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Activation of Host Mitogen-Activated Protein Kinases by Secreted *Legionella pneumophila* Effectors That Inhibit Host Protein Translation

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Studies of innate immunity in metazoans have largely focused on detection of microbial molecules by host pattern recognition receptors (PRRs). A complementary mode of innate immune recognition, based on detection of pathogen-encoded activities, has long been recognized in plants, where it is termed effector-triggered immunity; however, little is known about the possibility of effector-triggered immunity in metazoans. *Legionella pneumophila* is an intracellular bacterial pathogen that causes Legionnaires' disease, an inflammatory pneumonia. We recently demonstrated that macrophages infected with *L. pneumophila* exhibit mitogen-activated protein (MAP) kinase (MAPK) activation that is independent of known PRRs but dependent on a functional bacterial secretion system. Here, we show that five secreted *L. pneumophila* effectors are responsible for the activation of host MAP kinases. These five effectors inhibit host translation, and their activity is required for host MAPK activation. We demonstrate that MAPK activation by these effectors shapes the host transcriptional response to *L. pneumophila*. Furthermore, we find that uninfected macrophages treated with two different translation inhibitors exhibit activation of MAP kinases and upregulation of target genes, indicating that translation inhibition alone is sufficient to elicit this response in macrophages. MAP kinase pathways are crucial in many aspects of the immune response, including inflammation and cell motility. Our results demonstrate that this important host pathway can be activated in response to a pathogen-encoded activity, adding to an emerging body of evidence in support of this novel mode of innate immune detection in metazoans.

Historically, studies of innate immune surveillance have centered on pattern recognition receptors (PRRs), which are germ-line-encoded host proteins that recognize molecules that are highly conserved among microbes but absent in the host (29). These microbial molecules—called pathogen-associated molecular patterns (PAMPs)—may be unique to a certain class of microbe, as in the case of viral double-stranded RNA or bacterial lipopolysaccharide (LPS), allowing the generation of an immune response that is tailored to the pathogen at hand. While the PRR-PAMP model is fundamental to our understanding of innate immune surveillance, it does not provide a straightforward mechanism for immune discrimination between harmless and harmful microbes, since PAMPs are found on pathogens and nonpathogens alike. Therefore, it has been suggested that in addition to detection of microbial molecules, the host might also screen for patterns of pathogenesis (49), the activities that pathogens utilize to invade, replicate, and spread within the host (16, 38, 49). Indeed, several reports of host responses to such pathogen-encoded activities have recently emerged in the literature. Examples in mammals include activation of the Nlrp3 inflammasome by a viral ion channel (26) and activation of host mitogen-activated protein (MAP) kinases (MAPKs) by *Salmonella* (9, 42) or *Escherichia coli* (8, 41) effectors that target host Rho family GTPases. Additionally, disruptions of conserved host pathways have recently been reported to elicit immune responses in the nematode *Caenorhabditis elegans* (13, 37, 39) and in the fruit fly *Drosophila melanogaster* (8). Together, these host responses appear to represent a novel mode of immunosurveillance that relies not on ligand-receptor interaction but instead on the host's detection of the disruption of a crucial cellular process.

The intracellular pathogen *Legionella pneumophila* has proven an effective tool for probing novel mechanisms of innate immune sensing (16). *L. pneumophila* is a Gram-negative, motile bacterium that evolved as a parasite of freshwater amoebae. It can also infect macrophages in the mammalian lung, causing a severe inflammatory pneumonia known as Legionnaires' disease (14). Because *L. pneumophila* does not appear to be transmitted between mammals (31), it has not evolved significant immune evasion mechanisms. Thus, it can be used to reveal immunosurveillance pathways in the absence of the manipulation or evasion that is common with other, better-adapted pathogens.

Upon phagocytosis by the host amoeba or macrophage, *L. pneumophila* employs a type IV secretion system, called the Dot/Icm system, to translocate over 200 effector proteins into the host cytosol (51). These effectors manipulate host cell processes to remodel the *Legionella*-containing vacuole, preventing fusion with lysosomes and resulting in creation of a specialized compartment in which the bacteria can replicate (25, 28). The Dot/Icm apparatus is essential for bacterial replication and virulence, but its deployment also exposes the bacteria to cytosolic host surveillance pathways (discussed below).

