboratory (Bar Harbor, ME), with specific pathogen-free conditions at the University of Michigan animal facility. Differentiation of macrophages from mouse bone marrow cells has been previously described (23, 31). Briefly, marrow cells extracted from mouse femurs were cultured for 6 d in the presence of M-CSF, which was provided from 30% L-cell-conditioned medium. Bone marrow-derived macrophages (BMM) were frozen and stored at −130°C as aliquots in M-CSF–containing medium with 10% DMSO and thawed as needed for experiments.

Loading macrophage lysosomes and stimulation of cells

After allowing BMM to attach for several hours in RPMI 1640 with 10% FCS and penicillin/streptomycin, cells were incubated overnight in medium containing 150 μg/ml Fdx. BMM were then rinsed and chased in unlabeled medium for at least 3 h to allow Fdx trafficking into late compartments of the endolysosomal network (32). Penicillin/streptomycin was omitted from BMM cultures to be infected with L. monocytogenes (23, 31). To determine which other agents could induce this effect, BMM were stimulated with LPS, IFN-γ, and IL-6, IL-10, IL-1β, IL-1β, (10 ng/ml), TNF-α (10 ng/ml), IL-6 (5 ng/ml), IL-10 (10 ng/ml), IL-1β (10 ng/ml), or IFN-β (10 ng/ml) were included in both the Fdx pulse medium and the subsequent Fdx-free chase medium. Uncoated SMS were fed to BMM in serum-free medium or PLL-coated SMS were fed to cells in medium containing 10% FCS and incubated 1 h before imaging.

Measurement of damage to endolysosomal compartments

Endolysosomal membrane damage was measured in live BMM by epi-fluorescence ratiometric microscopy, as described previously (23). Cells loaded with Fdx were rinsed and imaged in Ringer’s buffer (155 mM NaCl, 5 mM KCl, 2 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 2 mM NaH<sub>2</sub>PO<sub>4</sub>, 10 mM HEPES, and 10 mM glucose) using a Nikon TE300 inverted microscope equipped with a mercury arc lamp, ×60 plan-apochromat 1.4-numerical aperture objective, a cooled digital CCD camera (Quantix Photometrics, Tucson, AZ), a temperature-controlled stage, and a Fura/FITC ratiometric dichoric mirror (Omega Optical, Brattleboro, VT). Three images were acquired for each field of cells: one phase-contrast image and two fluorescence images, which used a 510-nm emission filter and two different excitation band-pass filters (centered at 440 nm and 485 nm), mounted in computer-controlled filter wheels (Sutter Instruments, Novato, CA). Metamorph software (Molecular Devices, Downingtown, PA) was used for image capture and analysis.

The Fdx signal in the 440-nm excitation channel is relatively insensitive to pH, whereas the signal in the 485-nm excitation channel varies between pH 4.5 and 7.5. The ratio of Fdx fluorescence in the 485-nm channel divided by that in the 440-nm channel is related to pH in any subregion of the cell. Ratios were converted to pH, as described previously (17, 23, 33).

Results

Macrophage activation stabilizes lysosomes

Previous studies established that particle-mediated lysosome damage was reduced after macrophages were stimulated with LPS (23). To determine which other agents could induce this effect, BMM late endosomes and lysosomes were loaded with Fdx and stimulated with cytokines or TLR ligands, then the damage induced by phagocytosis of SMS was measured as the release of Fdx from endolysosomal compartments into cytoplasm (Fig. 1A, 1B). LPS, PGN, IFN-γ, and TNF-α protected endolysosomes from SMS challenge, whereas IL-6, IL-10, IL-1β, and IFN-β did not. Time-course measurements indicated that full lysosome protection...
required 18 h of LPS stimulation, although significant protection was observed after 5 h (Fig. 1C). We termed this inducible membrane resistance or repair mechanism renitence: resistance to pressure.

