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Maturation of the *Legionella pneumophila*-Containing Phagosome into a Phagolysosome within Gamma Interferon-Activated Macrophages

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Legionella pneumophila **is an intracellular pathogen that modulates the biogenesis of its phagosome to evade endocytic vesicle traffic. The *Legionella*-containing phagosome (LCP) does not acquire any endocytic markers and is remodeled by the endoplasmic reticulum during early stages. Here we show that intracellular replication of *L. pneumophila* is inhibited in gamma interferon (IFN-γ)-activated, bone marrow-derived mouse macrophages and IFN-γ-activated, human monocyte-derived macrophages in a dose-dependent manner. This inhibition of intracellular replication is associated with the maturation of the LCP into a phagolysosome, as documented by the acquisition of LAMP-2, cathepsin D, and lysosomal tracer Texas Red ovalbumin, and with the failure of the LCP to be remodeled by the rough endoplasmic reticulum. We conclude that IFN-γ-activated macrophages override the ability of *L. pneumophila* to evade endocytic fusion and that the LCP is processed through the "default" endosomal-lysosomal degradation pathway.**

Legionella pneumophila is an environmental organism that is ubiquitous in the aquatic environment, and its invasion and intracellular replication within protozoa are essential for its ecology and infectivity to humans (32, 33, 44, 48). This organism is the causative agent of Legionnaires' disease, which is manifested as pneumonia as a result of intracellular replication within phagocytic cells, primarily alveolar macrophages (25, 36). Upon entry into macrophages, *L. pneumophila* resides in a phagosome that is excluded from endocytic traffic (22, 23, 38). During early stages of infection, the *Legionella*-containing phagosome (LCP) excludes all endocytic and lysosomal markers such as LAMP-1, LAMP-2, and the lysosomal acid protease cathepsin D (11, 38, 40, 47). In addition to evading endocytic traffic, the LCP is remodeled by the rough endoplasmic reticulum (RER), and vesicles and mitochondria are recruited around the LCP within minutes of infection (26, 45, 46). Modulation of the biogenesis of the LCP, its evasion of endocytic traffic, and its remodeling by the RER are all controlled by the Dot/Icm type IV export machinery (26, 40, 46, 47). The Dot/Icm system is also essential for activation of caspase-3 during early stages of the infection, which is associated with cleavage of the Rab5 early endosome effector rabaptin-5 (15, 16, 50). During late stages of intracellular replication, the LCP membrane becomes disrupted, followed by bacterial escape into the cytoplasm where the bacteria continue to replicate (31). This transient replication in the cytoplasm is followed by lysis of the plasma membrane and bacterial egress from the host cell (2, 5, 28–30).

Interferon gamma (IFN-γ) has been demonstrated to activate host cells to control infection by *L. pneumophila* and other intracellular pathogens (4, 8, 9, 24, 27, 34, 35, 42). In vivo experiments have shown that *L. pneumophila* causes severe infection in IFN-γ-deficient mice. Treatment of *L. pneumophila*-infected mice with IFN-γ results in increased clearance of the bacteria from the lungs of the mice (41). The mechanisms by which IFN-γ-activated macrophages block intracellular replication of *L. pneumophila* are not well understood. These mechanisms may be partially mediated by limiting the availability of intracellular iron and the production of nitric oxide and reactive oxygen intermediates (20, 21). Whether activated macrophages alter trafficking of *L. pneumophila* and target it through a different endocytic route has never been examined. Interestingly, IFN-γ-activated macrophages prevent the escape of *Listeria monocytogenes* from the phagosome and lead to the killing of the organism (13). In addition, IFN-γ-activated macrophages override the ability of *Mycobacterium avium* to block acidification of the phagosome, leading to phagosome acidification and inhibition of intracellular replication (39). Interestingly, recent studies have shown that while *Francisella tularensis* modulates biogenesis of its phagosome and subsequently escapes into the cytoplasm of quiescent macrophages, the bacterial phagosome fuses to lysosomes within IFN-γ-activated macrophages (38b). Therefore, we tested the hypothesis that IFN-γ-activated macrophages may interfere with the ability of *L. pneumophila* to modulate the biogenesis of its phagosome.

The virulent clinical isolate of *L. pneumophila* strain AA100 and its isogenic *dotA* mutant (GL10) have been described previously (50). The bacteria were grown on buffered charcoal-yeast extract agar for 72 h. The wild-type *L. pneumophila* and *dotA* mutant strains harbored the plasmid pAM239, which encodes green fluorescent protein (37). The plates for *gfp*-transformed AA100 and *dotA* were supplemented with 5 μg/ml chloramphenicol.