Various PRRs are known to recognize *L. pneumophila*, leading

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to activation of multiple downstream pathways. Several Toll-like receptors (TLRs), especially the acylated lipoprotein sensor TLR2, recognize both Dot/Icm-positive (Dot/Icm$^+$) and Dot/Icm-negative (Dot/Icm$^-$) *L. pneumophila* isolates in the extracellular or endosomal compartment (1–3, 12, 22, 23). In addition, multiple cytosolic PRRs respond specifically to Dot/Icm$^+$ *L. pneumophila* isolates that can access the cytosol through type IV secretion. These include the peptidoglycan sensors Nod1 and Nod2 (6, 15, 17, 45), the inflammasome proteins Naip5 and Nlrc4 (20, 32, 35, 43, 50), and an additional pathway that utilizes the adaptor protein Mavs (11, 40). The outputs of these various sensing pathways are distinct: Naip5 and Nlrc4 initiate cytokine processing and caspase-1-dependent macrophage death in response to bacterial flagellin (32, 35), while the Mavs-dependent pathway results in transcription of type I interferons and coregulated genes (40). The TLRs and Nod1/Nod2 both induce overlapping transcriptional responses resulting from activation of host MAP kinases and the proinflammatory transcription factor NF-κB (15, 45).

Interestingly, we recently reported that macrophages deficient in both TLR and Nod signaling still exhibit MAP kinase activation when infected with Dot/Icm$^+$ *L. pneumophila* bacteria (45). This activation appeared to involve secreted bacterial effectors, since host MAPK activation was abrogated during infection with a bacterial mutant lacking IcmS (45), a chaperone protein required for secretion of many Dot/Icm effectors (10). The MAPK activation could not be explained by the Mavs-dependent cytosolic pathway, as the Δ*icmS* mutant robustly induces interferon (45, 46). Thus, a previously unknown, IcmS-dependent signal led to activation of host MAP kinases in macrophages infected with *L. pneumophila*.

In a separate publication, we also demonstrated a specific innate immune response to five secreted *L. pneumophila* effector enzymes (Lgt1, Lgt2, Lgt3, SidI, SidL) that inhibit host translation through inactivation of the host elongation factor eEF1a (4, 5, 44). Induction of the immune response required the activity of the effectors, prompting us to call it the effector-triggered response (ETR) (15). We characterized several downstream consequences of the ETR, including NF-κB activation; robust transcription of a subset of genes, including stress response genes and proinflammatory cytokines; and production of cytokine protein. Mutant *L. pneumophila* lacking the five effectors was defective in induction of the ETR, as was the Δ*icmS* mutant (15).

The immune response to translation inhibition resembled the previously observed TLR/Nod-independent MAP kinase activation, in that both responses required type IV secretion and the chaperone protein IcmS (15, 45). Furthermore, we noted that some transcriptional targets in the effector-triggered response to translation inhibition have been reported to be induced downstream of MAPK signaling (7, 30, 45). Therefore, we hypothesized that the five effectors might also be responsible for the activation of host MAP kinases in TLR/Nod-deficient macrophages. However, a connection between these two findings—the PRR-independent activation of host MAP kinases and the response to the five bacterial effectors that inhibit protein translation—had not been established.

Here we find that activation of host MAP kinases in macrophages lacking TLR and Nod signaling is indeed due to the activity of the five *L. pneumophila* effectors that inhibit host translation. We demonstrate that these effectors shape the transcriptional response to *L. pneumophila* via MAP kinase activation. Finally, we show that other diverse translation inhibitors induce similar MAPK and transcriptional responses in macrophages, suggesting that translation inhibition itself—rather than direct detection of bacterial molecules—can elicit this response. These results provide an important link between two previously published reports, giving us a clearer picture of how the immune system may recognize and respond to pathogen-associated activities independently of PRR-PAMP interactions.

MATERIALS AND METHODS

Ethics statement. This study was carried out in strict accordance with the recommendations in the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health (41a). The protocol was approved by the Animal Care and Use Committee at the University of California, Berkeley (protocol number R301-0311BRC).

Mice and cell culture. Macrophages were derived from the bone marrow of C57BL/6J mice (Jackson Laboratory) or *Myd88*$^{-/-}$ *Nod1*$^{-/-}$ *Nod2*$^{-/-}$ mice on the B6 background (generated from crosses at the University of California, Berkeley). Macrophages were derived by 8 days of culture in RPMI supplemented with 10% serum, 100 μM streptomycin, 100 U/ml penicillin, 2 mM L-glutamine, and 10% supernatant from 3T3–macrophage colony-stimulating factor cells, with feeding on day 5.

