Mast cells elicit proinflammatory but not type I interferon responses upon activation of TLRs by bacteria

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Balanced induction of proinflammatory and type I IFN responses upon activation of Toll-like receptors (TLRs) determines the outcome of microbial infections and the pathogenesis of autoimmune and other inflammatory diseases. Mast cells, key components of the innate immune system, are known for their debilitating role in allergy and autoimmunity. However, their role in antimicrobial host defenses is being acknowledged increasingly. How mast cells interact with microbes and the nature of responses triggered thereby is not well characterized. Here we show that in response to TLR activation by Gram-positive and -negative bacteria or their components, mast cells elicit proinflammatory but not type I IFN responses. We demonstrate that in mast cells, bound bacteria and TLR ligands remain trapped at the cell surface and do not undergo internalization, a prerequisite for type I IFN induction. Such cells, however, can elicit type I IFNs in response to vesicular stomatitis virus which accesses the cytosolic retinoic acid-inducible gene I receptor. Although important for antiviral immunity, a strong I IFN response is known to contribute to pathogenesis of several bacterial pathogens such as *Listeria monocytogenes*. Interestingly, we observed that the mast cell-dependent neutrophil mobilization upon *L. monocytogenes* infection is highly impaired by IFN-β. Thus, the fact that mast cells, although endowed with the capacity to elicit type I IFNs in response to viral infection, elicit only proinflammatory responses upon bacterial infection shows that mast cells, key effector cells of the innate immune system, are well adjusted for optimal antibacterial and antiviral responses.

endolysosome | innate immunity | *Listeria* | *Salmonella* | vesicular stomatitis virus

Pattern-recognition receptors (PRR) such as Toll-like receptors (TLRs) are key to innate immune detection and response against foreign agents and in induction of tissue injury. PRRs sense microbial-associated or damage-associated molecular patterns released by stressed or dying host cells (1–3). Activation of TLRs results in the production of a large set of NF-κB–dependent proinflammatory cytokines and type I IFNs induced via IFN regulatory factors (1, 4). Type I IFNs represent a large family of proteins composed of IFN-α, -β, -ω, -ε, and -κ. All signal through a common type I IFN receptor (IFNAR) and activate a large set of IFN-stimulated genes which mediate antiviral responses and exert a broad range of immunomodulatory effects that influence the outcome of nonviral infections as well as the pathology of inflammatory diseases and cancer (2).

Although principally meant to protect the host, production of proinflammatory cytokines and type I IFNs, when excessive or dysregulated, can cause toxicity, leucopenia, and autoimmunity and even can render the host more susceptible to certain infections. A strong proinflammatory response generally is associated with the rapid resolution of bacterial infection; however, depending on the bacterial pathogen and the experimental system used (e.g., in vitro versus in vivo), type I IFNs can play a protective role (5–9) or can enhance host susceptibility to bacterial infection (10–14). Furthermore, type I IFN signaling has been identified as one of the key pathologic events promoting bacteria-associated inflammatory disorders such as sepsis (15). The biological explanation for the ability of type I IFNs to mediate opposing immunological consequences is not clear. Different bacterial pathogens target different pathways leading to type I IFN responses of variable strength. Arguably, the broad spectrum of host responses variably triggered by type I IFNs might be dependent on the strength of type I IFN signaling. Therefore, whether type I IFN signaling serves a beneficial or injurious function could well come down to the strength of type I IFN response elicited. For example, although ample data suggest that a functional type I IFN system is required for optimal immunity against *Streptococcus pneumoniae* infection (16), recent studies also indicate that the robust type I IFN response following influenza is the key susceptibility factor responsible for postinfluenza pneumococcal superinfection (12). These findings suggest that, although moderate type I IFN responses such as those elicited by extracellular bacteria such as *Streptococcus* could be favorable to the host, strong type I IFN responses such as those induced by intracellular pathogens such as *Listeria monocytogenes* or viruses could be detrimental. In any case, for efficient antimicrobial defense, both proinflammatory and type I IFN responses must be regulated tightly. How the host balances the production of proinflammatory and type I IFN responses to optimize protection against a wide range of microbes, on the one hand, while guarding against self injury, on the other hand, is not well understood. The regulatory mechanisms underlying this balance, which obviously are dependent on the type of microbes encountered, most likely vary from one cell type to another.

Mast cells are among the key effector cells of the innate immune system strategically localized along host–environment interfaces such as mucosal surfaces, skin, and in the vicinity of blood and lymphatic vessels where pathogens and other environmental agents are encountered frequently. Thus, in addition to their well-established role in the pathogenesis of allergy and autoimmune disorders, mast cells now are recognized as key effector cells in the induction of protective immune responses to a wide range of microbial pathogens (17–21).