The studies of MacKaness (14), which first described a role for activated macrophages in host defense, showed that infection of mice with sublethal doses of *L. monocytogenes* led to differentiation of macrophages with increased resistance to subsequent *L. monocytogenes* infections. Although activation was eventually attributed to the action of IFN-γ and TNF-α (34), we postulated that some of the increased resistance may be induced directly by bacteria. We therefore measured the extent to which phagocytosis of hemolysin-deficient (∆hly) *L. monocytogenes*, which do not damage vacuoles (17, 35, 36), increased macrophage resistance to subsequent challenge by phagocytosed SMS. BMM that ingested ∆hly *L. monocytogenes* became resistant to lysosome damage after phagocytosis of SMS (Fig. 1D). The subset of BMM that were exposed to ∆hly *L. monocytogenes* but which did not ingest bacteria showed lysosome damage levels comparable to unstimulated cells (Fig. 1E), indicating that the ∆hly *L. monocytogenes* activated this protective mechanism inside the cells that ingested them, rather than through some secreted factor. This suggests that microbes ingested by macrophages initiate a cytokine-independent differentiation to a more resistant phenotype.

**ROS contribute to particle-mediated lysosome damage**

Inducible renitence could interfere with the mechanism(s) by which particles damage membranes. As ROS have been implicated in NLRP3 inflammasome activation by lysosome damage (37, 38), we examined the contribution of ROS to phagolysosome damage.

Macrophages were pretreated with DPI, which is a potent inhibitor of ROS-generating activities in macrophages, including the phagocyte oxidase (NOX2) and the mitochondrial electron transport chain (39–41). Macrophages loaded with Fdx and incubated in DPI or control medium were fed ground silica or SMS, and the extent of damage was assessed. DPI reduced phagolysosome damage after phagocytosis of both kinds of particle (Fig. 2A, 2B). Similarly, BMM preincubated in the antioxidant NAC showed less SMS-mediated lysosome damage than that of control cells (Fig. 2C). This reduced damage was independent of any DPI effect on the efficiency of phagocytosis, as DPI-treated BMM that had phagocytosed SMS showed less lysosome damage than control cells that had ingested equivalent numbers of particles. These data indicate that phagocyte oxidase-independent ROS contribute to silica-induced lysosome damage. Renitence may therefore consist of mechanisms that interfere with ROS generation in cytoplasm.

A major source of ROS in the phagosomal lumen is the phagocyte oxidase. We examined the contribution of phagocyte oxidase-generated ROS to damage by comparing wild-type and phagocyte oxidase (nox2)-deficient BMM and observed similar levels of phagolysosome damage after phagocytosis of ground silica (Fig. 2D) or SMS (Fig. 2E). This indicates that the phagocyte oxidase does not contribute to particle-mediated lysosome damage.

**L. monocytogenes hemolysin damages endolysosomes**

The relationship between *L. monocytogenes* vacuolar escape and endolysosome damage was investigated. A previous study suggested that at a low multiplicity of infection (MOI), *L. monocytogenes* escapes from LAMP-1–negative, late endosome-like vacuoles (16),...
which suggests that escape could occur without causing release of Fdx from late endocytic compartments. To determine if infection at higher MOI damages late endocytic compartments, Fdx-loaded BMM were fed SNARF-1–labeled wild-type or \( \Delta hly \) L. monocytogenes and assayed for endolysosome damage. In cells containing fewer than seven bacteria, wild-type L. monocytogenes showed low levels of lysosome damage, consistent with a mechanism in which L. monocytogenes escapes from vacuoles at earlier stages of maturation. However, higher bacterial loads showed more damage, which correlated with the number of L. monocytogenes per BMM (Fig. 3A–D). BMM infected with \( \Delta hly \) L. monocytogenes showed neither lysosome damage nor L. monocytogenes escape, confirming that both activities required LLO. Thus, although at low MOI L. monocytogenes entered cytoplasm with negligible damage to late endocytic compartments, higher MOI resulted in LLO-dependent release of Fdx from those compartments into cytoplasm. 

