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Role of Salmonella enterica Lipopolysaccharide in Activation of Dendritic Cell Functions and Bacterial Containment

Sebastian F. Zenk,* Jonathan Jantsch,† and Michael Hensel2*

In contrast to nonpathogenic bacteria, the Gram-negative pathogen Salmonella enterica is not eradicated, but persists in murine dendritic cells (DC). The molecular basis of this phenotype is unknown. We set out to characterize bacterial and DC functions that are involved in Salmonella persistence. Our data prove that neither bacterial nor host cell de novo protein biosynthesis is required for Salmonella persistence in DC. We identified the Salmonella O-Ag of the LPS of Salmonella as an important factor for controlling the intracellular fate of Salmonella in DC. A Salmonella strain with entirely absent O-Ag showed an increased rate of uptake by DC, altered intracellular processing, and increased degradation, and also boosted the activation of immune functions of DC. These novel findings demonstrate that in addition to the multiple functions of the bacterial LPS in adaptation to the intestinal environment and protection against innate immune function, this molecule also has an important role in interaction of Salmonella with DC. The Journal of Immunology, 2009, 183: 2697–2707.

Dendritic cells (DC)3 are a population of phagocytic cells that provide an important link between innate and adaptive immunity (reviewed in Ref. 1). Intestinal DC, especially enriched in the subepithelial dome of the Peyer’s patches, sample and phagocytose luminal Ags (2) and are capable of killing internalized microbes. In particular, the phagocytosis of intestinal pathogens such as Salmonella enterica by DC is supposed to occur within the lamina propria after overcoming the gastrointestinal epithelial layer via M cells or enforced epithelial uptake. After phagocytosis of Ags, DC are thought to mature and migrate to secondary lymphoid organs (in this study: mesenteric lymph nodes), where processed antigenic material is presented to naive CD4+ or CD8+ T cells in the context of MHC II or I molecules, respectively (reviewed in Ref. 3). Despite the central role of DC in mediating adaptive immune responses, there is accumulating evidence that specific properties of DC might also be used by bacterial pathogens as “Trojan horse” or vehicle to systemically disseminate within an infected (4–6).

Salmonella enterica is an important, Gram-negative, gastrointestinal pathogen of humans and animals with the ability to colonize different niches in the host organism, resulting in different disease outcomes (7). Human infections with S. enterica serovars such as Typhimurium or Enteritidis result in gastrointestinal diseases with localized inflammation and diarrheal symptoms. In contrast, S. enterica Serovar Typhi is highly human adapted and can cause a life-threatening systemic infection known as typhoid fever. Infection of mice with S. enterica Serovar Typhimurium mimics human typhoid fever in many aspects of pathogenesis. Salmonella is a facultative intracellular pathogen that resides in a specialized compartment within eukaryotic host cells, termed Salmonella-containing vacuole (reviewed in Ref. 8). The ability to survive and eventually replicate within mammalian cells is considered as one important virulence strategy in the pathogenesis of systemic infections by Salmonella (9). Intracellular survival and proliferation of S. enterica in host cells is a multifactorial process that involves diverse bacterial traits as composition of the bacterial cell envelope, activation of stress response systems, and the function of highly evolved virulence factors.

Due to adaption to the intestinal habitat, Gram-negative bacteria as S. enterica as well as the closely related commensal Escherichia coli possess LPS as a main constituent of the outer membrane. LPS consists of a conserved lipid A moiety with strong immunomodulatory properties responsible for its endotoxin effects, as well as a conserved inner and outer core oligosaccharide (reviewed in Ref. 10). The more exposed parts of the LPS are composed of the O-Ag, which is a modular assembly of oligosaccharide units that can vary with respect to the sugar composition, as well as in the number of their modal repeats. The O-Ag of S. enterica is found in short, long, and very long forms with 1–16, up to 35, and more than 100 modal repeats of the oligosaccharide units. It has also been observed that the LPS of Salmonella is modified during intracellular life, for example with respect to the lipid A moiety (11–13) and the down-regulation of the number of O-Ag modal repeats (14–16).

Among the specific virulence factors for intracellular life, the type III secretion system (T3SS) encoded by Salmonella pathogenicity island 2 (SPI2) is of special interest, because effector proteins translocated by this system allow intracellular Salmonella to modify host cell transport processes and to maintain the Salmonella-containing vacuole (reviewed in Ref. 17). We and others have previously reported that S. enterica is able to survive within DC (18, 19). Other phagocytosed bacteria, however, are killed by DC and processed. In contrast to other host cells, Salmonella does not proliferate within DC. Intracellular survival of S. Typhimurium in murine DC was independent from the...
function of virulence factors known to be important for survival in macrophages, such as the SPI2-T3SS (20). However, virulence factors of Salmonella are induced and are active to modify DC functions such as Ag presentation (4, 21, 22).