Bacterial strains. LP02 is a streptomycin-resistant thymidine auxotroph derived from *L. pneumophila* LP01. The Δ*flaA*, Δ*5*, and Δ*5* Δ*flaA* strains were generated on the LP02 background and have been described previously (15, 43). Mutants were complemented with the wild-type effector Lgt3 or a catalytically inactive mutant with a point mutation (15) expressed from the *L. pneumophila sidF* promoter in the plasmid pJB908, which encodes thymidine synthetase as a selectable marker.

Infection and stimulation. For harvesting of RNA, macrophages were plated in 6-well dishes at a density of 1.5×10^6 cells per well and infected at a multiplicity of infection (MOI) of 1. For Western blot analyses, macrophages were plated in 6-well dishes at a density of 2×10^6 cells per well and infected at an MOI of 2. For enzyme-linked immunosorbent assay (ELISA), macrophages were plated in 24-well dishes at a density of 5×10^5 cells per well and infected at an MOI of 1. After infection, plates were centrifuged for 10 min at 400 × *g*. Where indicated, wells were treated with cycloheximide (10 μg/ml; Sigma), exotoxin A (500 ng/ml; List Biological Labs), or the p38 inhibitor SB203580 or the JNK inhibitor II (both at 1 μM; Calbiochem).

Quantitative RT-PCR. Macrophage RNA was isolated at 4 h postinfection using an RNeasy kit (Qiagen) according to the manufacturer's protocol. RNA samples were treated with RQ1 DNase (Promega) prior to reverse transcription (RT) with Superscript III (Invitrogen). cDNA reactions were primed with poly(dT). Quantitative PCR was performed as described previously (40) using a Step One Plus RT-PCR system (Applied Biosystems) with Platinum *Taq* DNA polymerase (Invitrogen) and EvaGreen (Biotium). Transcript levels were normalized to those of *Rps17*. The following primer sequences were used: for *Il1a*, 5′-ATGACCTGCAA CAGGAAGTAAAA and 3′-TGTGATGAGTTTTGGTGTTTCTG; for *Dusp1*, 5′-ACGGGGCTCAGCCTCCC and 3′-GTCAAGCATATCCTTC CGAGAA; for *Fos*, 5′-GAAGGGGCAAAGTAGAGCAG and 3′-CAACG CAGACTTCTCATCTTCA; and for *Rps17*, 5′-CGCCATTATCCCCAG CAAG and 3′-TGTCGGGATCCACCTCAATG.

Western blot analyses. At the indicated time points postinfection or poststimulation, macrophages were lysed in radioimmunoprecipitation assay buffer supplemented with 2 mM $NaVO_3$, 50 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, and 1× complete protease inhibitor cocktail (Roche). Protein levels were normalized using a micro-bicinchoninic acid kit (Pierce), and then lysates were separated on 10% NuPAGE bis-Tris gels (Invitrogen). Proteins were transferred to polyvinylidene difluoride membranes and immunoblotted with antibodies to phospho-p38, total p38, phospho-stress-activated protein kinase (SAPK)/Jun N-terminal protein kinase (JNK), or total SAPK/JNK (all from Cell Signaling). For phosphoprotein-specific blots, 2 mM $NaVO_3$ and 20 mM NaF were added to blocking and antibody solutions.

