Listeria monocytogenes Infection Induces Prosurvival Metabolic Signaling in Macrophages

Tie Zou, Oleg Garifulin, Robert Berland, and Victor L. Boyartchuk*  
Program in Gene Function and Expression and Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, Massachusetts 01605

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Host cells use metabolic signaling through the LXRα nuclear receptor to defend against Listeria monocytogenes infection. 25-Hydroxycholesterol is a natural ligand of LXRs that is produced by the enzyme cholesterol 25-hydroxylase (CH25H). We found that expression of Ch25h is upregulated following L. monocytogenes infection in a beta interferon (IFN-β)-dependent fashion. Moreover, increased Ch25h expression promotes survival of L. monocytogenes-infected cells and increases sensitivity of the host to infection. We determined that expression of Cd5l, a prosurvival gene, is controlled by CH25H. In addition, we found that Cd5l inhibits activation of caspase-1, promoting survival of infected macrophages. Our results reveal a mechanism by which an intracellular pathogen can prolong survival of infected cells, thus providing itself with a protected environment in which to replicate.

Signaling through liver X receptors (LXRs), members of the nuclear receptor family of transcription factors, maintains lipid homeostasis of mammalian cells (20, 46, 51). In macrophages, LXRs activate genes involved in the transport of cholesterol out of the cell and loading it onto carrier proteins for transport to the liver (11). There are two LXR isoforms, LXRα and LXRβ, encoded by two different genes (41, 48). Recently it has become apparent that LXR metabolic signaling also plays an important role in defense against bacterial infections (23, 51). Notably, the absence of LXRα, but not LXRβ, dramatically increases susceptibility of mice to L. monocytogenes infection (22). The Cd5l prosurvival gene has been identified as the downstream target of LXRα that is primarily responsible for control of host resistance to infection and atherosclerosis development (2, 32).

Oxidized intermediates of cholesterol metabolism, such as 25-hydroxycholesterol (25-HC), are natural endogenous ligands for LXRs (1, 12, 17, 21, 25). 25-HC is produced from cholesterol primarily by the action of cholesterol 25-hydroxylase (CH25H), which is the main enzyme in the cell that produces 25-HC. The ability to produce 25-HC from cholesterol was also reported for sterol 27-hydroxylase (27), a mitochondrial P450 enzyme, and cholesterol 24-hydroxylase (28).

25-HC is an important regulatory molecule that is also a substrate in bile synthesis. Its best-understood function is potent suppression of cholesterol biosynthesis (6, 24, 29). 25-HC inhibits the cleavage of sterol regulatory element binding proteins (SREBPs) (24) that control levels of key enzymes in cholesterol synthesis, such as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and HMG-CoA synthase. In addition to controlling lipid homeostasis through inhibition of SREBPs, 25-HC acts as a natural endogenous ligand of several orphan nuclear receptors, including Nr5a1 (SF-1) and LXRs (25). Notably, activation of LXRs leads to upregulation of metabolic genes, such as the ABCA1 pump, and of the prosurvival genes Cd5l, Bcl-xl, and Birc1a (45). ABCA1 is capable of removing both cholesterol and 25-HC from the cell, providing an important negative feedback control of intracellular 25-HC activity.

The role of 25-HC in immune function is still poorly understood. Nevertheless, recent studies found that Ch25h mRNA is highly induced by treatment of bone marrow-derived macrophages (BMMs) with lipopolysaccharide (LPS) (15). It has also been shown that exposure of naïve B cells to nanomolar concentrations of 25-HC inhibited production of IgA by reducing B cell proliferation and class switch recombination (4). Our results presented below provide further evidence of the link between innate immune function and 25-HC biosynthesis in macrophages.