In this study, we set out to characterize the molecular basis of the survival of Salmonella within DC. We found that preformed bacterial factors were most important for the intracellular bacterial survival, and the structure of Salmonella LPS determines the bacterial uptake, endosomal degradation, and activation of immune functions of DC (23).

Materials and Methods
Bacterial culture conditions
S. Typhimurium strain NCTC12023 was used as a wild-type (WT) strain, and all mutant strains were isogenic to this strain. Strains used in this study are listed in Table 1. Bacteria were routinely cultured in Luria-Bertani (LB) broth or on LB plates. If required to maintain plasmids or to select strains, carbenicillin or kanamycin (Sigma-Aldrich) was added to a final concentration of 25 or 30 μg/ml. If required to maintain plasmids or to select strains, carbenicillin or kanamycin (Sigma-Aldrich) was added to a final concentration of 25 or 30 μg/ml. If required to maintain plasmids or to select strains, carbenicillin or kanamycin (Sigma-Aldrich) was added to a final concentration of 25 or 30 μg/ml.

Construction of strains and plasmids
For the generation of isogenic mutant strains of S. Typhimurium, the one-step inactivation approach was used basically, as described before (24–27). Oligonucleotides (Metabion) used for the construction of deletion strains are listed in Table S1.1.

For the complementation of chromosomal deletions of LPS biosynthesis genes, low copy number plasmids were constructed as follows. A fragment containing the promoter PrafD and genes rfaDFCL was amplified from S. Typhimurium genomic DNA using primers Pro-rfaD-For-I and RfaL-KpnI and was cloned into pBluescript KS+ (Stratagene). A fragment containing PrfaQ and genes rfaDFQG was amplified using primers Pro-rfaQ-For-KpnI and RfaL-Rev-XhoI. The resulting fragments were digested with KpnI and XhoI and subcloned into pWSK29 to generate plasmids p3313 and p3314 for the complementation of deletion of rfaD and rfaQ deletion, respectively.

To control the effect of deletions and complementing plasmids on LPS structure, bacterial clones were analyzed by slide agglutination with diagnostic antisera O4,5. The LPS profiles of selected clones were further analyzed by SDS-PAGE and silver staining, as described below.

LPS analysis
For detection of LPS, 1.5-ml bacterial cultures were grown to stationary phase, cells from 1.5 ml of culture were harvested by centrifugation, and the pellet was dissolved in 50 μl of lysis buffer per OD600 nm−1.6 and lysed at 100°C for 10 min. Lysates were subsequently digested at 65°C for 1 h with proteinase K (25 μg/ml) and 2 μg/ml of RNase. Lysates (15 μl) were separated by electrophoresis on a 12% SDS-polyacrylamide gel at 80 V, followed by an overnight fixation in 25% isopropanol, 7% acetic acid. After oxidation with 0.3 M periodic acid, gels were washed six times for 20 min with ddH2O and then stained with an alkaline, AgNO3-saturated solution. Before development with a solution consisting of 1 mM citric acid, 0.1% formaldehyde, gels were washed four times with ddH2O. The staining reaction was stopped with 0.35% acetic acid.

Preparation and culture of DC and macrophages
DC were generated from bone marrow (BM) of C57BL/6 or BALB/c mice (Charles River Laboratories), according to the protocol of Lutz et al. (28), with modifications, as described (18, 29). Before infection experiments, cells were allowed to adhere to culture plates for at least 6 h. MACS technique using CD11c MicroBeads (Miltenyi Biotec) was used to enhance the purity of CD11c-positive compartment. These enriched cells were used for uptake/survival experiments with LPS mutant strains, where indicated.

The RAW264.7 macrophage-like cell line was obtained from American Type Culture Collection, and the cells between passage numbers 8 and 25 were used for the experiments. BM-DC and RAW264.7 Macrophages were cultured in RPMI 1640 and DMEM, respectively, each containing 10% FCS (PAA). Macrophages from murine BM were generated essentially as previously described (30). Briefly, total BM cells were cultured in hydrophobic Teflon bags (DuPont, purchased via Cadillac Plastic) with 10% CO2/90% humidified air in DMEM (Invitrogen) supplemented with 0.05 mMmoll/L 2-ME, 1% nonessential amino acids, 10% FCS (PAA), 5% horse serum (Cell Concepts), and 10% L929 cell-conditioned medium. At day 8, the cells were harvested, routinely yielding a population of >90% CD11bhigh, F4/80high positive cells.