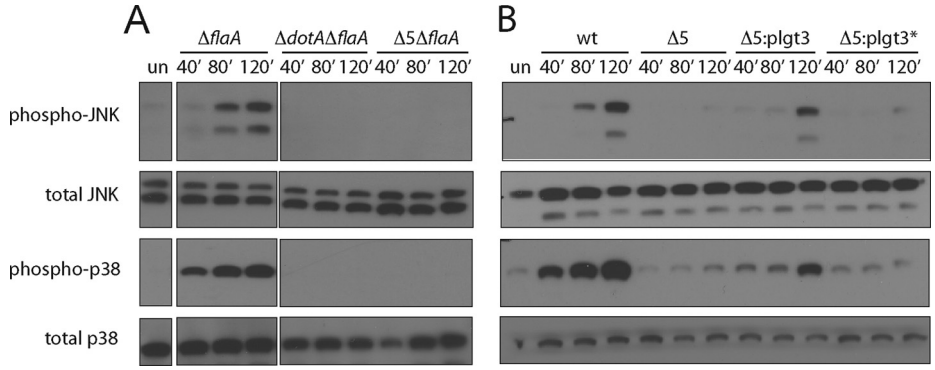

FIG 1 *L. pneumophila* effectors activate host MAP kinases in *Myd88*$^{-/-}$ *Nod1*$^{-/-}$ *Nod2*$^{-/-}$ (TKO) macrophages. (A) TKO macrophages were infected with the indicated *L. pneumophila* strains for the designated times. Cell lysates were blotted for phosphorylated and total p38 and SAPK/JNK. All panels in each row are from the same gel and exposure; intervening irrelevant lanes have been spliced out. (B) TKO macrophages were infected and blotted as described for panel A. Indicated strains were complemented with a plasmid expressing either a functional effector (plgt3) or a mutant with a point mutation lacking catalytic activity (plgt3*). wt, wild type; un, uninfected. Results shown are representative of at least three experiments.

ELISA. After 24 h of infection, supernatants were collected, cleared by centrifugation, and analyzed by ELISA using paired interleukin-1α (IL-1α) antibodies (BD Biosciences). Recombinant IL-1α (eBioscience) was used as a standard.

RESULTS

Effector-triggered activation of host MAP kinases in *L. pneumophila* infection. Previously, we showed that Dot/Icm^{+} *L. pneumophila* bacteria (but not Dot/Icm^{-} bacteria) induced activation of both p38 and SAPK/JNK MAP kinases in *Myd88*$^{-/-}$ *Rip2*$^{-/-}$ macrophages, which lack TLR and Nod signaling in response to *L. pneumophila* (45). (While Toll/interleukin-1 receptor domain-containing adaptor inducing beta interferon [Trif]-dependent signaling downstream of TLR4 is intact in these macrophages, *L. pneumophila* LPS is a very poor substrate for TLR4; most TLR-dependent recognition of *L. pneumophila* occurs via TLR2 [18].) We confirmed this finding in *Myd88*$^{-/-}$ *Nod1*$^{-/-}$ *Nod2*$^{-/-}$ (triple-knockout [TKO]) macrophages (Fig. 1A), using flagellin-deficient bacteria to avoid Naip5/Nlrc4-dependent host cell death. To determine whether the recently described bacterial effectors that inhibit host protein translation (15) were responsible for MAPK activation, we infected the TKO macrophages with a mutant strain of *L. pneumophila*, called Δ*5*, that lacks these five secreted effectors (Lgt1, Lgt2, Lgt3, SidI, SidL [4, 5, 44]). MAP kinase activation was abrogated during infection with the Δ*5* strain, as well as with the Dot/Icm^{-} mutant, the Δ*dotA* strain (Fig. 1A). MAPK activation could be restored by complementing the Δ*5* strain with one of the secreted effectors expressed constitutively from a plasmid (Fig. 1B). Importantly, however, complementation with a catalytically inactive effector did not rescue MAPK activation, indicating that the activity of the effector is required for MAPK activation (Fig. 1B).

Effector-triggered induction of MAPK-dependent host genes. It has been demonstrated that MAP kinase activation by *L. pneumophila* leads to upregulation of specific transcriptional targets, including *Il1a*, which encodes the proinflammatory cytokine IL-1α (45). We wondered whether induction of these targets was also dependent on the five effectors. Consistent with a role for these effectors in MAPK activation, we observed defective induction of *Il1a* in TKO macrophages infected with *L. pneumophila* Δ*5* (Fig. 2A). We also examined several other transcriptional targets of MAPK signaling, namely, *Dusp1*, which encodes a regulator of MAPK signaling (7), and the canonical MAPK target transcript *Fos* (30). As expected, a pharmacological p38 inhibitor abrogated induction of each of these genes (Fig. 2A to C). Furthermore, consistent with their role in MAP kinase activation (Fig. 1A and