Upon infection with L. monocytogenes, macrophages detect bacterial cyclic di-AMP using an as-yet-unknown cytoplasmic receptor and activate IRF3 to rapidly induce type I interferons (IFNs) (42, 44, 49). This induction has a detrimental effect on production of type I interferons (IFNs) (42, 44, 49). This induction has a detrimental effect on resistance of the organism to infection (3, 33, 43, 54). This is in part because type I interferons sensitize lymphocytes to infection-induced death, which, in turn, suppresses innate immune responses (9). Deletion of lymphocyte proapoptotic factors such as TRAIL or inhibition of lymphocyte formation by scid or rag mutations results in a significant increase in early resistance of animals to L. monocytogenes infection (8, 53). Therefore, control of lymphocyte cell death is an important aspect of L. monocytogenes infection.

Bacterial pathogens overcome host immune defenses by either destroying host immune cells or using them to provide a protected environment. L. monocytogenes uses cytoplasmic replication as a strategy to avoid detection and destruction by host immune cells (13, 14, 18). Therefore, death of invaded host cells might promote resistance of the organism to infection with L. monocytogenes. This is especially the case for death by pyroptosis, a proinflammatory process in which self-destruct-
tion not only exposes pathogens to immune surveillance but also generates inflammatory signals to stimulate immune defenses (5, 16). The ability of cytoplasmic pathogens such as L. monocytogenes to prevent pyroptosis of host cells would allow for extended intracellular replication and benefit the pathogen. Our results describe a novel mechanism by which L. monocytogenes may achieve this goal.

**MATERIALS AND METHODS**

Reagents and plasmids. 25-Hydroxycholesterol (25-HC), T909317, and 9-cis-retinoic acid were purchased from Sigma-Aldrich. The Ch25h expression vector was constructed by cloning the full-length C57BL/6J mouse cDNA in the pCS2 expression plasmid. The lentiviral construct (pGIPZ) carrying short hairpin RNA (shRNA) against C57BL/6J mouse Ch25h was obtained from Open Bio-systems.

Animals. Female mice, 10 to 14 weeks old, were used in all experiments. All mice were bred and maintained under specific-pathogen-free conditions in the animal facilities at the University of Massachusetts Medical School. All experiments involving live animals were carried out in accordance with the guidelines set forth by the University of Massachusetts Medical School Department of Animal Medicine and the Institute Animal Care and Use Committee.

**Transient transfections and infections.** Mice were injected in the tail vein with 1.5 ml Dulbecco’s phosphate-buffered saline (D-PBS) solution containing 5 μg of pCS2-Ch25h or Ch25h frameshift mutant expression construct. After 48 h, mice were infected intravenously with 5 × 10⁵ L. monocytogenes strain 10432a in 0.3 ml of PBS. After 24 h, infected animals were sacrificed by CO₂ asphyxiation. Harvested livers and spleens were weighed and homogenized in 0.02% Triton X-100. Aliquots of serial 5-fold dilutions in sterile water were plated in duplicate on tryptic soy broth (TSB) agar plates containing 10 μg/ml streptomycin. The number of bacteria per milliliter of tissue was determined by counting colonies at the appropriate dilution.

All cell lines and primary macrophages were infected ex vivo at a multiplicity of infection (MOI) of 5.

**Cell culture and generation of stable cell lines.** The macrophage-like ZBM2 cell line was generated from C57BL/6J bone marrow macrophages as previously described (34). ZBM2 cells and primary bone marrow macrophages were cultured in Dulbecco modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) (HyClone) and 10% L-cell-conditioned medium (LCCM). To produce recombinant lentiviruses, subconfluent HEK293T cells were cotransfected with 1.5 μg shRNA plasmid, 6 μg pMD2G, and 10 μg pCMV-Δ.E3 used for Fugene HD (Roche). At 72 h after transfection, the medium containing lentiviral particles was collected and stored at −80°C.

Stable cell lines producing Ch25h shRNA were generated by transduction of ZBM2 cells with lentiviral particles containing Ch25h shRNA or control shRNA sequence. Individual clones were isolated following selection with 2 μg/ml puromycin. The efficiency of knockdown was determined by quantitative reverse transcription PCR (qRT-PCR) analysis.