Immortalized macrophage cell lines were established by infecting bone marrow-derived macrophages from A/J mice with the murine recombinant J2 retrovirus as previously described (6, 12). After a 6-day culture period in L-cell-conditioned me-

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dium, macrophages were collected by centrifugation and suspended in RPMI 1640 containing 10% heat-inactivated fetal bovine serum (Gibco BRL). Peripheral blood monocytes were isolated from adult volunteers who had no history of Legionnaires' disease, and informed consent was obtained from each volunteer as approved by the University of Louisville. The monocytes were obtained using the Ficoll-Hypaque gradient as described previously (18). The monocytes were allowed to mature to quiescent macrophages in Teflon tissue culture flasks as described previously (49).

The human monocyte-derived macrophages (hMDMs) (5×10^5 cells per well in 96-, 12-, or 24-well plates) and A/J mouse macrophages (5×10^5 cells per well in 96-well plates) were incubated for 24 h at 37°C in 5% CO_2 with RPMI 1640 containing 10% fetal bovine serum and different concentrations (0.2, 1, or 2 μg/ml) of IFN-γ. Unless specified, all infections were carried out for 1 h followed by a washing off of the extracellular bacteria, and the infected cells were incubated for the indicated periods of infections specific for each experiment. A multiplicity of infection of 10 was used for intracellular growth kinetics, confocal laser scanning microscopy, and electron microscopy. For growth kinetics, 10^5 hMDMs and A/J mouse macrophages per well of 96-well plates were treated with IFN-γ and infected as described above. After 1, 15, 24, and 48 h of incubation, the cells were washed and either lysed immediately or incubated further. The number of *L. pneumophila* CFU in each well was determined on buffered charcoal-yeast extract agar as described previously (1).

Confocal microscopy to examine colocalization with endocytic markers was performed as we described previously (17). The anti-LAMP-2 (H4B4) (1:2,000) monoclonal antibody (developed by J. T. August and J. E. K. Hildreth) was obtained from the Developmental Studies Hybridoma Bank (University of Iowa). Monoclonal antibodies anti-cathepsin D (1:200) (BD Transduction) and anti-KDEL (1:200) were obtained from Stressgen. Anti-mouse secondary antibodies conjugated to Alexa fluor 594 were obtained from Molecular Probes (Oregon). To label the lysosomes, macrophages were incubated for 1.5 h with 100 μg/ml Trov (Molecular Probes), followed by a 30-min chase prior to infection with *L. pneumophila*. The cells were fixed, permeabilized, and stained with secondary antibody as described above. At least 100 infected cells from multiple coverslips were analyzed, and the experiments were performed at least three times. Transmission electron microscopy was performed as we described previously (31).

Inhibition of intracellular replication of *L. pneumophila* in IFN-γ-activated macrophages. The role of IFN-γ in intracellular replication of the clinical isolate of *L. pneumophila* strain AA100 was examined in bone marrow-derived macrophages from A/J mice and in hMDMs. Macrophages were pretreated for 24 h with different concentrations of IFN-γ and infected by *L. pneumophila* for 1 h, washed, and either lysed immediately or lysed after an incubation period of 1, 15, 24, or 48 h. Control hMDMs and control mouse macrophages were infected with the *dotA* mutant (3). IFN-γ pretreatment of mouse macrophages and hMDMs inhibited intracellular replication of *L. pneumophila* in a dose-dependent manner (Fig. 1). In quiescent mouse and human macrophages, the number of *L. pneumophila* CFU increased by ~10-fold 15 h after infection, while in activated macrophages, bacterial replication was inhibited. The maximum number of bacteria in quiescent macrophages was reached at 48 h after infection. Pretreatment with IFN-γ (1 μg/ml or higher) was essential to achieve maximal inhibition of *L. pneumophila* multiplication within macrophages. Addition of IFN-γ to the macrophages at the time of infection partially reduced intracellular replication (data not shown). Therefore, activation of macrophages by IFN-γ blocks intracellular replication of *L. pneumophila* in human and mouse macrophages, consistent with previous observations.

Previous studies have shown that simultaneous pretreatment of macrophages with IFN-γ and iron for 24 h prior to infection overcomes inhibition of intracellular replication of *L. pneumophila* (8, 35). We thought overloading the cells with iron for such an extended period of time may be nonphysiologic and may interfere with endocytic fusion events. Therefore, we examined whether a supplement of iron (8, 35) at the time of infection or 1 h or 24 h prior to infection would overcome inhibition of intracellular replication in IFN-γ-activated macrophages. Our data showed that the supplement of excess iron 24 h prior to infection abolished the effect of IFN-γ on inhibition of intracellular replication, consistent with previous observations (8, 35) (data not shown). In contrast, supplementation of IFN-γ-activated macrophages with excess iron at the time of infection or 1 h prior to infection did not rescue the inhibition of intracellular replication (data not shown). We conclude that iron supplementation of IFN-γ-activated macrophages does not reverse the ability of activated macrophages to inhibit intracellular replication of *L. pneumophila*.