Mast cells are endowed with several unique properties that enable them to initiate rapid and sustained immune reactions.

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Data deposition: We have deposited microarray data in the public database http://www.ncbi.nlm.nih.gov/geo/ under GEO series accession number GSE18500.

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First, mast cells are equipped with a large set of receptors such as TLRs and thus can recognize a wide range of microbial products as well as damage-associated molecular patterns (17–21). Second, being long-lived cells that can reenter cell cycle and proliferate locally, mast cells are able to respond repeatedly to stimulation (21). Third, and most importantly, in addition to de novo synthesis, mast cells are unique in that they are packed with presynthesized mediators such as histamine, proteases, cytokines, and chemokines. Upon appropriate triggering, such mediators are released immediately to initiate innate immune reactions (17–21). Although these aspects of mast cell biology are well recognized, not much is known about the spectrum of mediators induced in response to different microbial stimuli. In particular, although mast cells are known to be a ready source of several proinflammatory mediators such as TNF-α, whether these cells also elicit type I IFN responses and how the balance between proinflammatory and type I IFN responses is regulated to achieve an optimal antimicrobial defense is unclear.

Here we show that, in response to TLR activation by Gram-positive and -negative bacteria, mast cells elicit only proinflammatory but not type I IFN responses. Our results suggest that this phenomenon could be attributed to a spatial regulation of proinflammatory and type I IFN responses from different subcellular sites. We show that TLR-induced proinflammatory responses occur from the cell surface, whereas type I IFN responses are induced from endolysosomal compartments. Although bacteria or bacterial TLR ligands such as LPS can bind cell-surface receptors to trigger proinflammatory responses, such ligand–receptor complexes in mast cells, unlike those in macrophages, are not trafficked into intracellular compartments, a prerequisite for the induction of type I IFNs.

Results

IFN-β Impairs Mast Cell-Dependent Mobilization of Neutrophils in Response to *L. Monocytogenes* Infection. Infection with bacteria is accompanied by a rapid influx of neutrophils that mediate bacterial clearance from the site of infection. Previously we showed that, in the case of *L. monocytogenes*, this early innate immune response is caused by proinflammatory cytokines such as TNF-α rapidly released from mast cell granules (22). In addition to proinflammatory cytokines, type I IFNs constitute a key host response induced by *L. monocytogenes*. So far, studies indicate that type I IFN production is detrimental to the host during *L. monocytogenes* infection (10, 11, 13). However, whether mast cells also contribute to type I IFN production and how type I IFNs modulate early inflammatory responses such as neutrophil mobilization is undetermined. As shown previously (22), mice depleted of mast cells were impaired in the production of the proinflammatory cytokine TNF-α (Fig. S1) and exhibited diminished ability to mount neutrophil recruitment (Fig. 1 *A* and *B*). Interestingly, when *L. monocytogenes* was inoculated into mice i.p. together with recombinant IFN-β, neutrophil mobilization was impaired to an extent comparable to that in mast cell-depleted mice (Fig. 1 *A* and *B*). Although mast cells were crucial for optimal neutrophil mobilization, *L. monocytogenes* still could elicit neutrophil recruitment in the absence of mast cells, albeit only partially (Fig. 1 *A* and *B*). Intriguingly, this partial mast cell-independent neutrophil response was not impaired by IFN-β, suggesting that type I IFNs impair mast cell-dependent neutrophil mobilization.

Given the crucial role of mast cells in neutrophil mobilization and the apparent inhibitory effect of IFN-β, we wondered whether mast cells also elicit IFN-β production and, if so, how these opposing immunological responses are regulated to ensure optimal antibacterial immunity. To determine whether mast cells contribute to IFN-β response during bacterial infection in vivo, we used the IFN-β–luciferase reporter mouse (*Δβ-luc*) (23). Following i.p. infection, mast cells and macrophages were sorted by flow cytometry as previously described (22) and were analyzed for IFN-β induction