Stable cell lines expressing full-length Ch25h cDNA were generated by transfection of ZBM2 cells with pCS2-Ch25h plasmid using Fugene HD. Individual clones were selected as described above. Levels of Ch25h expression were analyzed by qRT-PCR.

**Cell viability assays.** Cell viability was assessed using Trypan Blue dye exclusion assay (Promega). ZBM2 cells (4 × 10⁵) were seeded in 96-well tissue culture plates, and following overnight incubation, the cells were infected with L. monocytogenes at defined MOIs. After 1 h, 10 μg/ml of gentamicin was added to kill extracellular bacteria. At defined time points, cellular viability assays were performed according to the manufacturer’s protocol. The relative cell viability was calculated as follows: % of relative cellular viability = (ODc × 100)/ODc, where ODc means the experimental absorbance and OΔc means the absorbance of untreated control. The means and standard deviations (SD) were calculated based on results from three independent experiments.

The terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end-labeling (TUNEL) staining assay was carried out using the In Situ Cell Death Detection kit (Roche). The percentage of TUNEL-positive cells was calculated as follows: % of TUNEL-positive cells = number of TUNEL-positive cells × 100/total number of DAPI-positive cells. The means and standard deviations were calculated based on results from three independent experiments.

**RNA isolation and real-time PCR.** Total RNA was isolated from cultured cells or tissue using Trizol reagent according to the manufacturer’s instructions. Relative mRNA levels were quantified by qRT-PCR on an ABI 7300 real-time PCR system utilizing SYBR green chemistry. Ribosomal protein s17 (Rps17) was used as a loading control. The following primers were used: Ch25h-F, 5’-GAC CTT CTG CAG GTT GA-3’; Ch25h-R, 5’-CCA CGG ACA GCC AGA TTA-3’; Cd5l-F, 5’-CCT TCG TGT TGG CTT TTG GA-3’; Cd5l-R, 5’-GTG TCT CTT CCC ACC AGC TT-3’; Abca1-F, 5’-GAG CAT CTT GGA CTC TT-3’; Abca1-R, 5’-GGG CAC GAC GAC ATC TT-3’; Rps17-F, 5’-TGG CAG GAT CCA CCT AA-3’; Rps17-R, 5’-GCC CATT TAT CCC CAG CAA G-3’. Each experiment included at least two biological and three experimental replicates.

**Western blot assays.** Combined cell and supernatant lysates were separated on a 10% acrylamide gel, transferred on polyvinylidene difluoride (PVDF) membranes, and probed with primary antibodies against caspase-1 p10 (1:200 dilution, SC 514, Santa Cruz), followed by appropriate secondary HRP-conjugated antibodies. In all cases, the blots were stripped and reprobed with anti-tubulin antibody (EMD).

**Statistical analysis.** All data, expressed as means ± SD, were analyzed with the Student’s t test. Differences were considered statistically significant at P < 0.05.

**RESULTS**

**Ch25h is an IFN-β-inducible gene.** Type I interferons have pleiotropic effects, as evidenced by their ability to induce the expression of a large number of genes (26). Existing inbred mouse strains differ in their susceptibilities to L. monocytogenes and in their abilities to induce IFN-β in response to infection (19). To gain insight into the function of IFN-β in the course of L. monocytogenes infection, we examined differences in infection-induced gene expression in macrophages from several inbred mouse strains. Using this approach, we identified the Ch25h gene as a candidate IFN-β-inducible gene that affects the response to L. monocytogenes.