Alteration of intracellular trafficking of *L. pneumophila* within IFN-γ-activated macrophages. We examined, by confocal laser scanning microscopy, colocalization of the LCP with the late endosomal-lysosomal marker LAMP-2 and the lysosomal acid protease enzyme cathepsin D. For a positive control, we utilized the *dotA* mutant that is defective in the Dot/Icm export machinery, and its phagosome was trafficked through the endosomal-lysosomal degradation pathway (3, 40, 47). The hMDMs were pretreated with IFN-γ for 24 h and then infected by *L. pneumophila* for 1 h. The cells were fixed and processed for confocal microscopy at 1 h postinfection. The data showed that in quiescent hMDMs, LAMP-2 did not colocalize with the LCPs of the wild-type (WT) strain AA100 (Fig. 2 and data not shown). In contrast, 90% of the LCPs within IFN-γ-activated macrophages colocalized with the late endosomal-lysosomal marker LAMP-2 (Fig. 2 and data not shown). Approximately 80% of *dotA* mutant phagosomes acquired LAMP-2, consistent with previous observations (Fig. 2 and data not shown).

While 95% of WT strain AA100 phagosomes excluded the lysosomal acid protease cathepsin D (14) in quiescent hMDMs, 80% of the WT LCPs colocalized with cathepsin D at 1 h postinfection of IFN-γ-activated hMDMs (Fig. 2 and data not shown). For the *dotA* mutant, used as a positive control, 78% of LCPs colocalized with cathepsin D (Fig. 2 and data not shown).

We next examined whether the LCP acquired the lysosomal tracer Trov, which was preloaded into the lysosomes of quiescent and IFN-γ-activated hMDMs, followed by a chase prior to infection. The data showed that in IFN-γ-activated hMDMs, 70% of the LCPs acquired Trov. In contrast, only 12% of the LCPs in quiescent hMDMs acquired Trov (Fig. 2 and data not