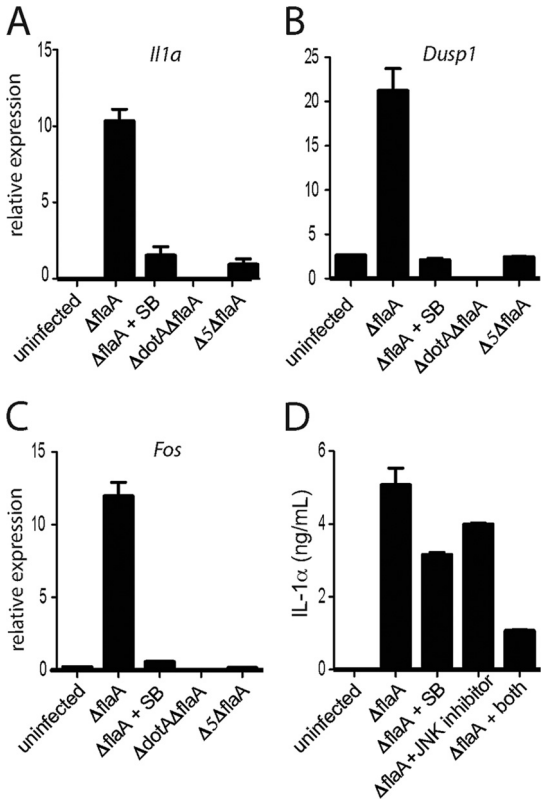

FIG 2 The five *L. pneumophila* effectors cause upregulation of MAPK-dependent host genes. (A to C) TKO macrophages were infected for 4 h with the indicated strains of *L. pneumophila*. A p38 inhibitor (SB203580 [SB]; 1 μM) was added 30 min before infection, where specified. Levels of *Il1a* (A), *Dusp1* (B), and *Fos* (C) mRNA were measured by quantitative RT-PCR. (D) B6 macrophages were infected with *L. pneumophila* Δ*flaA* for 24 h alone or in the presence of p38 inhibitor (1 μM), JNK II inhibitor (1 μM), or both (1 μM each). IL-1α protein in the supernatant was measured by ELISA. Data shown are representative of three experiments (mean ± SD for panels A to C).

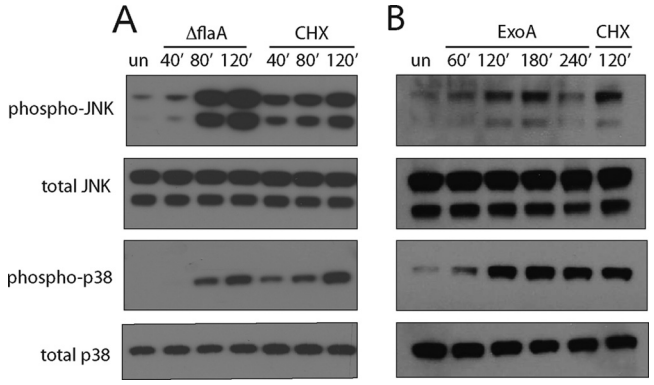

FIG 3 Other inhibitors of translation activate MAP kinases in macrophages. TKO macrophages were infected with *L. pneumophila* or treated with cycloheximide (CHX; 10 μg/ml) (A) or exotoxin A (ExoA; 500 ng/ml) (B) for the indicated time points. Cell lysates were blotted for phosphorylated and total p38 and SAPK/JNK. Results shown are representative of three experiments. un, untreated.

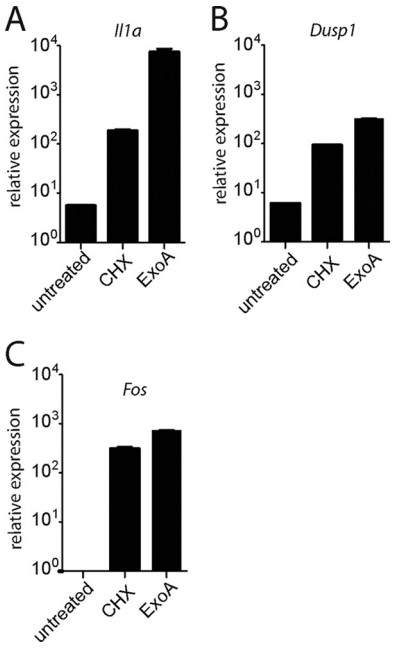

FIG 4 Translation inhibition is sufficient to induce transcription of target genes. Uninfected TKO macrophages were treated with cycloheximide (CHX; 10 μg/ml) or exotoxin A (ExoA; 500 ng/ml) for 4 h. Levels of *Il1a* (A), *Dusp1* (B), and *Fos* (C) mRNA were measured by quantitative RT-PCR. Data shown are representative of 3 experiments (mean ± SD). Note that in contrast to the graphs in Fig. 2, data are expressed in log scale to accommodate the wide range of values (e.g., >1-log-unit difference between cycloheximide and ExoA in panel A).