Basal Ch25h mRNA expression was highest in macrophages from the BALB/cByJ mouse strain (Fig. 1A). BALB/c mice are very sensitive to L. monocytogenes infection and exhibit uniform mortality after intravenous infection with as little as 1 × 10⁶ CFU, whereas all infected C57BL/6J mice survive this dose. Mouse strains that have either a mutation in Ifr3 (C57BL/6ByJ) or a deletion of Ifr3 (C57BL/6J Ifr3⁻/⁻) are hyperresistant and can survive infection with as many as 1 × 10⁸ L. monocytogenes cells. Macrophages from all C57BL/6 lines had similar basal Ch25h mRNA levels. However, at 2 h postinfection, Ch25h expression began to increase in cells from C57BL/6J mice but not in cells from C57BL/6ByJ or C57BL/6J Ifr3⁻/⁻ mice. By 4 h postinfection, Ch25h levels had increased 3.5-fold in C57BL/6J macrophages and 2-fold in BALB/cByJ macrophages. There was no detectable increase in macrophages from C57BL/6ByJ and C57BL/6J Ifr3⁻/⁻ mice. This experiment revealed that basal levels of Ch25h RNA are inversely correlated with resistance to L. monocytogenes. Furthermore, Ch25h expression is induced in response to infection, and this induction is dependent on IFR3.

The dependence of Ch25h induction on IFR3 suggested that it is an IFN-β-inducible gene. To test this, we treated macrophages from BALB/cByJ, C57BL/6J, and C57BL/6ByJ mice with recombinant IFN-β for up to 12 h. We used cells from C57BL/6J Ifnar⁻/⁻ mice which lack the IFN-I receptor as a negative control. We found that Ch25h was significantly up-regulated in macrophages that have an intact type 1 interferon signaling cascade (Fig. 1B) whereas there was no induction in cells from C57BL/6J Ifnar⁻/⁻ mice. Together, these observations demonstrate that Ch25h expression is induced by L. monocytogenes and this induction is mediated by type 1 interferons such as IFN-β. A recent study of macrophage and den-
dritic cell (DC) response to Toll-like receptor (TLR) ligands provides an independent confirmation of our results (35).

**Ch25h affects L. monocytogenes replication in infected macrophages.** To investigate the role of Ch25h in macrophage function, we generated a series of mouse macrophage-like cell lines that mimic the differences in Ch25h expression observed in the inbred strains of mice. To elevate Ch25h mRNA levels, we stably introduced a C57BL/6 Ch25h cDNA under the control of the constitutive cytomegalovirus (CMV) promoter into the C57BL/6J-derived ZBM2 macrophage cell line. To reduce Ch25h expression levels, we stably introduced Ch25h shRNA (Open Biosystems). qRT-PCR analysis of two independent overexpressing cell lines (hiC25-1 and hiC25-3) revealed a 5- to 10-fold increase in Ch25h expression in these lines compared to that in vector-transfected cells (Fig. 1C). In two independent Ch25h knockdown cell lines (shC25-B, shC25H-D) Ch25h mRNA levels were reduced to ~20% of levels in cells transduced with lentivirus expressing scrambled control shRNA (Fig. 1E).

![Graphs showing Ch25h expression and L. monocytogenes replication](http://iai.asm.org/)