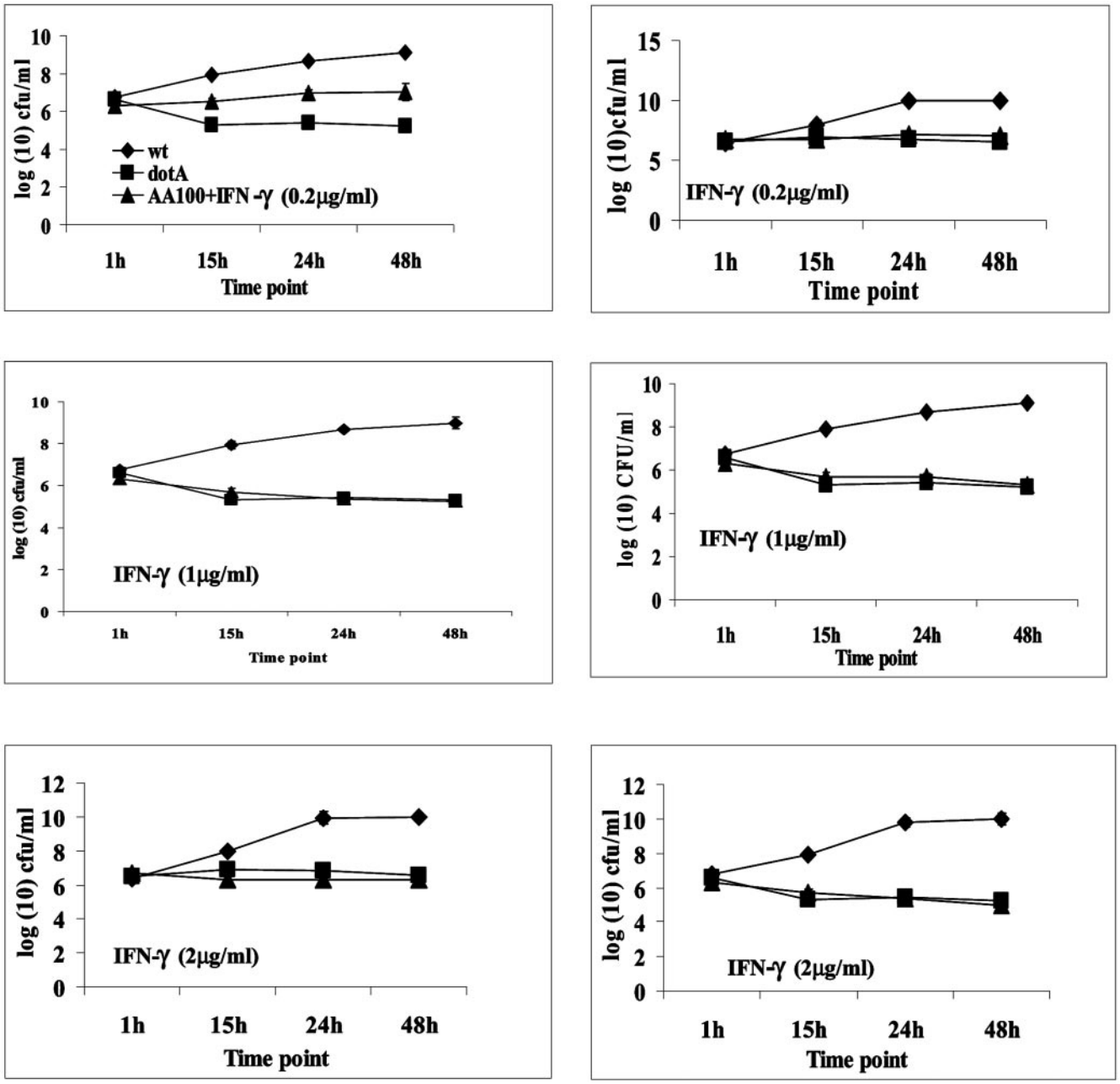

FIG. 1. IFN-γ-activated A/J mouse macrophages (left panels) and hMDMs (right panels) inhibit intracellular replication of *L. pneumophila.* Macrophages were pretreated with the indicated concentrations of IFN-γ. The cells were infected for 1 h, followed by a wash of extracellular bacteria, and a determination was made of the number of intracellular bacteria at the indicated time points. The *dotA* mutant was used as a control for a replication-defective mutant. The error bars represent standard deviations of triplicate samples, and the results shown are representative of three independent experiments.

shown). More than 70% of the phagosomes harboring paraformaldehyde-killed bacteria, used as a positive control, acquired Trov. Considering these findings, we conclude that the LCP is trafficked through the endosomal-lysosomal degradation pathway within IFN-γ-activated macrophages.

Effect of IFN-γ on remodeling the LCP into an RER-derived phagosome. We examined whether the *L. pneumophila* phagosome within IFN-γ-activated hMDMs colocalized with the RER. Confocal microscopy was used to examine colocalization of the LCP with the endoplasmic reticulum (ER) marker KDEL by the use of specific antibodies. The *dotA* mutant was used as a negative control, since it fails to recruit the ER. Within quiescent hMDMs, 95% of the LCPs colocalized with the ER marker KDEL. In contrast, only 19% of the LCPs colocalized with the ER within IFN-γ-activated hMDMs. Similarly, less then 20% of *dotA* mutant phagosomes colocalized

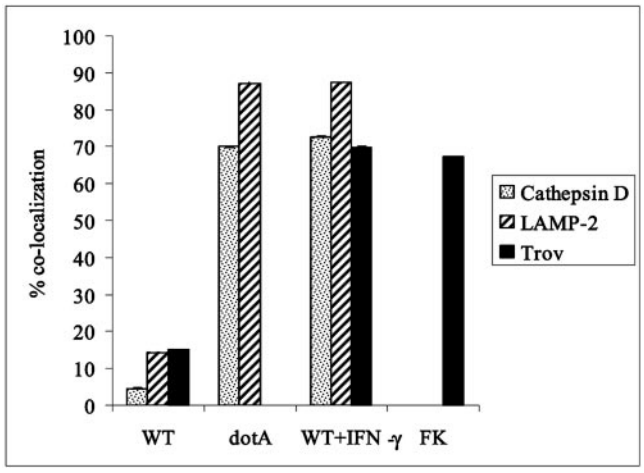

FIG. 2. Quantitation of acquisition of late endosomal and lysosomal markers by the LCP within quiescent and IFN-γ-activated hMDMs. FK, Formalin-killed *L. pneumophila*. At least 100 infected cells from multiple coverslips were examined in each experiment. Results are representative of three independent experiments performed in triplicate. Some of the error bars are too small to be displayed.

with the ER (Fig. 3 and data not shown). These results were confirmed by transmission electron microscopy (Fig. 3 and data not shown). We conclude that failure of *L. pneumophila* to evade endocytic fusion in IFN-γ-activated macrophages was associated with the failure to remodel its phagosome by the RER.