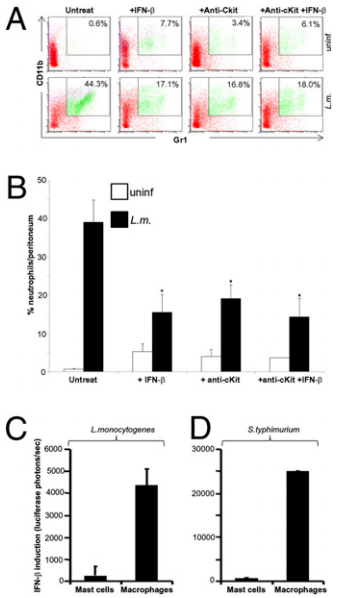

Fig. 1. Mast cell-dependent neutrophil recruitment during infection with *L. monocytogenes* is inhibited by IFN-β. Balb/C mice (four per group) were left untreated or were treated with 1.5 mg anti-c-kit for 24 h to deplete mast cells. Thereafter they were infected (or not) i.p. with 10^6 *L. monocytogenes* with or without 5,000 U recombinant *IFN-β*. (*A*) FACS dot plots of neutrophils ($CD11b^+GR1^+$) in peritoneum after 3 h. (*B*) Quantification of % neutrophils in the peritoneum as shown in *A*. *, $P < 0.001$ infected vs. untreated mice. *L.m.*, *Listeria monocytogenes*; uninf, untreated. (*C* and *D*) Mast cells do not contribute to *IFN-β* induction upon bacterial infection in vivo. After 3 h of infection with 10^6 *L. monocytogenes* (*C*) or 5×10^6 *S. typhimurium* (*D*), peritoneal mast cells ($c\text{-}kit^+$) and macrophages ($CD11b^+Gr1^-$) were sorted from *Δβ-luc* mice, and *IFN-β* induction (luciferase expression) was quantified. Cells were sorted from pooled peritoneal cells from five mice per group.

by luminescence measurement. As shown in Fig. 1 *C* and *D*, IFN-β response was detected in macrophages but not in mast cells. In addition to *L. monocytogenes,* we also analyzed whether mast cells elicit IFN-β responses upon infection with the Gram-negative bacterium *Salmonella typhimurium*. Similarly, macrophages but not mast cells were found to elicit an IFN-β response upon *S. typhimurium* infection (Fig. 1*D*). Together, the data suggest that during bacterial infection in vivo, mast cells are biased toward an acute proinflammatory response devoid of type I IFN production.

Mast Cells Exposed to *L. monocytogenes* or *S. typhimurium* Elicit a Transcriptional Program Devoid of Type I IFN-Stimulated Genes. Next, to get an overview of the general responsiveness of mast cells against bacterial pathogens, we used microarrays to define the global transcriptional program elicited by the Gram-positive bacterium *L. monocytogenes* and the Gram-negative *S. typhimurium* in bone marrow-derived mast cells (BMMC) (Fig. S2) and compared it with that of macrophages. The resulting data revealed 1,421 and 248 genes to be up-regulated (at least 1.5 log two-fold) by both *L. monocytogenes* and *S. typhimurium* in macrophages and mast cells, respectively (Fig. 2*A*). Some of the genes up-regulated in both cell types included classical NF-κB–dependent proinflammatory genes such as *NFkbia*, *TNF-α*, *IL-1α*, *IL-1β*, and *IL-6* (Fig. 2*B*). Further analysis demonstrated that of the genes up-regulated in WT macrophages, 235 genes were not up-regulated in IFNAR1-deficient macrophages. Interestingly, this set of genes which we designated as "type I IFN response genes" and which included *IFN-β*, *Mx-1*, *Mx-2, Stat1*, and *Ifrg5*, showed no up-regulation in WT mast cells exposed to *L. monocytogenes* or *S. typhimurium* (Fig. 2*B*). This finding again suggested that, although mast cells can transcribe proinflamma-

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tory genes in response to bacteria, their transcriptional response is devoid of type I IFN-regulated genes. We hereafter used *TNF-α* and *IL-1β* as representatives for proinflammatory genes, whereas *IFN-β* and *Mx-2* represent type I IFN response genes. It is noteworthy that, in spite of the similarity in the general transcriptional pattern induced by *S. typhimurium* and *L. monocytogenes*, the strength of transcriptional responses induced by these microorganisms varied from one gene to another, possibly emphasizing the differences in PRRs they target.

To validate the microarray data, we did quantitative reverse transcription-PCR (RT-PCR). As shown in Fig. 3 *A–H*, BMMCs incubated with *L. monocytogenes* or *S. typhimurium* elicited the transcription of proinflammatory cytokines, such as *TNF-α* and *IL-1β*, but not *IFN-β* or the IFN-inducible gene *Mx-2*. In macrophages, however, *L. monocytogenes* and *S. typhimurium* induced both proinflammatory and type I IFN genes (Fig. 3 *A–H*). Furthermore, analysis of culture supernatants revealed that, in contrast to macrophages which secreted both TNF-α and IFN-β, mast cells secreted TNF-α but not IFN-β in response to *L. monocytogenes* or *S. typhimurium* stimulation (Fig. S3).

To monitor induction of IFN-β in more detail, we also used mast cells and macrophages matured from bone marrows of the *Δβ-luc* mice (23). Consistently, *L. monocytogenes* and *S. typhimurium* elicited IFN-β responses in macrophages but not in mast cells (Fig. 3 *I* and *J*), showing again that, on encountering bacteria, mast cells produce only proinflammatory but not type I IFNs and type I IFN-dependent mediators.

