Listeria monocytogenes multidrug resistance transporters activate a cytosolic surveillance pathway of innate immunity

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To gain insight into the interaction of intracellular pathogens with host innate immune pathways, we performed an unbiased genetic screen of *Listeria monocytogenes* mutants that induced an enhanced or diminished host innate immune response. Here, we show that the major facilitator superfamily of bacterial multidrug resistance transporters (MDRs) controlled the magnitude of a host cytosolic surveillance pathway, leading to the production of several cytokines, including type I IFN. Mutations mapping to repressors of MDRs resulted in ectopic expression of their cognate transporters, leading to host responses that were increased up to 20-fold over wild-type bacteria, and a 20-fold decrease in bacterial growth *in vivo*. Mutation of one of the MDRs, MdrM, led to a 3-fold reduction in the IFN-β response to *L. monocytogenes* infection, indicating a pivotal role for MdrM in activation of the host cytosolic surveillance system. Bacterial MDRs had previously been associated with resistance to antibiotics and other toxic compounds. This report links bacterial MDRs and host immunity. Understanding the mechanisms through which live pathogens activate innate immune signaling pathways should lead to the discovery of adjuvants, vaccines, and perhaps new classes of therapeutics. Indeed, we show that the mutants identified in this screen induced vastly altered type I IFN response *in vivo* as well.

bacterial genetic screen | immune response | interferon-beta | intracellular pathogen

Intracellular pathogens have evolved exquisite mechanisms that lead to their compartmentalization and replication within host cells (1). Conversely, the mammalian innate immune system has evolved to recognize microbial infection within different cellular compartments by using a variety of surface, vacuolar, and cytosolic receptors that recognize conserved molecules of microbial origin (2, 3). Not surprisingly, pathogens acquired counter measures to avoid and/or exploit the host innate immune system to promote their pathogenesis (2, 4). Although the field of innate immunity has received enormous attention from those interested in infectious diseases, inflammation, and adaptive immunity, much of our current understanding is still derived from studying the interaction of host cells with purified components derived from microorganisms. How host cells distinguish and respond to live, replicating, pathogenic microorganisms and nonvirulent microbes is still unclear and is central to the understanding of host–pathogen interactions.

Intracellular pathogens fall into two broad classes: those living in vacuole-like compartments and those living directly in the host cell cytosol. *Listeria monocytogenes* is an example of the latter that has been used as a model organism for decades to study basic aspects of both innate and acquired immunity. On entry into the host cytosol, wild-type (w.t.) strains of *L. monocytogenes* activate a MyD88/TRIF-independent, IRF-3/TBK1-dependent host transcriptional response that leads to transcription of dozens of genes, including robust expression and synthesis of the cytokine IFN-β (5–8). *L. monocytogenes* mutants that fail to access the cytosol do not activate the cytosolic surveillance pathway and do not induce IFN-β (5–8). Virulent strains of other intracellular bacterial pathogens, such as *Mycobacterium tuberculosis*, *Legionella pneumophila*, *Brucellae* and *Francisella tularensis*, also activate this pathway, whereas, in each case, avirulent mutants do not (9–12). Currently, the nature of the bacterial ligand(s) or the host receptor(s) that are involved in recognition of bacteria in the host cytosol are unknown, although nucleic acids, as well as flavone-related compounds, can activate a similar host response (9, 13, 14). To begin to understand how pathogenic bacteria activate the cytosolic surveillance pathway and its role during infection and immunity, we performed an unbiased forward genetic screen and identified *L. monocytogenes* mutants that induced either elevated or diminished host responses to replicating cytosolic bacteria. We found that expression of *L. monocytogenes* multidrug resistance transporters (MDRs) of the major facilitator superfamily of bacterial MDRs controlled activation of the host cytosolic surveillance pathway both *in vitro* and *in vivo*.

Results and Discussion

Identification of *L. monocytogenes* Mutants That Differentially Induce IFN-β in Macrophages. To address how *L. monocytogenes* activates the host cytosolic surveillance system, we screened a *L. monocytogenes* Tn*917* transposon library (15) for mutants that exhibited an enhanced or diminished type I IFN response upon infection of macrophages. Approximately 5,000 *L. monocytogenes*::Tn*917* mutants were used to infect bone marrow-derived macrophages (BMM) in 96-well plates. The amount of type I IFN (i.e., IFN-β and/or IFN-α) secreted by macrophages during infection was measured by transferring macrophage culture supernatant onto a type I IFN reporter cell line that produces luciferase in response to type I IFN (16). We identified 17 mutants that induced altered induction of type I IFN compared with w.t. bacteria. Among these, six mutants, with transposon insertions located in three different genes, behaved like w.t. in their ability to infect, escape from a vacuole, and grow inside macrophages as shown by their intracellular growth curves (Fig. 1*a*). Transposon insertions in these mutants were located in genes encoding predicted transcription regulators (Fig. 1*b*): *ladR*, previously shown to be a negative