There are several potential mechanisms by which IFN-γ-activated macrophages inhibit the intracellular replication of *L. pneumophila*, such as activation of reactive oxygen intermediates and reactive nitrogen intermediates (7, 20, 21, 43), restriction of iron availability (8–10, 19, 21), and down-regulation of the transferrin receptors (8). However, in our current studies, when excess iron is added at 1 h prior to infection of IFN-γ-activated macrophages, the effect on inhibition of intracellular replication is not reversed by the presence of excess iron. Our data show alteration in trafficking of *L. pneumophila* in IFN-γ-activated macrophages where the ability of the organism to modulate the biogenesis of its phagosome into an RER-derived replicative niche is completely abolished. Instead, the phagosome harboring the organism matures into a phagolysosome within IFN-γ-activated macrophages. It is possible that the targeting of *L. pneumophila* into the phagolyso-

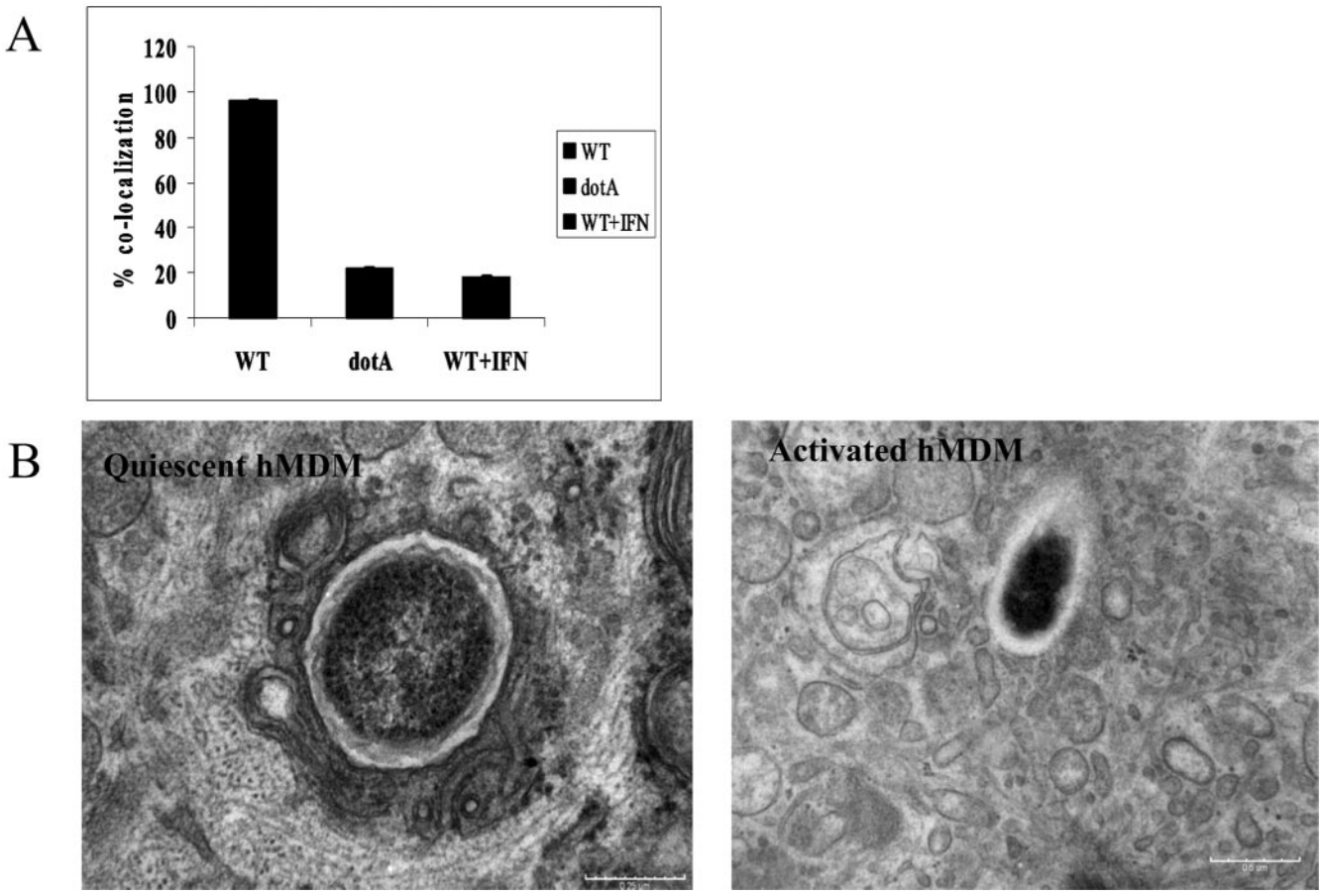

FIG. 3. Failure of the LCP to be remodeled by the ER in activated macrophages. A) Quantification of the acquisition of the KDEL marker was determined by confocal microscopy at 4 h postinfection. At least 100 infected cells from multiple coverslips were examined in each experiment. Results are representative of three independent experiments performed in triplicate. Some of the error bars are too small to be displayed. B) Representative transmission electron micrographs of remodeling of the LCP by the RER in quiescent but not IFN-γ-activated hMDMs at 4 h postinfection.

somes is a primary effect of activation of macrophages by IFN-γ. We speculate that multiple processes are triggered within IFN-γ-activated macrophages to restrict intracellular replication of *L. pneumophila*, and some of these processes lead to alteration in trafficking of the organism. It is also likely that these processes may be distinct between human and mouse macrophages, particularly the generation of reactive nitrogen intermediates.