**FIG. 1.** Levels of Ch25h expression impact the course of L. monocytogenes infection. Differences in Ch25h expression observed in vivo are sufficient to alter the course of bacterial infection of macrophages. (A) Mouse strain-specific differences in Ch25h expression following L. monocytogenes infection of bone marrow-derived macrophages (BMMs) from sensitive BALB/cByJ, resistant C57BL/6J, and superresistant C57BL/6ByJ and C57BL/6J IRF3−/− mice (n = 3 ± SD). (B) Treatment of BMMs with 100 U/ml of recombinant IFN-β leads to rapid induction of Ch25h expression in BMMs from all strains except those lacking the alpha/beta interferon receptor (Ifnar−/−) (n = 3 ± SD). (C and E) C57BL/6J-derived ZBM2 macrophage-like cell lines that express either Ch25h cDNA (C) or Ch25h shRNA (E) model differences in Ch25h expression found in inbred mouse strains. By 12 h following infection of these cell lines with L. monocytogenes (MOI, 5) there were significant differences in the numbers of viable intracellular bacteria (D and F). Lower Ch25h mRNA levels corresponded to a lower number of recovered bacteria (D), while a higher level of expression of Ch25h led to an increase in the number of recovered bacteria (F). (n = 3 ± SD, representative of results of three independent experiments. NS, no significant difference. *, P < 0.05; **, P < 0.01.)
To elucidate the role of \textit{Ch25h} in control of \textit{L. monocytogenes} infection we used our cell lines to determine the number of viable intracellular bacteria at various times after infection. Knockdown, overexpression, and respective control cell lines were infected with \textit{L. monocytogenes} at an MOI of 5. At early (4- and 8-h) time points there was no significant difference in recovered bacterial numbers among all cell lines examined (Fig. 1D and F). This observation indicates that there is no effect of \textit{Ch25h} expression levels on the uptake of \textit{L. monocytogenes} by macrophages. At 12-h and 24-h time points there were significant differences in numbers of recovered bacteria that correlated with \textit{Ch25h} expression levels. Cells that had higher levels of \textit{Ch25h} had more bacteria than the vector-transfected controls (Fig. 1D). Conversely, there were fewer \textit{L. monocytogenes} cells recovered from cell lines with reduced \textit{Ch25h} mRNA levels (Fig. 1F). Therefore, we conclude that changes in \textit{Ch25h} expression levels affect the course of \textit{L. monocytogenes} infection of macrophages.

\textbf{Ch25h mRNA levels modulate survival of infected macrophages.} Our cell culture assay of \textit{L. monocytogenes} infection measures numbers of intracellular bacteria that are protected from gentamicin in the medium. Host cells that die as a result of infection expose intracellular bacteria to the antibiotic. Therefore, differences in the number of recovered bacteria can reflect either a difference in the rate of bacterial replication or differences in the viability of host cells. To differentiate between these alternative explanations, we analyzed the viability of our cell lines following \textit{L. monocytogenes} infection. We used a CellTiter-Fluor assay to establish a direct correlation between the levels of \textit{Ch25h} expression and the viability of infected macrophages.

\begin{figure}[h]
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\caption{Increase in \textit{Ch25h} expression promotes survival of \textit{L. monocytogenes}-infected macrophages. Macrophages that express higher levels of \textit{Ch25h} (A) have improved survival after 12 h of \textit{L. monocytogenes} infection (MOI, 5) as measured by the CellTiter-Fluor assay. Reduction in \textit{Ch25h} expression leads to an increase in infection-induced death (B). The extent of infection-induced DNA fragmentation was evaluated by TUNEL staining (C). Cells that overexpress \textit{Ch25h} (HiC25-1) have fewer TUNEL-positive cells than the control cell lines or cell lines that express \textit{Ch25h} shRNA (shC25-B). The ratio of TUNEL-positive to DAPI-stained cells (D and E) was determined by counting 200 DAPI-positive cells in each group. Objective magnification is 100\times. (\textit{n} = 3 \pm SD, representative of results of three independent experiments. *, \textit{P} < 0.05; **, \textit{P} < 0.01.)}
\end{figure}
fected cells. This assay is not affected by the presence of intracellular bacteria since it measures a conserved and constitutive protease activity present within live mammalian cells. We found that cell lines expressing high levels of Ch25h mRNA had a higher rate of survival than the control cell line (Fig. 2A). Furthermore, the lines carrying Ch25h shRNA were very sensitive to L. monocytogenes infection (Fig. 2B). This indicates that an increase in Ch25h mRNA levels promotes survival of infected macrophages. This conclusion is further supported by our analysis of infection-induced DNA fragmentation that is indicative of either apoptotic or pyroptotic cell death. TUNEL staining of infected cells revealed that an increase in Ch25h levels resulted in a significant reduction in the number of TUNEL-positive cells from 17% in control cell lines to 7% as early as 8 h after infection (Fig. 2C and D). Conversely, in the shC25-B cell line that carried Ch25h shRNA there were significantly more (24% versus 12% in vector controls) TUNEL-positive cells at the 8-h time point (Fig. 2C and E). Significant differences in the number of TUNEL-positive cells were maintained throughout the 12-h course of infection examined. At the 12-h time point Ch25h overexpression reduced by more than 2-fold the percentage of TUNEL-positive cells, from the 40% found in vector controls to 15%, while reduction in Ch25h expression led to an increase in the percentage of TUNEL-positive cells to 69%. These results establish that changes in Ch25h expression modulate survival of infected cells.