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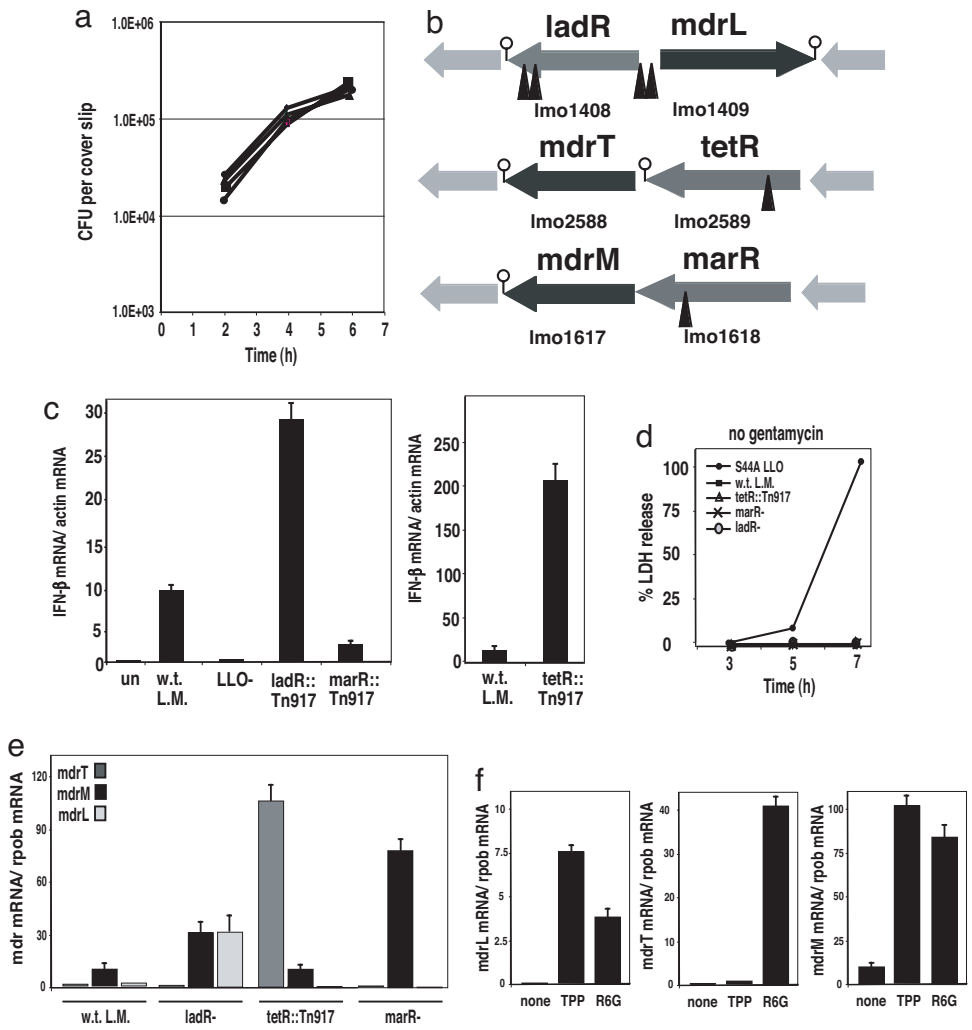

Fig. 1. *L. monocytogenes* strains with mutations in regulators of multidrug resistance transporters induce altered host IFN-β responses. (*a*) Intracellular growth curves of w.t. *L. monocytogenes* (filled square), *ladR*::Tn*917* (filled triangle), *tetR*::Tn*917* (large filled circle), and *marR*::Tn*917* (small filled circle), in bone marrow-derived macrophages (BMMs) (31). (*b*) Schematic presentation of site of transposon insertions (marked with triangles), mapped to genes predicted to be transcription regulators. (*c*) Quantitative RT-PCR (qRT-PCR) analysis of IFN-β gene induction in BMM in response to infection with w.t. *L. monocytogenes*, *ladR*::Tn*917*, *tetR*::Tn*917*, and *marR*::Tn*917*. (*d*) Lactate dehydrogenase (LDH) release assay was performed on macrophages infected with w.t. *L. monocytogenes*, *tetR*::Tn*917*, *marR*−, and *ladR*− mutants at various time points postinfection. *L. monocytogenes* cytotoxic LLO mutant *S44A* (32, 33) was used as a positive control. (*e*) qRT-PCR analysis of *L. monocytogenes* MDR transporters expression in *w*.t. bacteria, *ladR*−, *tetR*::Tn*917*, and *marR*− mutants grown to midlog in BHI broth. (*f*) qRT-PCR analysis of MDRs expression by w.t. *L. monocytogenes* in the presence of the toxic compounds tetraphenylphosphonium (TPP) or rhodamine 6G (R6G). All error bars represent one standard deviation; $n = 2$ or 3.

regulator of its adjacent multidrug resistance transporter, MdrL (17); *lmo2589* encoding a TetR-like protein; and *lmo1618* encoding a MarR-like protein (18). Real-time qRT-PCR analysis of IFN-β induction in macrophages infected with these mutants confirmed that the *ladR* mutant induced 3-fold more IFN-β, the *tetR* mutant induced 20-fold more IFN-β, and the *marR* mutant induced 3-fold less IFN-β compared with the level of IFN-β induced by w.t. bacteria [Fig. 1*c* and supporting information (SI) Fig. S1]. Although the three mutants affected the level of IFN-β in macrophages, none of them induced macrophage cell death as shown by a lactate dehydrogenase (LDH) release assay (Fig. 1*d*).