In summary, our data clearly show that the ability of *L. pneumophila* to evade endocytic fusion and to remodel the LCP into an ER-derived vesicle is completely inhibited in IFN-γ-activated macrophages, similar to that of the *dot/icm* mutants. Within IFN-γ-activated macrophages, the LCPs are processed through the "default" endosomal-lysosomal degradation pathway similar to the pathway of *dot/icm* mutants and inert particles as well.

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REFERENCES

1. **Abu Kwaik, Y., L.-Y. Gao, B. J. Stone, and O. S. Harb.** 1998. Invasion of mammalian and protozoan cells by *Legionella pneumophila.* Bull. Inst. Pasteur **96:**235–245.
2. **Alli, O. A. T., L.-Y. Gao, L. L. Pedersen, S. Zink, M. Radulic, M. Doric, and Y. Abu Kwaik.** 2000. Temporal pore formation-mediated egress from macrophages and alveolar epithelial cells by *Legionella pneumophila.* Infect. Immun. **68:**6431–6440.
3. **Berger, K. H., and R. R. Isberg.** 1993. Two distinct defects in intracellular growth complemented by a single genetic locus in *Legionella pneumophila.* Mol. Microbiol. **7:**7–19.
4. **Bhardwaj, N., T. W. Nash, and M. A. Horwitz.** 1986. Interferon-gamma-activated human monocytes inhibit the intracellular multiplication of Legionella pneumophila. J. Immunol. **137:**2662–2669.
5. **Bitar, D. M., M. Molmeret, and Y. A. Kwaik.** 2005. Structure-function analysis of the C-terminus of IcmT of Legionella pneumophila in pore formation-mediated egress from macrophages. FEMS Microbiol. Lett. **242:**177–184.
6. **Blasi, E., B. J. Mathieson, L. Varesio, J. L. Cleveland, P. A. Borchert, and U. R. Rapp.** 1985. Selective immortalization of murine macrophages from fresh bone marrow by a raf/myc recombinant murine retrovirus. Nature **318:**667–670.
7. **Brieland, J. K., D. G. Remick, P. T. Freeman, M. C. Hurley, J. C. Fantone, and N. C. Engleberg.** 1995. In vivo regulation of replicative *Legionella pneumophila* lung infection by endogenous tumor necrosis factor alpha and nitric oxide. Infect. Immun. **63:**3253–3258.
8. **Byrd, T. F., and M. A. Horwitz.** 1989. Interferon gamma-activated human monocytes downregulate transferrin receptors and inhibit the intracellular multiplication of *Legionella pneumophila* by limiting the availability of iron. J. Clin. Investig. **83:**1457–1465.
9. **Byrd, T. F., and M. A. Horwitz.** 1991. Lactoferrin inhibits or promotes *Legionella pneumophila* intracellular multiplication in nonactivated and interferon gamma-activated human monocytes depending upon its degree of iron saturation. Iron-lactoferrin and nonphysiologic iron chelates reverse monocyte activation against *Legionella pneumophila.* J. Clin. Investig. **88:** 1103–1112.
10. **Byrd, T. F., and M. A. Horwitz.** 1993. Regulation of transferrin receptor expression and ferritin content in human mononuclear phagocytes. Coordinate upregulation by iron transferrin and downregulation by interferon gamma. J. Clin. Investig. **91:**969–976.
11. **Clemens, D. L.** 1996. Characterization of the *Mycobacterium tuberculosis* phagosome. Trends Microbiol. **4:**113–118.
12. **Clemons-Miller, A. R., G. W. Cox, J. Suttles, and R. D. Stout.** 2000. LPS stimulation of TNF-receptor deficient macrophages: a differential role for TNF-alpha autocrine signaling in the induction of cytokine and nitric oxide production. Immunobiology **202:**477–492.
13. **Dai, W. J., W. Bartens, G. Kohler, M. Hufnagel, M. Kopf, and F. Brombacher.** 1997. Impaired macrophage listericidal and cytokine activities are responsible for the rapid death of Listeria monocytogenes-infected IFN-gamma receptor-deficient mice. J. Immunol. **158:**5297–5304.
14. **Diment, S., M. S. Leech, and P. D. Stahl.** 1988. Cathepsin D is membrane-associated in macrophage endosomes. J. Biol. Chem. **263:**6901–6907.
15. **Gao, L.-Y., and Y. Abu Kwaik.** 1999. Activation of caspase 3 during *Legionella pneumophila*-induced apoptosis. Infect. Immun. **67:**4886–4894.
16. **Gao, L.-Y., and Y. Abu Kwaik.** 1999. Apoptosis in macrophages and alveolar epithelial cells during early stages of infection by *Legionella pneumophila* and its role in cytopathogenicity. Infect. Immun. **67:**862–870.
17. **Gao, L.-Y., and Y. Abu Kwaik.** 2000. The mechanism of killing and exiting the protozoan host *Acanthamoeba polyphaga* by *Legionella pneumophila.* Environ. Microbiol. **2:**79–90.
18. **Gao, L.-Y., M. Susa, B. Ticac, and Y. Abu Kwaik.** 1999. Heterogeneity in intracellular replication and cytopathogenicity of *Legionella pneumophila* and *Legionella micdadei* in mammalian and protozoan cells. Microb. Pathog. **27:**273–287.