B), the five effectors were required for induction of these genes (Fig. 2B and C). Thus, in the absence of TLR and Nod signaling, MAP kinase activation by *L. pneumophila*-secreted effectors shapes the host transcriptional response to this pathogen.

Since *L. pneumophila* inhibits host translation during infection, it was important to examine whether the transcriptional response downstream of MAP kinase activation could actually result in production of protein. To that end, we measured IL-1α protein in the supernatant of infected wild-type macrophages. Consistent with previous reports of cytokine induction by wild-type *L. pneumophila* (15), we found that infected macrophages did indeed secrete substantial levels of IL-1α (Fig. 2D), despite a greatly reduced translational capacity (15). This protein production could be partially inhibited by either a p38 or an SAPK/JNK inhibitor and almost completely inhibited by the combination of both inhibitors (Fig. 2D). We note that transcription of *Il1a*, measured at 4 h postinfection, can be abrogated by a p38 inhibitor alone (Fig. 2A), while full suppression of IL-1α protein production, measured at 24 h postinfection, requires inhibition of both p38 and SAPK/JNK (Fig. 2D). Taken together, these results suggest that p38 and SAPK/JNK can both mediate upregulation of IL-1α, but with different kinetics. Thus, host MAPK activation can result in production and secretion of protein and may therefore impact the course of infection and the development of the immune response.

Recapitulation of MAPK activation by noninfectious inhibitors of translation. We wished to further investigate whether translation inhibition itself was the precise signal that led to activation of host MAP kinases. To do this, we treated uninfected macrophages with other inhibitors of translation: the pharmacological agent cycloheximide and the *Pseudomonas aeruginosa*-secreted toxin exotoxin A (ExoA). Interestingly, we found that treatment with each translation inhibitor resulted in activation of p38 and SAPK/JNK in TKO macrophages (Fig. 3A and B). We also observed robust upregulation of MAPK-dependent transcriptional targets in uninfected macrophages treated with cycloheximide or ExoA (Fig. 4A to C; note the log scale), indicating that translation inhibition alone is sufficient to induce these genes. Importantly, each agent that we tested—the *L. pneumophila*-secreted effectors, cycloheximide, and ExoA—has a unique structure and inhibits protein translation by a distinct mechanism. Thus, direct molecular recognition or nonspecific effects are unlikely to account for the common activation of MAP kinases. Instead, these data suggest that the block in translation itself leads to MAPK activation and transcription of downstream target genes.

Possible mechanisms. We attempted to determine the mechanism of MAPK activation by translation inhibition in macrophages. Studies in other cell types have implicated the RNA-dependent protein kinase PKR and the ribotoxic stress response in activation of MAP kinases by certain translation inhibitors, for example, Shiga toxin (19, 27, 34). However, we were unable to block MAP kinase activation with a PKR inhibitor (CAS 608512-97-6; Calbiochem; data not shown), suggesting that a ribotoxic stress response is not involved in this cell type or with this stimulus. In addition, we examined whether translation inhibition might prevent resynthesis of some unidentified short-lived inhibitor of MAPK signaling, resulting in activation of MAP kinases. We reasoned that this hypothetical mechanism would resemble the previously reported activation of NF-κB by *L. pneumophila*, in which translation inhibition results in loss of the labile inhibitor of NF-κB, IκB, allowing NF-κB activation and transcription of target genes (15). However, contrary to our hypothesis, we observed intact MAP kinase activation in macrophages infected with *L. pneumophila* in the presence of two different proteasome inhibitors (data not shown). This suggests that MAPK activation does not require degradation of an inhibitor, although we have not definitely ruled out this possibility; proteasome inhibition may be incomplete, or a putative MAPK inhibitor could be degraded or inactivated in a proteasome-independent manner.