**Inducible renitence inhibits L. monocytogenes escape and associated lysosome damage**

The phagocyte oxidase inhibits L. monocytogenes escape in activated macrophages (21, 22). To determine if inducible renitence also contributes to the inhibition of L. monocytogenes escape in activated macrophages, wild-type and nox2-deficient BMM were loaded with Fdx, activated with IFN-\( \gamma \), infected with wild-type

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**FIGURE 2.** Nonphagosomal reactive oxygen intermediates contribute to silica-mediated lysosome damage. BMM were loaded with Fdx then fed ground silica (A, D) or PLL-coated SMS (B, C, E). Then, 5 \( \mu \)M DPI (A, B) or 50 mM NAC (C) was added to the medium with the particles. (D and E) BMM from wild-type or nox2-deficient (Nox2\( ^{-/-} \)) mice were loaded with Fdx, fed particles, and scored for lysosome damage. All data points represent mean \( \pm \) SEM Fdx release with \( n > 200 \) BMM for (A), (B), (D), and (E) and \( n > 50 \) BMM for (C). *\( p < 0.05 \), **\( p < 0.001 \).

**FIGURE 3.** Inducible renitence protects IFN-\( \gamma \)-activated macrophages from L. monocytogenes escape and damage. (A–C) Damage by infection with L. monocytogenes. C57/B6L BMM were loaded with Fdx overnight, chased in dye and antibiotic-free medium for at least 3 h, infected with SNARF-1–labeled wild-type or \( \Delta hly \) L. monocytogenes, chased in gentamicin-containing medium for 90 min, and imaged to measure lysosome damage. (A) Fdx pH map of a BMM infected with wild-type L. monocytogenes. Blue pixels indicate lysosome damage (i.e., cytoplasmic Fdx). (B) Fdx pH map of a BMM infected with \( \Delta hly \) L. monocytogenes indicates that the lysosomes are intact. Scale bar, 10 \( \mu \)m. Green pixels in (A) and (B) indicate SNARF-1–labeled L. monocytogenes. (C) Average \( \pm \) SEM percent Fdx release for BMM (\( n > 100 \) for each group) plotted in groups of cells containing similar numbers of total wild-type (filled bars) or \( \Delta hly \) (open bars) L. monocytogenes. (D) Fdx release data replotted from (C) showing average number of escaped (phalloidin-positive) L. monocytogenes \( \pm \) SEM (\( n > 100 \) for each group) plotted on the x-axis for each category of BMM grouped by total number of L. monocytogenes per cell (in parentheses). Filled circles represent groups of BMM infected with wild-type L. monocytogenes; open symbol near the origin represents all groups of \( \Delta hly \) L. monocytogenes. (E and F) C57/B6L (WT) and nox2-deficient (Nox2\( ^{-/-} \)) BMM were stimulated with IFN-\( \gamma \) (open bars) or left unstimulated (dark bars) and loaded with Fdx. BMM infected 120 min with wild-type L. monocytogenes were fixed and stained with phalloidin to measure L. monocytogenes escape (E) or imaged for Fdx release (F). Bars in (E) are average percent of phalloidin-positive L. monocytogenes and error bars are SEM (\( n \approx 350 \) cells). Bars in (F) indicate average percent of Fdx released \( \pm \) SEM of BMM containing one to seven L. monocytogenes per cell (\( n \approx 130 \) cells). *\( p < 0.05 \), **\( p < 0.001 \).
L. monocytogenes, and scored for actin-positive cytoplasmic bacteria. IFN-γ treatment limited L. monocytogenes escape in both wild-type and nox2-deficient BMM. Although IFN-γ-activated, nox2-deficient BMM did not inhibit escape as efficiently as IFN-γ-activated wild-type BMM, they were significantly better than unactivated, nox2-deficient BMM (Fig. 3E). IFN-γ-activated, nox2-deficient BMM also showed reduced levels of endolysosome damage after L. monocytogenes infection compared with unactivated nox2-deficient cells (Fig. 3F). This nox2-independent resistance suggests that inducible renitence inhibits L. monocytogenes escape from vacuoles of activated macrophages. However, contributions of other IFN-γ-inducible activities to macrophage resistance to L. monocytogenes escape cannot be excluded.