Bacterial Activation of Mast Cells via TLR4 Results in Proinflammatory but Not Type I IFN Responses. To investigate the role of TLRs in the responses observed during bacterial infections, we used LPS as a representative TLR ligand. Fig. S4 shows that macrophages stimulated with LPS could elicit *TNF-α*, *IL-1β*, and the type I IFN genes *IFN-β* and *Mx-2*. In contrast mast cells activated only proinflammatory but not type I IFN genes. This finding suggests that, in contrast to macrophages in which TLR4 signaling is associated with a balanced induction of proinflammatory and type I IFN responses, in mast cells TLR4 signaling is characterized by a skewed response devoid of type I IFN induction.

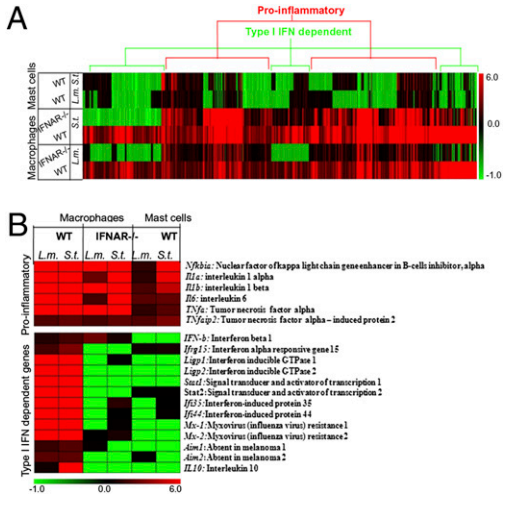

Fig. 2. Mast cells exposed to *L. monocytogenes* or *S. typhimurium* elicit a transcriptional program devoid of type I IFN response genes. WT BMMC and WT or IFNAR$^{-/-}$ macrophages were infected with *L. monocytogenes* or *S. typhimurium*, respectively, and after 6 h gene expression microarrays were done. (*A*) Pearson uncentered hierarchical cluster analysis of genes with at least a 1.5 $\log_2$-fold difference in inducible gene expression compared with unstimulated cells. (*B*) Representative proinflammatory and type I IFN-dependent response genes.

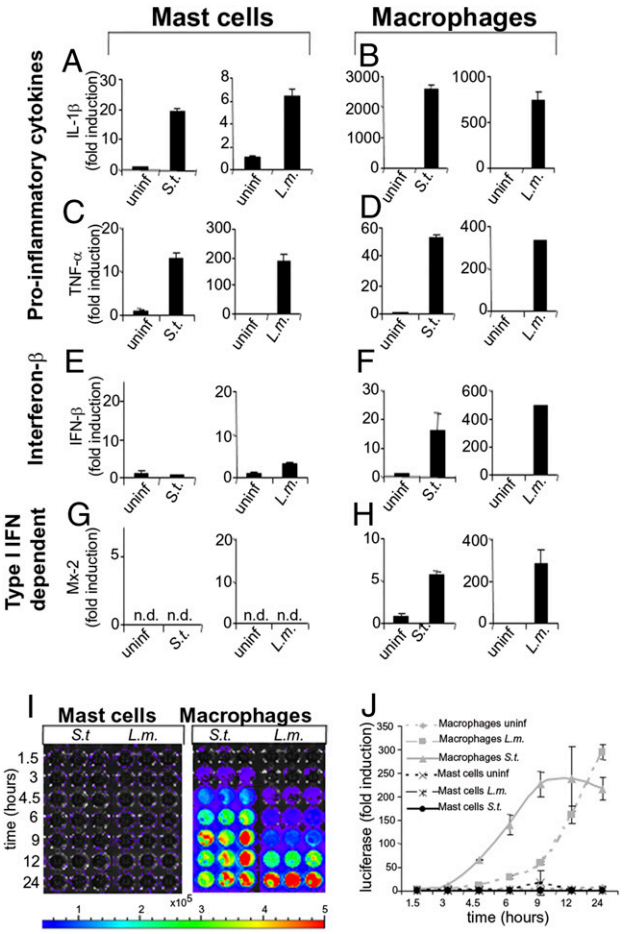

Fig. 3. Proinflammatory but no type I IFN responses are seen in mast cells upon bacterial infection. (*A–G*) BMMC and macrophages were infected with *L. monocytogenes* or *S. typhimurium* After 6 h, cytokine induction was assayed by RT-PCR. (*A–D*) Induction of proinflammatory cytokines. (*E–H*) Induction of type I *IFN-β* and IFN-dependent gene *Mx-2* in mast cells and macrophages. Data are representative of three independent experiments (mean ± SEM). (*I* and *J*) IFN-β induction was monitored over time using cells derived from *Δβ-luc* mice. *I* shows representative luminescence images of mast cells and macrophages; *J* depicts corresponding fold induction over time.