LadR, TetR, and MarR Are Repressors of MDRs. This is the first description of the *tetR* and *marR* genes in *L. monocytogenes*. Interestingly, like the *ladR* transcription regulator, the *tetR* and *marR* regulators were located adjacent to putative multidrug resistance transporters of the major facilitator superfamily, named here *mdrT* and *mdrM*, respectively (*lmo2588*, *lmo1617*) (Fig. 1*b*). Among the three MDRs, MdrM and MdrT are highly similar (46% amino acid identity and 64% similarity) and share similarity with the well studied multidrug efflux transporter system, QacA-QacR, of *Staphylococcus aureus* (18). In *S. aureus*, QacR represses expression of the MDR *qacA*. To study the regulation of *mdrL*, *mdrT*, and *mdrM* expression by their adjacent regulators and their effect on the cytosolic innate immune response, we generated a series of in-frame deletions (19) of the regulator genes, the MDR genes, and a double deletion of each MDR-regulator set of genes (Table 1). Unfortunately, for reasons that are still not clear, we were unable to generate in-frame deletions of the *tetR* gene or the double *tetR-mdrT* genes; thus, for the rest of the study, we used the original transposon *tetR*::Tn*917* mutant. The expression level of each MDR was analyzed by real-time qRT-PCR from bacteria grown in broth. We found that w.t. *L. monocytogenes* did not express *mdrL* or *mdrT*, but expressed a measurable level of *mdrM* (Fig. 1*e*). In the *ladR* mutant the multidrug transporter, *mdrL*, was highly in-

Table 1. *Listeria monocytogenes* strains generated in this study

L. monocytogenes strain	Description	IFN-β induction/w.t. *L. monocytogenes*
10403S	Wild type	1
DP-L5396	*ladR*::Tn*917*	3
DP-L5418	*marR*::Tn*917*	0.3
DP-L5397	*tetR*::Tn*917*	20
DP-L5523	w.t. 10403S + pLIV2:*mdrT*	3.5
DP-L5441	*ladR*−	3
DP-L5445	*marR*−	6
DP-L5444	*mdrM*−	0.3
DP-L5516	*mdrM*− + pLIV2:*mdrM*	1
DP-L5446	*mdrT*−	1
DP-L5442	*mdrL*−	1
DP-L5448	*marR*−/*mdrM*−	0.3
DP-L5443	*ladR*−/*mdrL*−	3

Listed for each strain is the relative level of IFN-β induced by host macrophages, compared with the level of IFN-β induced by w.t. *L. monocytogenes*.

duced (≈30-fold) (17). In addition, mutation in the *ladR* gene resulted in ≈3-fold induction of *mdrM*, compared with its basal level of expression (Fig. 1*e*). In the *tetR*::Tn*917* mutant, the adjacent multidrug transporter, *mdrT*, was specifically and highly induced (≈100-fold) (Fig. 1*e*). In the case of the *marR* regulator, the *mdrM* gene was located downstream of *marR*, and both genes were predicted to be part of an operon (Fig. 1*b*). Although *mdrM* was not expressed in the original *marR*::Tn*917* mutant (data not shown), it was highly induced in the *marR* in-frame deletion (≈70-fold) (Fig. 1*e*), suggesting that the transposon insertion blocked the expression of both genes because of polarity. These results clearly demonstrated that LadR, TetR, and MarR are negative regulators of the putative MDRs MdrL, MdrT, and MdrM, respectively.

One common property of MDRs is that their expression is often induced by the presence of their cognate drug substrates (18, 20). For example, in the QacA-QacR system, the repression of *qacA* imposed by QacR is relieved when QacR binds toxic drugs, leading to induction of *qacA* expression (18, 20). When w.t. *L. monocytogenes* was grown in the presence of the commonly used toxic drugs, tetraphenylphosphonium (TPP) or rhodamine 6G (R6G) (18), the transcription of the three MDRs was highly induced (Fig. 1*f*), suggesting that the regulator genes identified in this screen were involved in the regulation of MDR transporters.