Inducible renitence protects lysosomes against photooxidative damage

Inducible renitence was also measured in a nonparticulate endolysosome-damaging assay. Late endosomes and lysosomes were loaded by endocytosis with TRdx, as a photosensitizer, and Fdx, to monitor membrane integrity. Cells were stimulated with LPS or IFN-γ, then exposed to 580-nm light, which excites TRdx but not Fdx. The excited TRdx increases photooxidative chemistries that damage cell membranes (24, 42). Lysosome integrity was measured by imaging Fdx after each 580-nm exposure. In unstimulated BMM, lysosome damage was detectable after 25-s exposure and was nearly complete by 50 s (Fig. 4). Control BMM, in which endolysosomes were loaded with Fdx but not TRdx and exposed similarly to 580-nm light, showed no damage. Half-maximal lysosome damage in LPS-stimulated or IFN-γ-stimulated BMM required greater exposure to light than in unstimulated BMM (Fig. 4). Thus, the inducible resistance to membrane damage also affects nonphagosomal lysosomes.

HSP70 limits damage

As infection can increase synthesis and secretion of HSP70 from macrophages (11, 12) and exogenously added HSP70 can increase lysosomal resistance to photodamage (24), we hypothesized that HSP70 contributed to induced renitence. Macrophages were incubated with recombinant HSP70 or LPS, and endolysosome damage was measured after SMS phagocytosis. Exogenous HSP70 reduced lysosome damage to levels comparable to those in LPS-activated cells (Fig. 5A). To rule out endotoxin contamination as an alternative explanation for HSP70-mediated lysosome protection (43), damage was measured in TLR4-deficient BMM. These cells showed significant resistance to damage when treated with recombinant HSP70 or IFN-γ but not with LPS (Fig. 5B, 5C). HSP70-mediated protection was also inhibited by the inhibitors of HSP70 ATPase activity Ym1 (30, 44) and Myr (29, 45, 46) (Fig. 5B). HSC70 induced less protection than HSP70 (Fig. 5C), consistent with previous observations indicating the specificity of HSP70 for lysosome-protective effects (24). To test the hypothesis that endogenous HSP70 contributes to inducible lysosome renitence, macrophages were loaded with Fdx and stimulated with LPS for 0, 5, or 18 h. For the last 5 h before phagocytosis of SMS, cells were treated with HSP70 inhibitors Ym1 and Myr. Macrophages stimulated with LPS in the presence of inhibitors showed greater endolysosome damage than cells without drug (Fig. 5D), indicating that HSP70 mediates LPS-induced renitence. The contribution of HSP70 to inducible renitence was also tested in an L. monocytogenes infection model. TLR4-deficient BMM were treated with HSP70 or IFN-γ, infected with L. monocytogenes, and scored for escape. HSP70-treated BMM showed a reduction in L. monocytogenes escape comparable to that of IFN-γ-stimulated cells (Fig. 5E). Inhibitors of HSP70 partially reversed the IFN-γ-mediated reduction in L. monocytogenes vacuolar escape, indicating that HSP70-mediated membrane protection inhibits L. monocytogenes vacuolar escape and that HSP70 contributes to inducible renitence.