***L. monocytogenes*, *S. typhimurium*, and LPS Bound to Mast Cells Are Not Transported into Intracellular Compartments.** It had been proposed that the subcellular compartments where microorganisms localize may influence the nature of cellular responses triggered (24). Accordingly, all the TLRs associated with potent type I IFN responses, such as TLR3, TLR7/8, and TLR9, are intracellular receptors that recognize microbial products in endosomes (25). Furthermore, it has been proposed that, upon activation, TLR4 translocates from the plasma membrane into endosomal compartments in macrophages where it triggers type I IFN induction (26). On the other hand, bacteria such as *L. monocytogenes* also can trigger cytosolic signaling pathways leading to proinflammatory as well as type I IFN responses (27). Thus, to investigate the inability of mast cells to elicit type I IFN responses following TLR activation by bacteria, we compared the interaction and subcellular localization of bacteria in mast cells and macrophages. First, mast cells and macrophages isolated ex vivo by flow cytometry from peritoneal washouts of mice infected with *L. monocytogenes* or *S. typhimurium* were incubated with gentamycin to kill extracellular bacteria and then were plated on bacterial agar to determine the extent of bacterial internalization

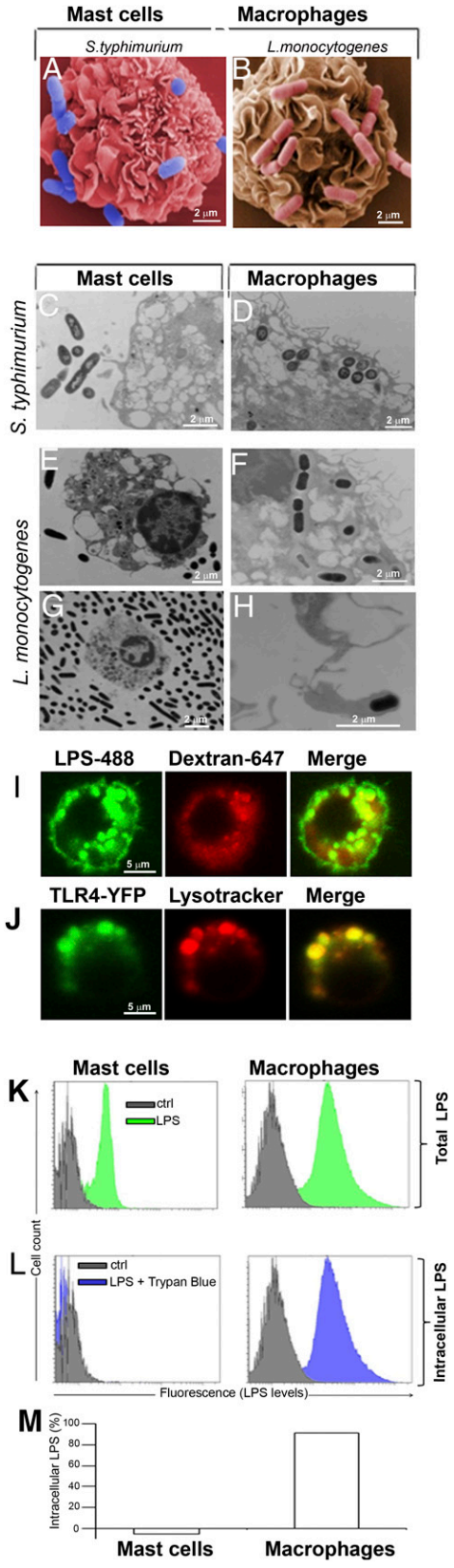

Fig. 4. Subcellular localization of bacteria and LPS in mast cells and macrophages. (*A–H*) Mast cells and macrophages were incubated with *L. monocytogenes* or *S. typhimurium* and processed 3 h later for electron microscopy. (*A* and *B*) Scanning electron micrographs of infected mast cells. (*C–H*) Transmission electron micrographs of ultrathin sections of mast cells and macrophages. (*I* and *J*) Internalization and trafficking of TLR4 into endolysosomal compartments. RAW 264.7 macrophages were loaded with fluorescent dextran-Alexa Fluor 647 (Dextran-647) and then were stimulated with 1 μg/mL LPS-Alexa 488; live confocal microscopic imaging was done 3 h later (*I*). RAW 264.7 macrophages transfected with TLR4-YFP were loaded with fluorescent LysoTracker and then were stimulated with 1 μg/mL LPS; live confocal microscopic imaging was done 3 h later (*J*). (*K–M*) Mast cells and macrophages were incubated with LPS-Alexa 488 (1 μg/mL) for 3 h. After washing, cells were subjected to flow cytometry analysis to measure total cell-bound LPS (*K*) or were incubated with trypan blue to quench cell surface-associated LPS before intracellular LPS was measured (*L*). The level of cell-associated LPS is shown as mean fluorescent intensity (MFI) of the cells. *M* shows the quantification of intracellular LPS in mast cells and macrophages calculated from the MFI of cells depicted in *K* and *L*.