Expression of *L. monocytogenes* Multidrug Resistant Transporters Controls the Induction of IFN-β in Macrophages. To evaluate the role of each MDR in the induction of type I IFN, we infected macrophages with w.t. *L. monocytogenes* and the MDR mutants,

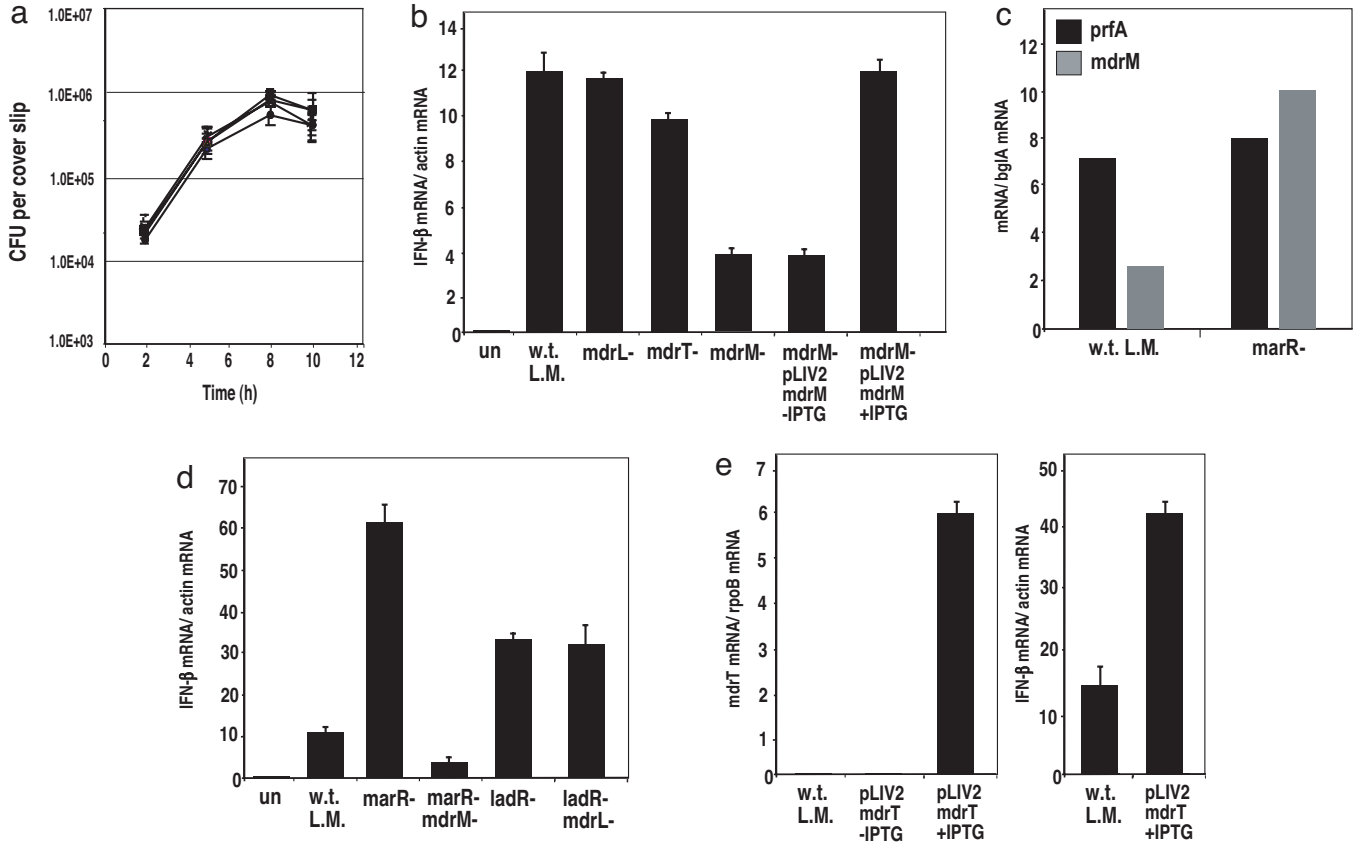

Fig. 2. Role of the *L. monocytogenes* multidrug resistance transporters *mdrL*, *mdrT*, and *mdrM* in the induction of IFN-β in macrophages. (*a*) Intracellular growth curves of w.t. *L. monocytogenes* (filled circle) and the deletion mutants: *mdrL*− (filled square), *mdrT*− (×), and *mdrM*− (filled diamond) in BMMs (31). (*b*) qRT-PCR analysis of IFN-β induction in BMMs infected with w.t. *L. monocytogenes*, *mdrL*−, *mdrT*−, *mdrM*−, and a complemented *mdrM*− strain expressing *mdrM* from the IPTG-inducible vector pLIV2 (21). (*c*) qRT-PCR analysis of MdrM expression by w.t. *L. monocytogenes* and *marR* mutant intracellularly at 4 h postinfection. (*d*) qRT-PCR analysis of IFN-β induction in BMMs infected with *ladR*−, *marR*− or the double deletions of *ladR*−/*mdrL*− or *marR*−/*mdrM*−. (*e*) qRT-PCR analysis of *mdrT* expression level in *w.*t. *L. monocytogenes* bacteria containing IPTG-inducible plasmid pLIV2::*mdrT*, and analysis of IFN-β induction by this strain in infected BMMs. All error bars represent one standard deviation; $n = 2$ or 3.

and analyzed the induction of IFN-β at 4 h postinfection by real-time qRT-PCR. The results clearly demonstrated that among the three MDRs, MdrM was the only one necessary for induction of IFN-β, because this mutant induced only a third of the IFN-β induced by w.t. bacteria (Fig. 2*b*). This was not due to a growth defect, because all 3 MDR deletion mutants were able to infect and replicate within macrophages like w.t. bacteria (Fig. 2*a*). Complementation of *mdrM* expression with an IPTG-inducible expression system (pLIV2 integration vector) (21) restored the induction of IFN-β to the level induced by w.t. *L. monocytogenes* (Fig. 2*b*). Further analysis of *mdrM* expression during infection revealed that *mdrM* is expressed intracellularly, but, as in broth, its expression is well below the level of the *marR* deletion mutant (Fig. 2*c*). These results suggested that this basal expression of *mdrM* accounted for the majority of IFN-β expression.