Abs, immunofluorescence, and molecular tracers
For immunostaining, the following Abs were used at the indicated dilutions: Armenian hamster anti-CD11c mAb (BD Biosciences), dilution 1/100; rat anti-hemagglutinin epitope mAb (Roche), dilution 1/250; and rabbit anti-Salmonella O4,5 antiserum (BD Biosciences), dilution 1/500. As secondary Abs (Dianova), we used goat anti-Armenian hamster conjugated to Cy5, dilution 1/100; goat anti-rabbit conjugated to Cy3, dilution 1/100; and goat anti-rabbit conjugated to Cy2, dilution 1/100.

OVA co-uptake assays were conducted with 100 μg/ml 1OVA Al- eka Fluor 647 conjugate (Molecular Probes, Invitrogen). The endocytic marker dextran-Texas Red, 70,000 m.w. (Molecular Probes, Invitrogen), was used at a concentration of 100 μg/ml and was added to the cells for 30 min, followed by washing for removal of noninternalized markers and incubation for 2 h before bacterial infection, to allow the partitioning of markers to the compartments.

For immunostaining, cells were fixed in 3% paraformaldehyde, stained, and subsequently mounted with Fluoprep (bioMérieux). Microscopy images were recorded with a Zeiss Axiosvert 200M equipped with ApoTome, AxioCam MRm, and ×20 and ×63 Plan Neofluar oil immersion objectives (Carl Zeiss). Taken images were processed with AxiOVISION 4.7 software (Carl Zeiss).

For surface phenotyping, the following fluorochrome (FITC, PE, or allophycocyanin)-labeled Abs were used (all from BD Biosciences, unless otherwise stated): anti-FcRγ-II (2.4G2), anti-CD11b (M170), anti-CD11c (HL3), anti-F4/80 (Cl:A3-1), and goat anti-MHC II (2G9), anti-CD80 (16-10A1), and anti-CD86 (GL1). The specificity of the staining was verified by the use of isotype control mAbs. All analyses were performed on a FACsCalibur (BD Biosciences), applying the CellQuest Pro software. For the stimulation of BM-derived macrophages or DC, PMA (Sigma-Aldrich) was added at final concentrations of 6 or 12 ng/ml.

Bacterial infection of cultured cells and pharmacological inhibitors
BM-DC and macrophages were infected with bacterial strains grown to stationary phase. The multiplicity of infection (MOI) was adjusted by resuspending the culture density at the OD of 600 nm. The actual MOI of each experiment was determined by plating dilutions of the inoculum onto agar plates for the determination of the number of CFU. For synchronization of

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Table I. Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype, Relevant Characteristics</th>
<th>Source/Reference</th>
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<tbody>
<tr>
<td>Salmonella enterica Serovar Typhimurium:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCTC12023 WT</td>
<td>Laboratory stock</td>
<td></td>
</tr>
<tr>
<td>NCTC12023 WT NaR</td>
<td>Laboratory stock</td>
<td></td>
</tr>
<tr>
<td>NCTC12023 rfaDFCL</td>
<td>Laboratory stock</td>
<td></td>
</tr>
<tr>
<td>MvP885 rfaDFCL</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>MvP885 rfaFL-Kpn</td>
<td>This study</td>
<td></td>
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<tr>
<td>MvP886 rfaFL-Kpn</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>MvP7590 rfaFL-Kpn</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>P2D6che rfaFL-Kpn</td>
<td>staV::mTn5, SPI2 defective</td>
<td>Laboratory stock</td>
</tr>
<tr>
<td>Enterobacter cloacae:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meratella (Nisse 1917)</td>
<td>Probiont strain, semirough LPS</td>
<td>DSM strain 6601</td>
</tr>
<tr>
<td>Serotype O6:K5:H1 (Provided by S. Schubert, Munich, Germany)</td>
<td></td>
<td></td>
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<tr>
<td>HB101 Plasmids:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pWSK29 Low copy number vector</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>pDK46 Expression of A Red genes</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>pFPV2.1 Constitutively expressing GFP, AmpR</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>pFPVmCherry Constitutively expressing mCherry, ampicillin resistance</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>p2777 pWSK PrafQ rfaDFCL</td>
<td>This study</td>
<td></td>
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<tr>
<td>p3313 PrafQ rfaDFCL</td>
<td>This study</td>
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<td>p3314 pWSK PrafQ</td>
<td>This study</td>
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The online version of this article contains supplemental material.
infection, a centrifugation at 500 × g for 5 min was performed. If not otherwise stated, BM-DC were infected at a MOI 25 for 60 min, and RAW264.7 macrophages were infected at a MOI 10 for 30 min at 37°C, 5% CO₂. If indicated, infected bacteria were opsonized by incubation with 20% normal mouse serum (The Jackson Laboratory, Dianova) for 30 min at 37°C. After infection, cells were washed to remove noninternalized bacteria. Gentamicin was added to prevent replication of remaining extracellular bacteria. For BM-DC, gentamicin was added for 1 h at 100 μg × ml⁻¹, followed by 25 μg × ml⁻¹ gentamicin for the rest of the experiment. For RAW264.7 macrophages, gentamicin was added at 100 μg × ml⁻¹ for 1 h, followed by 10 μg × ml⁻¹ gentamicin for the rest of the experiment. Infection was terminated by cellular lysis using 0.5% Triton X-100 in PBS, and the number of intracellular bacteria was determined by serial dilution in 0.05% Tween 20 in PBS and subsequent plating.