by such cells. As shown in Fig. S5, intracellular bacteria were associated with macrophages but not with mast cells.

For further analysis, we used microscopy. The scanning electron micrographs in Fig. 4 *A* and *B* show that *L. monocytogenes* and *S. typhimurium* do bind to mast cells. However, as shown in Fig. 4 *C* and *E*, when ultrathin sections were analyzed carefully by transmission electron microscopy, no bacteria were found inside mast cells. In fact, even when incubated with an extremely high dose of *L. monocytogenes* that would kill macrophages rapidly, no bacteria were found within mast cells (Fig. 4*G*). Interestingly, even at such high doses mast cells were still viable. In clear contrast, in macrophages most of the *L. monocytogenes* and *S. typhimurium* were found within the cells (Fig. 4 *D* and *F*). In macrophages, *S. typhimurium* was found mainly in endosomal vacuoles, whereas *L. monocytogenes* was found in endosomes as well as in the cytoplasm (Fig. 4 *F* and *H*). Cytoplasmic *L. monocytogenes* displayed the typical formation of actin tails within the cytosol (Fig. 4*H*).

The present findings, however, do not suggest that mast cells completely lack the ability to internalize bacteria. Previous studies from Malaviya et al. (28) and Abraham and coworkers (29) have shown that mast cells can mediate CD48-dependent bacterial internalization. So far, however, such internalization has been shown to be possible mainly for bacteria engineered to overexpress the mannose-binding subunit of type I fimbriae, FimH, indicating that, except for such notable exceptions, mast cells, unlike macrophages, generally are not well adapted for bacterial internalization. Next we compared the internalization of bacterial TLR ligand LPS or its receptor TLR4 in mast cells and macrophages. To do so, we analyzed the subcellular localization of TLR4-YFP or cell-bound fluorescent LPS. Confocal live imaging of macrophages incubated with LPS depicted that TLR4-YFP and bound LPS clustered mainly in intracellular vesicles that highly colocalized with the endolysosomal markers LysoTracker or dextran (Fig. 4 *I* and *J*). In contrast to macrophages, these intracellular clusters of activated TLR4 could not be observed in mast cells (Fig. S6). To quantify cell surface-bound and internalized LPS, we resorted to flow cytometry. Mast cells and macrophages were incubated with LPS-Alexa 488 for 3 h and then were analyzed by flow cytometry. As expected, both cell types bound the TLR4 ligand strongly (Fig. 4*K*). However, quenching the fluorescence of cell surface-bound LPS using trypan blue completely abolished the signal observed for mast cells but not for macrophages (Fig. 4*L*). This finding clearly indicates that LPS bound to mast cells remains extracellular, whereas that bound to macrophages is endocytosed with an efficiency of almost 100% (Fig. 4*M*) and hence is not amenable to quenching by trypan blue. Thus, despite binding much like that seen in bacteria, LPS in mast cells remains trapped at the cell surface and is not internalized.

Internalization and Endosomal Acidification Is a Prerequisite for Induction of Type I IFN Responses by Bacteria or LPS. Next, we blocked bacterial internalization and endolysosome maturation in macrophages and tested whether in such situations bacterial TLR

activation could elicit a transcriptional response similar to that in mast cells. We used cytochalasin D, which prevents receptor internalization by disrupting the actin cytoskeleton, and the proton pump inhibitor bafilomycin A, which impairs the phagosome maturation pathway by blocking endosomal acidification. In cells treated with either of the inhibitors, bacteria were found on the cell surface and did not colocalize with the endolysosomal marker LysoTracker (Fig. S7 *A–C*). Importantly, although cells treated with either of the inhibitors could elicit proinflammatory responses (TNF-α), they were profoundly impaired in *IFN-β* and *Mx-2* induction upon bacterial exposure (Fig. S7 *F–H*). Comparable results also were observed using the TLR4 ligand LPS (Fig. S7 *D* and *E*). Thus, in the absence of internalization and translocation into endolysosomal compartments, macrophages respond to bacteria or TLR ligand only with proinflammatory cytokines but with not type I IFNs, much like mast cells.