Overexpression of *mdrM*, or its related MDR, *mdrT*, caused massive expression of IFN-β. The *marR* deletion mutant, which overexpressed *mdrM* (Fig. 1*e*), induced 6-fold more IFN-β than w.t. bacteria (Fig. 2*d*). This level of IFN-β induction was completely dependent on *mdrM* expression because it was not observed with the *marR-mdrM* double-deletion mutant, which induced the same level of IFN-β as the *mdrM* mutant alone, thereby excluding a potential role for other MarR-inducible genes (Fig. 2 *b* and *d*). Further support for the role of MdrM in IFN-β induction came from infecting macrophages with the *ladR*− mutant. As shown in Fig. 1*e*, LadR also repressed the expression of *mdrM*, although to a lesser extent than the *MdrM* repressor, MarR. Infecting macrophages with the *ladR*− mutant resulted in 3-fold higher induction of IFN-β than with w.t. bacteria; however, infection with the double-deletion *ladR-mdrL* mutant still induced 3-fold more IFN-β then w.t. bacteria, suggesting that this induction was not due to the expression of *mdrL* (Fig. 2*d*). Microarray analysis comparing total gene expression of w.t. bacteria versus the *ladR*− mutant revealed that, besides *mdrL*, *mdrM* was the most differentially expressed gene in the *ladR*− mutant (Table S1 and *SI Methods*). Because mdrM overexpression in the *marR*− mutant resulted in enhanced host IFN-β expression (Figs. 1*e* and 2*d*), it is most likely that the induction of IFN-β by the *ladR*− mutant was because of overexpression of *mdrM* and not *mdrL*. Overall, these results demonstrated a direct role for MdrM in activation of IFN-β in response to *L. monocytogenes* infection. Interestingly, w.t. bacteria expressing IPTG-inducible MdrT (MdrM homolog) also resulted in increased induction of IFN-β in infected macrophages (Fig. 2*e*). These observations strongly suggest that the induction of IFN-β was not restricted to MdrM, but could be recapitulated by expression of homologous MDRs, likely with similar substrate specificity.

***L. monocytogenes* MDRs Control the Magnitude of the Host Cytosolic Response.** How immune cells recognize intracellular pathogens such as *L. monocytogenes* is not fully understood. The cytosolic innate immune response to *L. monocytogenes* is generally described as independent of Toll-like receptors (TLRs) and their signaling adaptors, Myd88 and Trif, and dependent on p38 MAPK signaling and the IFN regulatory factor 3 (IRF-3) (5, 7). To test whether induction of IFN-β by the mutants identified in this screen activated the same pathway, we infected *Myd88-Trif* double-knockout macrophages, *IRF-3* deficient (22) macrophages, and macrophages treated with a p38 MAPK inhibitor with *ladR*, *marR*, and *tetR* mutants. We found that the increased induction of IFN-β by these mutants was almost entirely independent of TLRs, largely dependent on p38 MAPK, and absolutely dependent on IRF-3 (Fig. 3 *a*, *b*, and *d*). One well characterized cytosolic pathway that leads to IRF-3 activation and IFN-β expression depends on the cytosolic receptors RIG-I and MDA-5 and their adaptor, MAVS (23–25). We infected