19. **Gebran, S. J., C. Newton, Y. Yamamoto, R. Widen, T. W. Klein, and H. Friedman.** 1994. Macrophage permissiveness for *Legionella pneumophila* growth modulated by iron. Infect. Immun. **62:**564–568.
20. **Gebran, S. J., Y. Yamamoto, C. Newton, T. W. Klein, and H. Friedman.** 1994. Inhibition of *Legionella pneumophila* growth by gamma interferon in permissive A/J mouse macrophages: role of reactive oxygen species, nitric oxide, tryptophan, and iron(III). Infect. Immun. **62:**3197–3205.
21. **Heath, L., C. Chrisp, G. Huffnagle, M. LeGendre, Y. Osawa, M. Hurley, C. Engleberg, J. Fantone, and J. Brieland.** 1996. Effector mechanisms responsible for gamma interferon-mediated host resistance to *Legionella pneumophila* lung infection: the role of endogenous nitric oxide differs in susceptible and resistant murine hosts. Infect. Immun. **64:**5151–5160.
22. **Horwitz, M. A.** 1983. Formation of a novel phagosome by the Legionnaires' disease bacterium (*Legionella pneumophila*) in human monocytes. J. Exp. Med. **158:**1319–1331.
23. **Horwitz, M. A.** 1983. The Legionnaires' disease bacterium (*Legionella pneumophila*) inhibits phagosome-lysosome fusion in human monocytes. J. Exp. Med. **158:**2108–2126.
24. **Horwitz, M. A., and S. C. Silverstein.** 1981. Activated human monocytes inhibit the intracellular multiplication of Legionnaires' disease bacteria. J. Exp. Med. **154:**1618–1635.
25. **Horwitz, M. A., and S. C. Silverstein.** 1980. Legionnaires' disease bacterium (*Legionella pneumophila*) multiples intracellularly in human monocytes. J. Clin. Investig. **66:**441–450.
26. **Kagan, J. C., and C. R. Roy.** 2002. Legionella phagosomes intercept vesicular traffic from endoplasmic reticulum exit sites. Nat. Cell Biol. **4:**945–954.
27. **Klein, T. W., Y. Yamamoto, H. K. Brown, and H. Friedman.** 1991. Interferon-gamma induced resistance to Legionella pneumophila in susceptible A/J mouse macrophages. J. Leukoc. Biol. **49:**98–103.
28. **Molmeret, M., and Y. Abu Kwaik.** 2002. How does Legionella pneumophila exit the host cell? Trends Microbiol. **10:**258–260.
29. **Molmeret, M., O. A. T. Alli, M. Radulic, M. Susa, M. Doric, and Y. Abu Kwaik.** 2002. The C-terminus of IcmT is essential for pore formation and for intracellular trafficking of *Legionella pneumophila* within *Acanthamoeba polyphaga.* Mol. Microbiol. **43:**1139–1150.
30. **Molmeret, M., O. A. T. Alli, S. Zink, A. Flieger, N. P. Cianciotto, and Y. Abu Kwaik.** 2002. *icmT* is essential for pore formation-mediated egress of *Legionella pneumophila* from mammalian and protozoan cells. Infect. Immun. **70:**69–78.
31. **Molmeret, M., D. M. Bitar, L. Han, and Y. Abu Kwaik.** 2004. Disruption of the phagosomal membrane and egress of *Legionella pneumophila* into the cytoplasm during last stages of intracellular infection of macrophages and *Acanthamoeba polyphaga.* Infect. Immun. **72:**4040–4051.
32. **Molmeret, M., D. M. Bitar, L. Han, and Y. A. Kwaik.** 2004. Cell biology of the intracellular infection by Legionella pneumophila. Microbes Infect. **6:**129–139.
33. **Molmeret, M., M. Horn, M. Wagner, M. Santic, and Y. Abu Kwaik.** 2005. Amoebae as training grounds for intracellular bacterial pathogens. Appl. Environ. Microbiol. **71:**20–28.
34. **Murray, H. W., A. M. Granger, and R. F. Teitelbaum.** 1991. Gamma interferon-activated human macrophages and *Toxoplasma gondii*, *Chlamydia psittaci*, and *Leishmania donovani*: antimicrobial role of limiting intracellular iron. Infect. Immun. **59:**4684–4686.
35. **Nash, T. W., D. M. Libby, and M. A. Horwitz.** 1988. IFN-gamma-activated human alveolar macrophages inhibit the intracellular multiplication of *Legionella pneumophila.* J. Immunol. **140:**3978–3981.
36. **Nash, T. W., D. M. Libby, and M. A. Horwitz.** 1984. Interaction between the legionnaires' disease bacterium (*Legionella pneumophila*) and human alveolar macrophages. Influence of antibody, lymphokines, and hydrocortisone. J. Clin. Investig. **74:**771–782.
37. **Pedersen, L. L., M. Radulic, M. Doric, and Y. Abu Kwaik.** 2001. HtrA homologue of *Legionella pneumophila*: an indispensable element for intracellular infection of mammalian but not protozoan cells. Infect Immun. **69:**2569–2579.
38. **Roy, C. R., K. H. Berger, and R. R. Isberg.** 1998. *Legionella pneumophila* DotA protein is required for early phagosome trafficking decisions that occur within minutes of bacterial uptake. Mol. Microbiol. **28:**663–674.