DISCUSSION

In previous papers, we demonstrated (i) the PRR-independent activation of host MAP kinases by *L. pneumophila* (45) and (ii) a specific immune response resulting from inhibition of host protein synthesis by *L. pneumophila* effectors (15). However, it was not clear whether the observed MAP kinase activation was related to inhibition of host protein synthesis; the latter report focused instead on the mechanism of NF-κB activation in response to translation inhibition. Here we have investigated a possible link between these two published reports and found that, indeed, they describe distinct components of a single immunosurveillance pathway—namely, host monitoring of the integrity of its translational machinery. We have shown that host MAP kinases are activated by a specific pathogen-encoded activity, resulting in alteration of host transcriptional and translational responses. Our results therefore provide an important supplement to the literature and are crucial for understanding the multiple downstream signaling events that occur upon inhibition of translation by a pathogen. In macrophages, MAP kinases activate both stress-related and immune-related genes, including proinflammatory cytokines; thus, it is logical that they should be activated in response to pathogen-induced stress.

Several toxins and small molecules that inhibit translation have previously been reported to induce MAP kinase activation in diverse cell types, including intestinal epithelial, monocyte, and fibroblast cell lines (19, 24, 27, 48). However, in these cases, the precise disruption that leads to MAPK activation is thought to be not translation inhibition itself but a ribotoxic stress response resulting from damage to the 28S rRNA and transmitted by the RNA-dependent protein kinase PKR (27, 47). Evidence for this model stemmed from the observation that agents that targeted the 28S rRNA peptidyl transferase center, such as anisomycin and Shiga toxins, activated MAP kinases in a PKR-dependent manner in a fibroblast cell line, while other translation inhibitors such as ExoA did not activate MAP kinases (27).

In contrast to these reports, all translation inhibitors that we tested—including ExoA—were capable of activating MAP kinases in macrophages. Furthermore, the MAPK activation that we observed could not be abrogated by inhibition of PKR. These results imply that there are cell-type-specific differences in the mechanisms of MAP kinase activation in macrophages compared with those in other cell types. Furthermore, given that each of the translation inhibitors that we tested has a distinct structure and mode of action, we favor the hypothesis that the block in translation itself—rather than either direct molecular recognition or more indirect effects, such as ribosomal damage—gives rise to MAP kinase activation in macrophages. As with other cell types (19, 24, 27, 34, 48), further research is needed to elucidate the molecular interactions that lead to MAP kinase activation downstream of translation inhibition in macrophages. Nevertheless, this report provides a novel example of immune activation by an intracellular pathogen and identifies both the bacterial factors that elicit the response and the host pathway that is activated.

Because *L. pneumophila*'s natural hosts, amoebae, do possess MAP kinase pathways, it is possible that *L. pneumophila* has evolved to intentionally activate host MAP kinases to further its own survival (21). However, in the mammalian host, MAP kinase activation results in an immune response that does not exist in the amoebae and that may help restrict growth and spread of the bacteria (33). Translation inhibition by *L. pneumophila* also results in activation of the proinflammatory NF-κB pathway (15, 36), which is not present in amoebae and is therefore unlikely to be an intentional target of *L. pneumophila* survival strategies (21). In consideration of all these factors, we favor the hypothesis that MAP kinase activation in response to *L. pneumophila* represents a host mechanism that has evolved to detect disruptions in the vital process of protein synthesis. Indeed, such a mechanism may be conserved in metazoans: several recent papers report that translation inhibition also elicits an immune response in *C. elegans* and that this response is partially dependent on the p38 MAP kinase PMK-1 (13, 37, 39).

Conceptually, host detection of pathogen-associated activities bears resemblance to the well-established mode of PAMP-PRR recognition in several important ways. Since PRRs are relatively few in number and are hardwired in the germ line, they must target a relatively low number of slow-to-evolve microbial features. Unlike many microbial molecules, pathogen-associated activities cannot easily be mutated without negative consequences for the pathogen. Furthermore, many diverse pathogens—including both bacteria and viruses—perturb a relatively small set of host physiological processes, such as plasma membrane integrity, vesicle trafficking, host translation, and cytoskeletal dynamics (49). Thus, pathogen-associated activities are equivalent to a set of highly conserved, difficult-to-modify features that make attractive potential targets for innate immune recognition. While recognition of pathogenic activities may not provide information about the class of pathogen present, this information may be obtained by simultaneous detection of microbial molecules, chosen in turn for their high conservation and slow evolvability. Recognition of pathogenic activities therefore provides an important complement to the long-established recognition of microbial molecules that serves as the cornerstone of innate immunity.

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