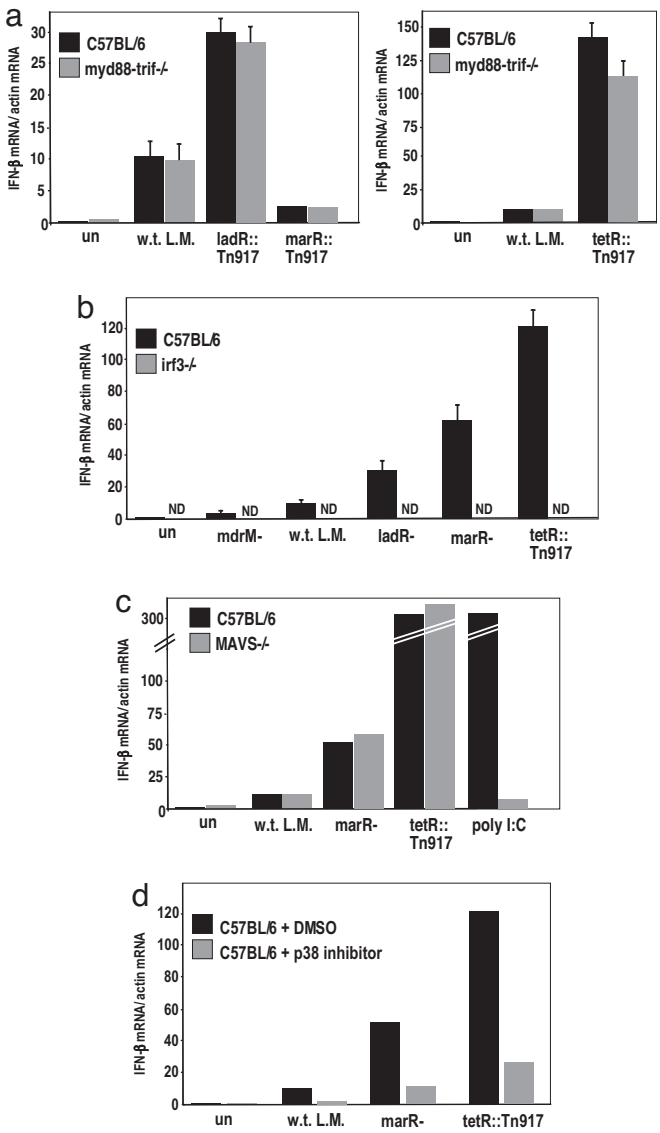

Fig. 3. Induction of IFN-β by *ladR*, *tetR*, and *marR* mutants is independent of MyD88/Trif and MAVS but dependent on IRF-3 and p38 MAPK. qRT-PCR analysis of IFN-β induction in C57BL/6 w.t. vs. *myd88/trif*−/− BMMs (*a*), and C57BL/6 w.t. vs. *irf3*−/− BMMs (22) (*b*) infected with w.t. *L. monocytogenes*, and *ladR*, *marR*, *tetR*::Tn*917* regulator mutants. (*c*) qRT-PCR analysis of IFN-β induction in C57BL/6 BMMs vs. *MAVS*−/− BMMs (25) infected with *w.t. L. monocytogenes*, *marR*−, *tetR*::Tn*917*, or transfected with poly[I:C] as a positive control. (*d*) qRT-PCR analysis of IFN-β induction in C57BL/6 BMMs treated with either DMSO or 10 μM SB202190 (p38 MAPK inhibitor) (5). All error bars represent one standard deviation; $n = 2$ or 3.

MAVS-deficient macrophages with w.t. *L. monocytogenes*, *marR*−, and *tetR*::Tn*917* mutants, and the induction of IFN-β by w.t. *L. monocytogenes* and the mutants was independent of MAVS (Fig. 3*c*) (25, 26). These results are consistent with the hypothesis that w.t. *L. monocytogenes* and the mutants induced altered levels of activation of the same host cytosolic surveillance pathway. To gain further insight into the host pathways and downstream genes activated by bacterial MDRs, we compared the macrophage response to infection with w.t. *L. monocytogenes*, *mdrM*− and *tetR*::Tn*917* mutants by using microarray analysis. We used type I IFN receptor minus (IFN$\alpha\beta$R−/−) macrophages to avoid the complication of IFN-β autocrine signaling. Macrophages infected with the *mdrM*− mutant, which

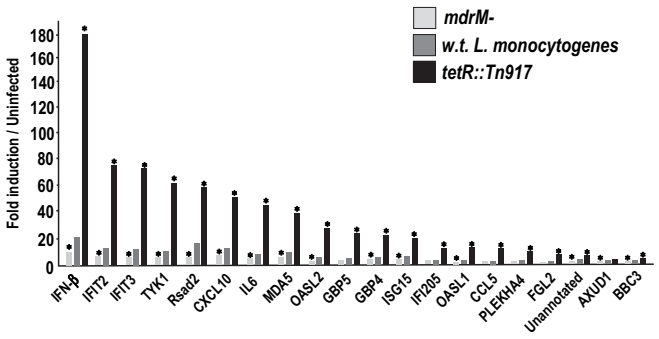

Fig. 4. *L. monocytogenes* MDR expression determines the magnitude of the host immune response. Genes identified by microarray analysis as having lower expression in *mdrM*− infected IFN$\alpha\beta$R−/− macrophages (by SAM) or higher expression in *tetR*::Tn*917* infected IFN$\alpha\beta$R−/− macrophages (by SAM and at least 4-fold higher than w.t.), as compared with their expression in *w.t. L. monocytogenes* infected IFN$\alpha\beta$R−/− macrophages. All data are represented as fold induction over uninfected macrophages and are the average of two experiments. * indicates that these values are significantly different from macrophages infected with w.t. *L. monocytogenes,* by SAM analysis with a false discovery rate of 10%.

induced a 3-fold lower host IFN-β response, had altered expression of only 16 genes (by SAM analysis), all of which were diminished compared with macrophages infected with w.t. *L. monocytogenes*. Macrophages infected with the *tetR*::Tn*917* mutant, which induced a 20-fold higher IFN-β response, had strongly increased induction (by SAM and at least 4-fold) of 13 genes, compared with macrophages infected with w.t. *L. monocytogenes*. Interestingly, the genes whose expression was affected by *mdrM*− and *tetR*::Tn*917* mutants largely overlapped and are presented in Fig. 4. Moreover, the vast majority of these genes were previously identified as "cytosolic response genes" (i.e., genes that are induced only by w.t. *L. monocytogenes* in the cytosol) and included IFN-β, IL-6, CCL5, and CXCL10 (13). Like IFN-β, the expression of these genes appears to be almost entirely IRF-3-dependent (Fig. S2). These experiments provided further evidence that bacterial MDR expression specifically controlled the magnitude of the host cytosolic response to *L. monocytogenes*, which includes a range of host genes, in addition to IFN-β.