38a. **Santic, M., M. Molmeret, and Y. Abu Kwaik.** Modulation of biogenesis of the *Francisella tularensis* subsp. *novicida*-containing phagosome in quiescent human macrophages and its maturation into a phagolysosome upon activation by IFN-γ. Cell. Microbiol., in press.

39. **Schaible, U. E., S. Sturgill-Koszycki, P. H. Schlesinger, and D. G. Russell.** 1998. Cytokine activation leads to acidification and increases maturation of

Mycobacterium avium-containing phagosomes in murine macrophages. J. Immunol. **160:**1290–1296.
40. **Segal, G., M. Purcell, and H. A. Shuman.** 1998. Host cell killing and bacterial conjugation require overlapping sets of genes within a 22-kb region of the *Legionella pneumophila* chromosome. Proc. Natl. Acad. Sci. USA **95:**1669–1674.
41. **Shinozawa, Y., T. Matsumoto, K. Uchida, S. Tsujimoto, Y. Iwakura, and K. Yamaguchi.** 2002. Role of interferon-gamma in inflammatory responses in murine respiratory infection with Legionella pneumophila. J. Med. Microbiol. **51:**225–230.
42. **Skerrett, S. J., and T. R. Martin.** 1991. Alveolar macrophage activation in experimental legionellosis. J. Immunol. **147:**337–345.
43. **Skerrett, S. J., and T. R. Martin.** 1996. Roles for tumor necrosis factor alpha and nitric oxide in resistance of rat alveolar macrophages to *Legionella pneumophila*. Infect. Immun. **64:**3236–3243.
44. **Swanson, M. S., and B. K. Hammer.** 2000. Legionella pneumophila pathogenesis: a fateful journey from amoebae to macrophages. Annu. Rev. Microbiol. **54:**567–613.
45. **Swanson, M. S., and R. R. Isberg.** 1995. Association of *Legionella pneumophila* with the macrophage endoplasmic reticulum. Infect. Immun. **63:**3609–3620.
46. **Tilney, L. G., O. S. Harb, P. S. Connelly, C. G. Robinson, and C. R. Roy.** 2001. How the parasitic bacterium Legionella pneumophila modifies its phagosome and transforms it into rough ER: implications for conversion of plasma membrane to the ER membrane. J. Cell Sci. **114:**4637–4650.
47. **Vogel, J. P., H. L. Andrews, S. K. Wong, and R. R. Isberg.** 1998. Conjugative transfer by the virulence system of *Legionella pneumophila.* Science **279:**873–876.
48. **Vogel, J. P., and R. R. Isberg.** 1999. Cell biology of Legionella pneumophila. Curr. Opin. Microbiol. **2:**30–34.
49. **Welsh, C. T., J. T. Summersgill, and R. D. Miller.** 2004. Increases in c-Jun N-terminal kinase/stress-activated protein kinase and p38 activity in monocyte-derived macrophages following the uptake of *Legionella pneumophila*. Infect. Immun. **72:**1512–1518.
50. **Zink, S. D., L. Pedersen, N. P. Cianciotto, and Y. Abu Kwaik.** 2002. The Dot/Icm type IV secretion system of *Legionella pneumophila* is essential for the induction of apoptosis in human macrophages. Infect. Immun. **70:**1657–1663.

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