Increased Ch25h expression leads to increased susceptibility to L. monocytogenes infection in vivo. To address the role of changes in Ch25h expression in the resistance of the whole organism to L. monocytogenes infection, we used transient transgenesis to overexpress Ch25h in mice. This approach uses hydrodynamic delivery of recombinant DNA mainly to liver and spleen cells, which are the primary sites of L. monocytogenes infection (7, 52). We compared the effect of injection of a Ch25h cDNA expression construct to that of the same construct containing a frameshift mutation. In five independent experiments we found that a transient increase in Ch25h ex-

FIG. 3. Elevated levels of Ch25h increases susceptibility to L. monocytogenes infection in vivo. Hydrodynamic delivery of the pCS2-CH25H construct led to a 6-fold increase in Ch25h expression in livers (A) and a 3-fold increase in spleens (C) of C57BL/6J mice 48 h after plasmid injection. At 24 h after infection with L. monocytogenes, mice injected with intact transgene had 7-fold higher numbers of bacteria in their livers (B) and similarly higher bacterial loads in their spleens (D) (total of 16 animals per treatment, combining the combined results of five independent experiments, each with 3 or 4 animals per treatment). Histological analysis (E) of tissue sections identified substantially more infectious foci (arrows) in livers of animals injected with the intact transgene than in livers of animals injected with the vehicle or the mutant construct.
pression levels for 48 h prior to infection with *L. monocytogenes* (Fig. 3A and C) resulted in a 7-fold increase in bacterial loads in livers of infected animals (Fig. 3B). Spleens of animals that overexpressed Ch25h also had 7-fold-higher bacterial counts (Fig. 3D). Histological examination revealed an increased number of infectious foci in liver sections from animals that had higher levels of Ch25h expression (Fig. 3E). All of these observations serve to support our hypothesis that elevated Ch25h expression prolongs survival of infected cells and therefore contributes to increased bacterial burdens.

**CH25H controls expression of Cd5l.** CH25H converts cholesterol to 25-HC, which can serve as an endogenous ligand for LXR nuclear receptors. CD5L, an LXR target gene, is a soluble protein belonging to group B of the scavenger receptor cysteine-rich (SRCR) superfamily. It is expressed by macrophages present in lymphoid tissues (spleen, lymph node, thymus, and bone marrow), and it binds to myelomonocytic and lymphoid cells, which suggests that it may play an important role in the regulation of the innate immune system and in control of survival of infected macrophages (22, 38). We therefore examined the effect of differential Ch25h expression on the levels of Cd5l mRNA in our cell lines. Cells that overexpressed Ch25h (Fig. 4A) had significantly higher levels of Cd5l transcripts that were further increased by treatment with LXR/RXR agonists. Conversely, in cells that have less Ch25h we observed reduced levels of Cd5l mRNA. This reduction was also evident when cells were treated with synthetic LXR/RXR agonists. In contrast to these effects of Ch25h expression on Cd5l levels, we observed no effect of Ch25h expression on either basal or induced levels of Abca1, which is induced by either LXRα or LXRβ (Fig. 4B). Because Cd5l is a target of LXRα (22), our observation suggests that 25-hydroxycholesterol acts as an endogenous ligand of LXRα. Thus, it appears that induction of IFN-β after *L. monocytogenes* infection leads to an increase in CD5L production that ultimately promotes macrophage survival.