***In Vivo* Analysis of *L. monocytogenes* MDR Mutants.** To test the role of the cytosolic surveillance response in the host's defense to *L. monocytogenes* infection, we infected mice with w.t. *L. monocytogenes*, *mdrM*−, *marR*−, and *tetR*::Tn*917* mutants. Mice infected with the mutant that induced 20 times more IFN-β, *tetR*::Tn*917*, had 20-fold lower bacterial loads in the liver, whereas the other mutants had w.t. levels of bacteria, suggesting that only a drastic change in activation of the cytosolic surveillance pathway had a measurable effect on host resistance (Fig. 5 *a* and *b*). Although the *tetR*::Tn*917* mutant induced stronger activation of the entire cytosolic response (Fig. 4 and Fig. S2), we specifically tested the role of IFN-β in the increased clearance of the *tetR*::Tn*917* mutant by using IFN$\alpha\beta$R−/− mice. Interestingly, IFN$\alpha\beta$R−/− mice were still more resistant to *tetR*::Tn*917* than w.t. *L. monocytogenes*, suggesting that the increased host resistance may not be solely because of the high expression of IFN-β. Instead, the increased host resistance is likely due to a combination of genes in the enhanced cytosolic response. Although previous studies suggested that host induction of IFN-β might be beneficial to *L. monocytogenes* (22, 27), the results in this study are consistent with a model in which *L. monocytogenes* avoids excessive activation of the host cytosolic surveillance system.

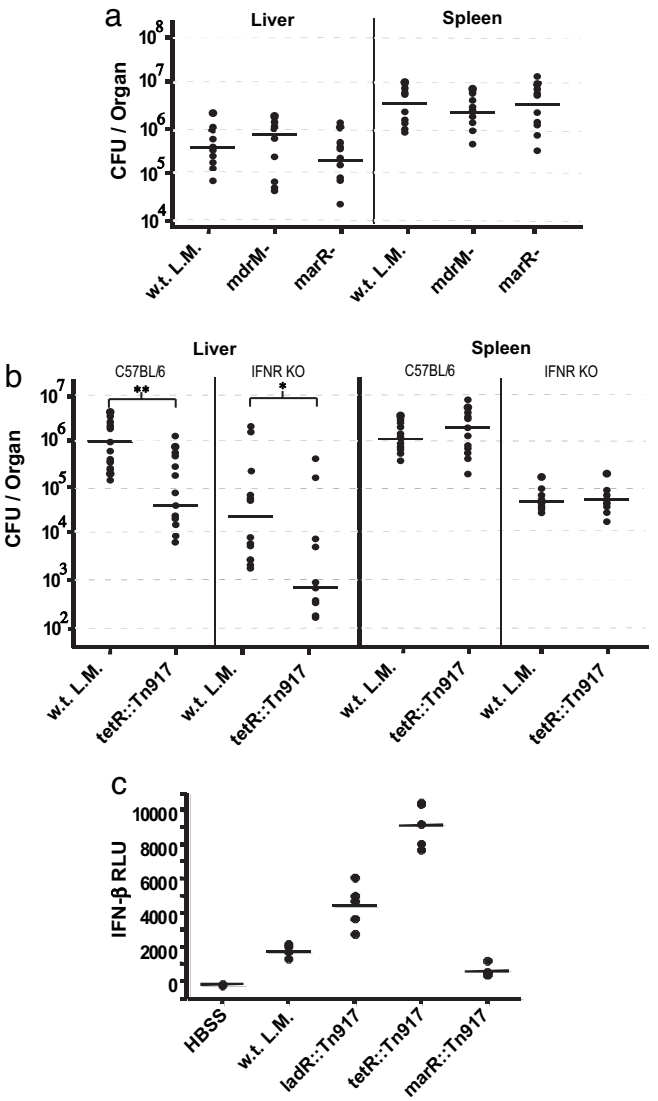

Fig. 5. Effect of MDR mutations on *in vivo* induction of IFN-β and *L. monocytogenes* virulence. (*a*) C57BL/6 mice infected with 1×10^4 ($0.1\ LD_{50}$) of *w.t.*, *mdrM*−, or *marR*− *L. monocytogenes*. Organs were collected 48 h postinfection, and bacterial numbers are represented as colony forming units (cfu) per organ, $n = 10$ mice per strain. (*b*) C57BL/6 or IFN$\alpha\beta$R−/− mice were infected with 1×10^4 of w.t. or *tetR*::Tn*917 L. monocytogenes* and processed and analyzed as described for *a*, $n = 15$ (C57BL/6) or 12 (IFN$\alpha\beta$R−/−) mice per strain. (*c*) Detection of type I IFN levels in serum of Balb/C mice infected i.v. (1×10^4 bacteria) with Hank's Buffered Salt Solution (HBSS), w.t. *L. monocytogenes*, *ladR*::Tn*917*, *tetR*::Tn*917*, or *marR*::Tn*917* for 24 h. Units are presented as relative light units (RLU), detected by luciferase reporter ISRE-L929 cell line assay (16), $n = 5$ mice per strain. All median values are represented by horizontal lines. Statistical significance was determined by nonparametric Mann–Whitney test. **, $P = 0.001$; *, $P = 0.007$.