Immunofluorescence microscopy indicated that total levels of HSP70 increased in BMM stimulated with LPS (Fig. 5F, 5G) and IFN-γ (data not shown) and that HSP70 localized to small, LAMP-1-negative vesicles. Lack of colocalization between HSP70 and

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**FIGURE 4.** Inducible renitence protects against photodamage. BMM lysosomes were loaded with Fdx and TRdx. Cells were exposed to 580-nm light from the microscope arc lamp in 5-s pulses; Fdx pH images were acquired between each pulse. (A) Sample pH maps of fields of photostimulated BMM, with pH color key on the right and a 50-μm scale bar below the images (right side). (B) Percent of Fdx released was calculated for each cell. Average percent release for each time point is plotted ± SEM. At least 50 cells were analyzed for each condition. Control macrophages were significantly different from both LPS-stimulated and IFN-γ-simulated cells with p < 0.001 for all data between 30 to 50 s and p < 0.05 for the data at 25 and 55 s (unpaired Student t test).
LAMP-1 suggests that HSP70 effects on renitence do not occur from within the endolyosomal network.

**Discussion**

This study identifies inducible renitence as a novel common feature of classical macrophage activation, which can be induced by the TLR ligands LPS and PGN, by whole bacteria, and by the cytokines IFN-γ and TNF-α. Renitence was observed in a variety of different membrane-damaging insults, including phagocytosis of ground or microsphere silica particles, LLO-dependent membrane perforation, and photooxidative chemistries. Thus, inducible renitence is unlikely to involve mechanisms specific to any one kind of damage but instead represents a set of activities that reinforce endolysosomal membranes. Although the assays used here allowed us to analyze renitence as a feature of late endosomes and lysosomes, it is possible that other membranous compartments, such as early endosomes or endoplasmic reticulum, may exhibit renitence, as well. It will be interesting to determine if renitence is inducible.

**FIGURE 5.** HSP70 contributes to inducible renitence. (A–D) HSP70 inhibits damage by SMS. (A) Fdx-loaded BMM were exposed to LPS or 300 nM recombinant HSP70 for 5 h, then cells were rinsed and fed SMS for 1 h and imaged for Fdx release (1–3 SMS/cell, n > 100 cells). (B) BMM from TLR4−/− mice were exposed to IFN-γ overnight, LPS for 5 h, or 300 nM HSP70 for 5 h, with or without HSP70 inhibitors Ym1 (20 μM) or Myr (25 μM). HSP70 inhibited lysosome damage (n > 300 cells). (C) TLR4−/− BMM were exposed to IFN-γ overnight or 300 nM recombinant HSP70 or HSC70 for 5 h, then assayed for SMS damage (n > 180 cells). (D) Wild-type BMM were exposed to LPS for 5 or 18 h or left untreated. Ym1 (20 μM) or Myr (25 μM) were included for the last 5 h of stimulation (n > 120 cells). (E) TLR4−/− BMM were exposed to recombinant HSP70 for 5 h or to IFN-γ overnight. Indicated IFN-γ-stimulated cells were also incubated with Ym1 or Myr for the last 5 h of stimulation. BMM were infected with wt or Δhly L. monocytogenes, and vacuolar escape was measured (n > 500 cells). Bars are population averages (± SEM). *p < 0.05, **p < 0.001. (F and G) Immunofluorescence localization of HSP70 and LAMP-1 in (F) unstimulated BMM and (G) IFN-γ-stimulated BMM. Overlay images show corresponding distributions of LAMP-1 (green) and Hsp70 (red). Scale bar, 10 μm.
in other common targets of infection, such as dendritic cells and epithelial cells.

Renitence was induced by mediators of classical activation in macrophages, indicating a role in inflammation and host defense against infections. Previous studies showed that resistance of lysosomes to various kinds of damage can be induced by LPS (23), TNF-α (47), or HSP70 (24), all of which are associated with infection. That infection by \( H. pylori \) could also induce renitence suggests that early signaling by TLRs induces renitence as a protection against subsequent infections. Local induction by \( H. pylori \) (Fig. 1E) suggests that at least some of the induction is cell autonomous. It remains to be determined if renitence is inducible by TLR signaling, by membrane damage itself, or by bacterial HSP70 (i.e., DnaK).