**Caspase-1 activation is controlled by CD5L.** CD5L has been previously shown to inhibit apoptosis of *L. monocytogenes*-infected macrophages by interfering with activation of caspase-3 (22). However, *L. monocytogenes* has also been reported to induce macrophage pyroptosis (10, 39), which is a caspase-3-independent, caspase-1-dependent proinflammatory mode of macrophage cell death (37, 47). To test the role of CD5L in inhibition of pyroptosis, we transfected control or Ch25h-overexpressing cell lines with either Cd5l siRNA or a scrambled small interfering RNA (siRNA) control. Following infection with *L. monocytogenes*, we measured the amount of the p10 cleavage product of caspase-1. In the absence of infection there was almost no detectable caspase-1 processing in our cell lines (Fig. 5A). Infection resulted in caspase-1 maturation but to a significantly reduced extent in the hiC25-1 cell line that overexpresses Ch25h (Fig. 5B, control siRNA-treated sample). Treatment of either the control or hiC25-1 cell line with Cd5l siRNA resulted in enhanced caspase-1 processing (Fig. 5B), indicating that CD5L inhibits *L. monocytogenes*-induced macrophage death in part by inhibiting caspase-1 cleavage and pyroptosis. Comparison of infection-induced caspase-1 cleavage in cells overexpressing Ch25h (Fig. 5C) and cells with reduced Ch25h expression (Fig. 5D) indicates that levels of Ch25h...
define the extent of caspase-1 cleavage by *L. monocytogenes* infection (Fig. 5C and D).

**Activation of the LXR pathway suppresses caspase-1 processing.** The effect of *Ch25h* expression on the activation of caspase-1 could be specific to 25-HC, or it could reflect the effect of the activation of LXRs. We therefore analyzed the effect of treatment of primary macrophages with either endogenous or synthetic LXR ligands. Cells treated with 25-HC or a mixture of RXR and LXR ligands displayed a similar reduction in the maturation of caspase-1 following infection with *L. monocytogenes* (Fig. 5E). There was no effect of pretreatment on survival of RAW264 macrophage-like cells that were reported to lack LXRα and not induce *Cd5l* in response to LXR/RXR agonists (Fig. 6A) (22). However, consistent with their role in promoting survival of infected macrophages, pretreatment of ZBM2 cells or BMMs with either LXR/RXR ligands or 25-HC resulted in a higher number of bacteria recovered at 12 h postinfection (Fig. 6B and C). Therefore, our data indicate that activation of LXRα by either endogenous or exogenous stimulation results in inhibition of *L. monocytogenes*-induced activation of caspase-1.

**DISCUSSION**

LXRα has been recently shown to play a significant role in resistance to *L. monocytogenes* infection by controlling survival of macrophages (22). Therefore, the identification of *Ch25h* as a differentially expressed IFN-β-inducible gene was striking because CH25H converts cholesterol to 25-HC, a natural ligand of LXRs. Results from earlier gene knockout studies indicated a requirement for LXRα signaling for resistance to infection and attributed the effects of LXRα disruption to impaired *Cd5l* expression. Our data similarly demonstrate that upregulation of *Cd5l* at the time of infection promotes survival
eloid-specific overexpression of Cd5l leads to increased numbers of macrophages, neutrophils, and dendritic cells, resulting in systemic inflammation and adenocarcinoma in the lung (36). Therefore, our study not only reveals new points of intersection of metabolic and inflammatory signaling but also underscores the delicate balance of cell survival that has to be maintained by the host to resist infection.