Inhibition of prokaryotic and eukaryotic protein biosynthesis was conducted with 5–80 μg × ml⁻¹ tetracycline and 0.01–10.0 μg × ml⁻¹ cycloheximide (both used at the indicated concentrations and ordered from Sigma-Aldrich), respectively.

TFN in cell supernatant was quantified with the BD OptEIA Mouse TNF (Mono/Poly) ELISA Kit II (BD Biosciences), according to the manufacturer’s instructions. To assay cell viability, the Live/Dead viability/cytotoxicity kit (Molecular Probes, Invitrogen) was used, according to the manufacturer’s instructions.

Statistical analyses
Statistical significance was calculated using Prism 4.0 software. One-way ANOVA was used for comparison of multiple data sets, and Student’s t-test for use of pairwise comparison. Statistical significance was indicated as follows: p ≤ 0.05; NS; p < 0.05; *, p < 0.01; **; p < 0.001; ***.

Results
The intracellular survival of Salmonella in DC is independent from bacterial and host cell de novo protein biosynthesis
We set out to identify the factor(s) that allows the intracellular survival and persistence of S. Typhimurium within murine DC. Because previous approaches failed to identify mutant strains with reduced survival in DC, we applied a more general approach and investigated whether bacterial proteins synthesized after bacterial uptake are required for the adaptation to intracellular life in DC. In this study, we infected BM-DC with S. Typhimurium WT and added the antibiotic tetracycline for various periods of time during the infection to inhibit bacterial protein biosynthesis. For comparison with a cell type that is permissive for intracellular replication, we used the murine macrophage-like cell line RAW264.7. In non-activated RAW264.7 cells, Salmonella WT consistently showed a 20- to 50-fold increase in viable intracellular bacteria over a period of 15 h (27) (this study), whereas the increase in bacterial counts in BM-DC was 1- to 2-fold.

To inhibit bacterial protein biosynthesis, the antibiotic tetracycline was applied at various concentrations (Figs. S1 and S2). If added 2 h after infection for 1 h, followed by subsequent removal, increasing concentrations of tetracycline did not inhibit replication of Salmonella WT in macrophages (up to 40 μg × ml⁻¹) and the survival in DC (Fig. S1). The presence of 20 or 40 μg × ml⁻¹ tetracycline for 1 h, followed by removal of the antibiotic, only led to minor reduction of intracellular proliferation in macrophages and had no significant effect on the survival of Salmonella in BM-DC (Fig. 1), or the synthesis and translocation of SseJ as a representative effector protein of the SPI2-T3SS (Fig. S2). The presence of tetracycline for a prolonged duration from 2 to 15 h after infection inhibited proliferation of Salmonella in macrophages, led to reduced intracellular survival in BM-DC (Fig. 1), and completely ablated translocation of SseJ (Fig. S2). By contrast, the same treatment of BM-DC with tetracycline 2–15 h after infection only had a minor effect on the intracellular survival, i.e., a 0.35- to 0.45-fold reduced bacterial recovery compared with the mock-treated control (Fig. 1C).