Mast Cells Elicit Strong Proinflammatory and Type I IFN Responses upon Viral Infection. To exclude the possibility that the type I IFN system of mast cells is generally unresponsive, we used vesicular stomatitis virus (VSV), which invades the cytoplasm, triggering the cytosolic receptor, retinoic acid inducible gene (*RIG*) (30–32). We used the VSV mutant AV2, which induces high IFN production in target cells. By scanning and transmission electron microscopy, we observed that VSV-AV2 can bind to, invade, and reside in intracellular endosomal compartments of mast cells (Fig. 5 *A* and *B*). Next, microarrays were used to compare the global gene transcriptional profile elicited by VSV-AV2 in mast cells and macrophages. Detailed analysis revealed that VSV-AV2 induced the up-regulation of both proinflammatory and type I IFN responses in mast cells and macrophages (Fig. S8). These findings were confirmed by quantitative RT-PCR of proinflammatory (*TNF-α*, *IL-1β*) and type I IFN (*IFN*-β, *Mx-2*) genes (Fig. 5 *C* and *D*) as well as by analyzing cells from IFN-β–luciferase reporter mice (Fig. S9). As depicted in Fig. 5 *C* and *D*, these genes could be induced by viable but not by heat-killed viral particles. Because, in principle, heat-killed viral particles should activate TLRs but cannot enter the cytoplasm, these results suggest that VSV-induced responses were caused largely by viral activation of cytosolic receptors and not of TLRs.

Virus-Induced Proinflammatory and Type I IFN Responses Require Endosomal Acidification. Next we investigated whether virus-induced responses require endosome maturation. Inhibition of endosomal acidification using bafilomycin A effectively abrogated VSV-AV2–induced responses in mast cells and macrophages (Fig. 5 *E* and *F*). This finding is consistent with recent studies showing that endosomal acidification is crucial for the membrane fusion process that delivers the VSV into the cytoplasm (30–32). However, unlike the selective inhibition of type I IFN responses observed during bacterial infection and TLR activation, inhibition of endosomal acidification abrogated both the type I IFN and the proinflammatory responses induced by VSV-AV2. This finding suggests that both proinflammatory and type I IFN responses are induced mainly by viable virus that is able to enter the cytoplasm via the endolysosomal compartments.

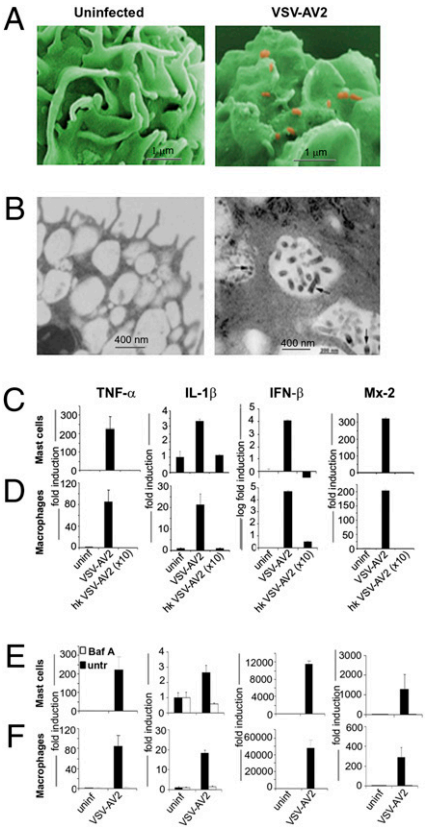

Fig. 5. Mast cells elicit proinflammatory and type I IFN responses upon viral infection. Scanning (*A*) and transmission (*B*) electron micrographs of uninfected and VSV-AV2–infected mast cells. (*C* and *D*) Mast cells and macrophages were stimulated with viable or heat-killed VSV-AV2 (hk VSV-AV2) and then were assayed 6 h later by RT-PCR for *TNF-α*, *IL-1β*, *IFN-β*, and *Mx-2*. (*E* and *F*) Induction of proinflammatory and type I IFN responses by VSV requires endosomal acidification. Mast cells and macrophages pretreated with 50 μM bafilomycin A or not pretreated were incubated with VSV-AV2 for 6 h and then were analyzed by RT-PCR for *TNF-α*, *IL-1β*, *IFN-β*, and *Mx-2*. Each bar represents average of triplicate stimulations. Data are representative of three independent experiments (mean ± SEM). Arrows in *B* indicate VSV particles in intracellular vesicles.