Our cell culture experiments revealed that changes in Ch25h expression lead to 2-fold differences in the viability of infected cells (Fig. 2A and B) and the percentage of TUNEL-positive cells (Fig. 2D and E). While it could be difficult to appreciate the significance of these differences, similar changes have been observed in other cell culture analyses of infected macrophages. For example, an analysis of the role of the Mcl-1 proapoptotic gene in Mycobacterium tuberculosis infection revealed that a reduction in Mcl-1 levels by transfection with antisense oligonucleotides that led to 2-fold differences in the viability of infected MDMs at day 1 and a 1.5-fold difference at day 4 had a significant effect on intracellular growth of the pathogen (40). Follow-up studies of pneumococcal infections provided further support for the importance of Mcl-1-controlled macrophage survival in the course of infectious diseases (30). In addition, a recent study demonstrated how macrophage cell death promotes resistance to Salmonella enterica serovar Typhimurium. Approximately 2-fold differences in macrophage viability translated into differences between life and death of infected animals (31). Similarly, in our study the importance of the effects observed ex vivo was confirmed by in vivo experiments.

Hydrodynamic delivery of recombinant DNA targets transient expression of transgenes mainly to livers and spleens of experimental animals (7, 52). Because these organs are primary sites of bacterial replication in the murine model of listeriosis, we used this approach to demonstrate that an increase in Ch25h expression increases the bacterial burdens of infected animals. This observation is consistent with our hypothesis that increased survival of infected macrophages promotes bacterial replication. However, considering that 25-HC can be secreted by macrophages, we cannot exclude the possibility that changes in Ch25h expression have paracrine effects that exacerbate the course of infection (15). In either case, our data clearly demonstrate a detrimental effect of increased Ch25h expression on resistance to L. monocytogenes infection.

Our results demonstrate that L. monocytogenes infection upregulates Ch25h expression through IFN-β signaling. This results in enhanced macrophage survival, presumably due to reduced levels of active caspase-1. Because caspase-1 is rapidly activated in response to infection (50), before Ch25h expression reaches its peak, it appears that Ch25h levels affect processing of this enzyme. We propose that this is due to a Ch25h-mediated increase in CD5L production, since CD5l siRNA is capable of reversing the effects of Ch25h overexpression on the processing of caspase-1 (Fig. 5). It is possible that these events are a part of a strategy evolved by the pathogen to maintain a protected intracellular environment for its replication and prevent immune activation by pyroptotic death of macrophages. However, CD5L is a survival factor for lymphocytes as well as macrophages. Given the profound detrimental effect of L. monocytogenes-induced splenocyte apoptosis on of infected macrophages. However, rather than promoting resistance to infection, signaling events that lead to CD5L induction, such as overexpression of Ch25h, result in increased recovery of L. monocytogenes from infected macrophages in vitro and in greater bacterial loads in livers and spleens of infected mice in vivo. We suggest that the different consequences for the host observed in LXRe-deficient mice and in mice transiently overexpressing Ch25h may reflect the different effects of a transient induction of macrophage survival at the time of infection compared to the constitutive reduction of a macrophage survival factor for the life of the animal. Steady-state viability of macrophages must be maintained to ensure detection of invading pathogens, but increased survival of L. monocytogenes-infected macrophages could intensify the disease. A detrimental effect of prolonged macrophage survival is not unique to L. monocytogenes pathogenesis. For example, my-
host resistance (8), the induction of CD5L could be an attempt by the host to enhance survival of lymphocytes. The control of infectious disease relies on the integration of a multitude of host cellular signaling pathways. Cross talk between inflammatory and metabolic pathways has recently been recognized as an important interaction that shapes the immune response to infection (23, 51). Our studies reveal new points of intersection of metabolic and inflammatory signaling and define differences in Chh25h expression that affect both pathways. These findings are based on our analysis of L. monocytogenes infection in inbred strains of mice that we use to model the phenotypic diversity of mammalian populations.

Therefore, our findings underscore the importance of genetically defined variation in gene expression as a factor in the control of infectious diseases.

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