In cells containing low numbers of bacteria, \( L. monocytogenes \) escape occurred with negligible damage to late endosomes and lysosomes (Fig. 3D). As lysosomal perforation can activate inflammasomes (38), the escape of \( L. monocytogenes \) from prelysosomal compartments may function as an immune evasion strategy. Although macrophages containing few bacteria showed little damage, cells containing many bacteria showed extensive endolysosomal damage, which suggests that the mechanisms that allow \( L. monocytogenes \) to escape prior to phagosome–lysosome fusion may break down later in infection when more bacteria are present in an area. LLO secreted by \( L. monocytogenes \) prior to escape may be trafficked to later compartments and perforate membranes there. Alternatively, LLO secreted by cytosolic \( L. monocytogenes \) could damage lysosomal membranes.

The mechanisms underlying renitence remain unknown but could include reduced susceptibility to membrane-damaging chemistries, increased resistance of membranes to damage, or the upregulation of a membrane-repair process. Nonphagosomal ROS contributed to damage induced by silica particles (Fig. 2), and ROS generated in the lysosome lumen likely account for phototoxic damage by TRDx photostimulation (Fig. 4). Increased synthesis of glutathione was implicated in the TNF-α–induced resistance to lysosome damage (47), consistent with a role for cellular antioxidants in limiting damage. In cells infected with \( L. monocytogenes \), phagosome-derived ROS inhibited escape of \( L. monocytogenes \) from the macrophage phagosome (21). Thus, while modulation of ROS remains a possible mechanism for limiting damage, this is unlikely to be the main mechanism of renitence. Decreased sensitivity to photodamage induced by LPS or IFN-γ (Fig. 4) indicated that endolysosomal membranes become more resistant to damage.

The potential roles for membrane damage-repair mechanisms in renitence remain to be tested.

Vacular membrane damage can have significant physiological and immunological consequences. Aside from allowing access of microbes or virulence factors into cytoplasm, membrane damage induces a proinflammatory cell death program in macrophages (38). Lysosome damage is implicated in several diseases of crystal or particle accumulation, such as silicosis, arteriosclerosis, gout, and asbestosis (37, 38, 48). Lysosome damage in unactivated cells results in noninflammatory cell death (49). These different consequences in activated and nonactivated cells may explain why renitence is an inducible activity. A low level of endocytic membrane leakage into cytoplasm (23, 50) may be beneficial in some circumstances, as it may allow surveillance by cytoplasmic Nod-like receptors (51) or facilitate the cross-presentation of exogenous Ags on MHC class I molecules (52).

The mechanism of endolysosome protection by HSP70 is unknown. Bacterial infection of macrophages can lead to increased synthesis and secretion of HSP70 via nonclassical routes, including exosomes (12). Exogenous HSP70 activates lysosomal acid sphingomyelinase, which, in turn, catalyzes phospholipid chemistries that increase damage-resistance of lysosomal membranes in fibroblasts (24). The evidence presented in this study indicates that activated macrophages induce a similar state in their lysosomes (Fig. 5). HSP70 levels were increased by LPS and IFN-γ, but HSP70 did not localize to lysosomes. This suggests that the effect of HSP70 on renitence may be autocrine. As HSP70 inhibitors only partially reversed lysosome renitence induced by LPS (Fig. 5D) or IFN-γ (Fig. 5E), we hypothesize that HSP70-mediated protection is one of several membrane-stabilizing activities that compose inducible renitence.

Thus, renitence is a novel pathway induced in activated macrophages that reduces damage to endolysosomal membranes caused by mechanical forces or by \( L. monocytogenes \). This suggests that therapeutic interventions that increase renitence could be efficacious against infection by intracellular pathogens or in reducing inflammation due to microparticles or nanoparticles.

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Disclosures

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