Next, we investigated the role of host cell protein biosynthesis and performed similar experiments with cycloheximide, an inhibitor of eukaryotic protein biosynthesis. Treatment of macrophages with cycloheximide resulted in increased intracellular proliferation (1.4- to 1.5-fold; Fig. 2A). By contrast, no difference in proliferation of intracellular Salmonella was observed in BM-DC treated with cycloheximide, and a slightly decreased bacterial recovery was observed (0.19- to 0.23-fold; Fig. 2B). The effect of the inhibitor was confirmed by quantification of the release of the TNF by BM-DC (Fig. 2D). Higher concentrations of cycloheximide caused eukaryotic cell death (data not shown). Application of cycloheximide at various time points before infection also resulted in increased Salmonella replication in RAW264.7 macrophages, but did not affect the intracellular bacterial survival in BM-DC (data not shown).

Taken together, inhibitor experiments suggest that neither bacterial de novo protein biosynthesis nor de novo host cell protein biosynthesis affects the intracellular fate of Salmonella in BM-DC. This unexpected result prompted us to investigate in more detail the role of preformed bacterial factors for intracellular survival in BM-DC.

FIGURE 1. Bacterial de novo protein biosynthesis is dispensable for intracellular survival of Salmonella in BM-DC, but not for replication in macrophages. A, Schematic representation of the experimental set-up of assays for intracellular survival. RAW264.7 macrophages (B) or BM-DC (C) were infected with S. Typhimurium WT at a MOI of 10 or 25, respectively. To kill residual extracellular bacteria, gentamicin (G) was added at 100 μg × ml⁻¹ for 1 h and maintained at 10 μg × ml⁻¹ for the rest of the experiment. Tetracycline (Tet) was added at a final concentration of 20 or 40 μg × ml⁻¹ at various time points, as indicated. The number of viable intracellular bacteria at 2 and 15 h postinfection was determined by lysis of host cells by addition of 0.1% Triton X-100 for 10 min and plating of serial dilutions of the lysate onto agar plates to enumerate CFU. Relative survival or replication is calculated as the ratio of CFU at 15 and 2 h after infection. Intracellular replication of S. Typhimurium WT in RAW264.7 macrophages (B) and survival in BM-DC (C) without or with addition of various amounts of tetracycline was quantified, and means and SD of three assays are shown. The inhibitory effect of tetracycline on bacterial protein biosynthesis was controlled by analyzing translocation of a Salmonella effector protein (SseJ) at various antibiotic concentrations and application time point conditions (Fig. S2). Statistical analysis, antibiotic-treated cells vs mock-treated cells: *, p < 0.05; ***, p < 0.001.
The intracellular fate of bacteria in DC is determined by the bacterial cell envelope

To approach the specific adaptation of *Salmonella enterica* to the intracellular life in DC experimentally, we first compared the uptake and intracellular fate of *S. enterica* with closely (E. coli, *Shigella flexneri*) or distantly (Yersinia enterocolitica) related Gram-negative bacteria, as well as typical Gram-positive pathogens (Staphylococcus aureus, Streptococcus pyogenes). We observed that the intracellular survival in BM-DC of the various bacteria varied substantially (data not shown). We also observed that the rates of uptake and intracellular survival varied between closely related *S. Typhimurium* and *E. coli* strains. Although genetically closely related, the *E. coli* strains used for comparison of the intracellular fate are commonly laboratory strains that differ from *S. Typhimurium* in the absence of a complete O-Ag. For a more detailed analyses, the fate of *S. Typhimurium* and two representative *E. coli* strains, i.e., the laboratory strain HB101 and the probiotic strain Nissle Mutaflor, was analyzed (Fig. 3). The phagocytic uptake of *E. coli* Mutaflor by macrophages and BM-DC in general was lower than that of *S. Typhimurium* WT in RAW264.7 macrophages (B) and the survival in BM-DC (C) were determined, as described in Fig. 1, B and C. The effect of CHX on host cell protein biosynthesis was controlled by quantification of TNF secretion (D). Culture supernatants of BM-DC from the experiment shown in C were subjected to an ELISA to quantify the amounts of TNF secreted by BM-DC. Means and SDs for three experiments are shown. Statistical analysis, antibiotic-treated cells vs mock-treated cells: *** p < 0.001.