This report demonstrates a role for bacterial MDR transporters in the activation of a host immune response. MDRs are known to bind and transport a broad range of structurally unrelated compounds. We propose that MDR-mediated transport of bacterial ligands to the host cytosol triggers the host cytosolic surveillance system. However, we cannot rule out that the MDR proteins by themselves are the stimulatory ligands for the host immune system, although we have shown that high expression of one MDR, *mdrL*, had no effect on the activation of the cytosolic surveillance system (Fig. 2*d*). The results of this study indicate that the host immune system can detect a live,

virulent intracellular pathogen by recognition of the pathogen's own defense mechanism to toxic molecules. Interestingly, other intracellular bacterial pathogens, including *Mycobacterium tuberculosis*, *Brucella*, and *Legionella pneumophila*, also activate a similar host response (9, 10, 12), although for these pathogens, activation requires an auxiliary secretion system. Perhaps *L. monocytogenes* MDRs and these auxiliary secretion systems release the same or related molecules into the host cytosol, resulting in activation of the host cytosolic surveillance pathway.

We generated *L. monocytogenes* strains that vary by 60-fold in the amount of IFN-β and >5- or 10-fold in the amount of IL-6, CCL5, and CXCL10 induced in infected macrophages, because of their levels of MDR expression (Figs. 3*b* and 4; Fig. S2). Importantly, the activation of the cytosolic surveillance system in infected animals, as measured by IFN-β in the serum, recapitulated the results observed in tissue culture (Fig. 5*c*). Type I interferons have wide ranging effects on innate and adaptive immune responses, and are used to treat multiple sclerosis, hepatitis C, and some malignancies (28, 29). The strains generated in this study may provide insight into the role of IFN-β and the cytosolic surveillance pathway in linking innate and adaptive immunity, thereby leading to the development of adjuvants and vaccines and, perhaps, to the discovery of new therapeutics.

Materials and Methods

Bacterial Genetic Screen. A total of 5,000 individual *L. monocytogenes* Tn*917-LTV3* transposon insertion mutants (15) were grown on BHI media in 96-well plates overnight at 30°C. Bone marrow-derived macrophages from C57BL/6 mice were plated on 96-well plates, 4×10^4 cells per well, and infected with 2×10^6 bacteria. 30 min postinfection, macrophages were washed and gentamicin was added (50 μg/ml) to prevent extracellular growth of bacteria. At 6 h postinfection 100 μl of macrophage culture media was frozen at −80°C. The amount of type I IFN in the media was detected by using a reporter cell line, ISRE-L929 (16). ISRE-L929 cells were grown in 96-well plates and incubated with 40 μl of infected macrophage culture media for 4 h. Cells were lysed and luciferase activity was detected by using Bright Glow Assay (Promega, E-2620) and measured with a luminescence counter (VICTOR3, PerkinElmer).

Infections and Analysis of Gene Expression in Macrophages. RNA was collected from infected macrophages at 4 h postinfection, and induction of IFN-β was analyzed by qRT-PCR, as described (30). Where indicated, 10 μM p38 MAPK inhibitor SB202190 (Calbiochem) was added to cells 30 min before infection, and kept on for the duration of the infection (5).

***L. monocytogenes* Gene Expression.** Expression of MDR genes by *L. monocytogenes* growing in BHI broth or intracellularly was analyzed by using real-time qRT-PCR analysis (30). Level of gene expression was normalized to the level of expression of the *rpoB* gene or *bglA* gene. To test for expression of MDR genes after treatment with toxic drugs, tetraphenylphosphonium (50 μM, Sigma) or rhodamine 6G (50 μM, Sigma) were added for 1 h at log phase, then total bacteria RNA was extracted and analyzed by qRT-PCR (30).

Microarray Analysis. Microarray analysis of IFN$\alpha\beta$R−/− macrophages infected with w.t., *mdrM*−, or *tetR*::Tn*917 L. monocytogenes* was done as described in refs. 13 and 30, with several modifications detailed in the *SI Methods*.

***In Vivo L. monocytogenes* Infections.** C57BL/6 or IFN$\alpha\beta$R−/− mice were infected with 0.1 LD50 (1×10^4) of w.t. *L. monocytogenes*, *mdrM*−, or *tetR*::Tn*917* mutants, and organs were harvested 48 h postinfection, as described (27).

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