Discussion

Mast cells are endowed with multiple receptors for several microbial components. By virtue of their strategic localization at host–environment interfaces where microbes are first encountered and because these cells are packed with presynthesized mediators ready for secretion, mast cells are among the first cells that initiate immune reactions. This activity could be beneficial against microbes or, in the worst case, could lead to the onset of hyperinflammatory diseases. In the present study, we investigated the nature of responses induced by these cells during bacterial and viral infection. In particular, we addressed how the balance between proinflammatory and type I IFN responses in these cells is regulated. We show that mast cells can interact with the Gram-positive and -negative bacteria *L. monocytogenes* and *S. typhimurium*. Although inducing a set of proinflammatory cytokines, this interaction is not accompanied by the induction of type I IFNs. Our results suggest that this partial response is caused by the inability of TLRs in mast cells to translocate into acidic endolysosomal compartments where signals leading to type I IFN responses are induced. Whereas bound bacteria or LPS in macrophages were localized mainly within endolysosomal compartments, we found that in mast cells they were restricted to the cell surface. Accordingly, macrophages in which internalization of bacteria and LPS or endosomal acidification was blocked elicited proinflammatory but not type I IFN responses, much like mast cells. However in response to VSV-AV2, which fuses with the cell membrane and enters the cytoplasm to activate the *RIG-I* receptor (31), mast cells could elicit the induction of proinflammatory as well as type I IFN responses.

The molecular explanation of why TLR triggering can induce only type I IFNs from acidic endolysosomal compartments is not clear. Aside from the endosomal compartments being home to TLR3, TLR7/8, and TLR9, which are associated with a strong type I IFN response and which recognize nucleotides generated from microbes degraded in such compartments, as evident from

the LPS-TLR4–induced responses, the importance of endolysosomal localization is obviously more than enabling the access of pathogen-associated molecular patterns to their cognate TLRs. One attractive hypothesis is that endolysosomal compartments serve as concentration platforms on which signaling molecules involved in type I IFN induction are assembled to mediate downstream signaling processes. We also surmise that the acidic environment in endolysosomal compartments might facilitate conformational changes within the TLR signaling complexes, thus enabling efficient interactions with downstream signaling molecules, such as TNF receptor-associated factor 3 (26, 33), required for type I IFN induction. Recent findings showing that distinct biological responses induced by TLR9 are associated with different conformational changes in distinct endocytic compartments (34, 35) are in concurrence with this idea.

Although it is clear that the ability to induce proinflammatory and type I IFN responses ensures protection of the host against a wide range of pathogens, it also has emerged that these sets of responses play distinct and even conflicting roles depending on the microbe encountered. Thus, any excesses or deregulation leading to an imbalance in these sets of responses may spell doom to the host. In this regard, although type I IFNs were found to play a protective role during some bacterial infections (16), recent findings suggest that strong type I IFN responses not only result in increased susceptibility to bacterial infections (10–14) but also are a central pathologic event in sepsis and other hyperinflammatory conditions associated with bacterial infections (2, 15).

In the race between host and pathogen, survival of the host depends on its ability to harness a response that is (*i*) rapid and (*ii*) well adjusted to achieve an optimal balance maximizing protection from infection while minimizing the pathology caused by an overzealous immune response. Mast cells are known to be ready sources of several proinflammatory cytokines that are released rapidly to initiate antibacterial host defenses (20, 22, 36–39).We now show that mast cells also can elicit type I IFNs but only in response to viral infection. As shown in this study, during *L. monocytogenes* infection strong type I IFN signaling attenuates mast cell-dependent neutrophil recruitment that is required for bacterial clearance. Therefore, the fact that mast cells are equipped with the ability to elicit type I IFNs but mount proinflammatory responses only upon TLR activation by bacteria illustrates how mast cells adjust their responses for optimal antibacterial and antiviral host defenses.

Materials and Methods

Bacterial and Viral Infections. BALB/c mice were infected i.p. with 10^6 *L. monocytogenes* or 5×10^6 *S. typhimurium* cfus. Mast cells were depleted by injecting 1.5 mg anti-c-kit for 24 h. BMMCs were infected with freshly cultured *L. monocytogenes* or *S. typhimurium* with a multiplicity of infection (MOI) of 50, whereas macrophages were infected with an MOI of 5. Extracellular bacteria were killed with gentamycin (Biochrom AG) after 1.5 h, and PCR analysis was carried out after 6 h. For microscopic analysis the bacteria were kept alive, and the subcellular location was investigated after 3 h.

BMMCs and bone marrow-derived macrophages were exposed to VSV-AV2 (MOI 2) or heat-killed VSV-AV2 (hi VSV-AV2) (MOI 20) in serum-free medium. After 4 h, complete FCS-containing medium was added, and cytokine induction was investigated after 6 h by PCR. Heat-killed virus was generated for 30 min at 96 °C and used at an MOI of 20.

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