**FIGURE 3.** Contribution of preformed bacterial factors to intracellular survival of *Salmonella* in BM-DC. Murine cell line RAW 264.7 macrophages (MΦ; A, C) or BM-DC (DC; B, D) were infected with *S. Typhimurium* WT, or *E. coli* strains Mutaflor or HB101, as indicated. A MOI of 10 and 25 was used for macrophages and DC, respectively. Noninternalized bacteria were removed by washing, and gentamicin was added to kill residual extracellular bacteria. To determine bacterial uptake (A and B), cells were lysed 2 h after infection by addition of 0.1% Triton X-100 for 10 min, and serial dilutions of the lysate were plated onto agar plates to enumerate the total number of intracellular bacteria. Relative uptake is expressed as the ratio of bacteria in the inoculum to intracellular bacteria. To determine intracellular replication in macrophages (C) or survival in BM-DC (C and D), infected cells were lysed 15 h after infection, and intracellular bacteria were quantified, as described above. Replication or survival is expressed by the ratio of intracellular bacteria at 15 and 2 h after infection. E. Analysis of the LPS of the bacterial strains used for infection. LPS extracts from equal numbers of bacterial cells (~3 × 10⁶ CFU) were loaded in each lane and analyzed by SDS-PAGE on 12% polyacrylamide gels, followed by silver staining. The relative positions of the core oligosaccharide and the O-Ag are indicated. Note the absence of the polymeric O-Ag species in LPS of *E. coli* Mutaflor and HB101. Statistical analysis, *E. coli* strains vs *S. Typhimurium* WT: *** p < 0.001.
novosynthesized factors as well as known virulence factors (19) were not required for the survival of Salmonella in BM-DC, we questioned which preformed bacterial factors might be responsible for the different outcome of the infection by S. Typhimurium and the E. coli strains. Laboratory strains of E. coli such as HB101 are known to harbor various mutations, including the lack of O-Ag. Furthermore, the probiotic strain Mutaflo has been characterized as possessing a semimorph LPS, i.e., partially lacking the polymeric O-Ag of the LPS (31). Our analysis indicated a strong difference in the LPS structure of the three strains (Fig. 3E), with E. coli Mutaflo and HB101 lacking the polymeric O-Ag, as well as the outer core oligosaccharides, respectively. These data gave a first indication for the role of the O-Ag in bacterial survival in BM-DC.

LPS O-Ag determines uptake and intracellular fate of Salmonella

To further investigate the role of the O-Ag for the intracellular survival of Salmonella in BM-DC, we generated an isogenic set of mutant strains with defined defects in the O-Ag biosynthesis. The LPS of S. Typhimurium has been studied in detail, and gene functions for the addition of the O-Ag and for the regulation of the O-Ag chain length are known (see Fig. 4A for a model). To generate a set of isogenic mutant strains with defined defects in the O-Ag, rfbP (O-Ag transferase; NP_461027) or rfaL (O-Ag ligase; NP_462613) was deleted, and the resulting strains should be defective in the addition of the polymeric O-Ag to the outer core. Deletion of rfaG (glucosyltransferase I; NP_462622) should result in a highly truncated LPS, because no outer core can be added to the essential inner core. rfaH (transcriptional activator; NP_462862) encodes a function for the stabilization of the long mRNA for LPS biosynthesis genes, and an overall reduction on the amount of the LPS species with polymeric O-Ag was anticipated (32). To analyze the effect of the various deletions on LPS structure, S. Typhimurium WT and various mutant strains were grown in liquid medium and processed for analyses of the LPS. The LPS profiles indicated that all mutant strains had the expected phenotypes with respect to O-Ag length (Fig. 4B). To restore O-Ag biosynthesis, the WT alleles of rfaL and rfaG were cloned in low copy number plasmids, and mutant strains harboring complementing plasmids showed restored O-Ag length of the LPS (Fig. 4B). We also analyzed growth rate of the various strains and did not observe differences in the growth in rich medium (LB broth) or in minimal medium (phosphate/carbon/nitrogen (pH 7.4 or 5.8); data not shown).

We next quantified phagocytic uptake of S. Typhimurium WT and various mutant strains by macrophages and BM-DC (Fig. 3F; Fig. 4C). Both cell types phagocytosed the rfaG strain with the highest rate, and uptake of the rfbP and rfaL mutant was also higher than that of the WT strain. For the intracellular replication, we observed a clear difference between macrophages and BM-DC. In macrophages, replication for rfbP, rfaL, and rfaH strains was only slightly reduced compared with Salmonella WT, whereas the rfaG strain was ~10-fold reduced in intracellular proliferation. In BM-DC, rfbP and rfaL were slightly reduced in survival (~2- to 3-fold), and a strong reduction of the intracellular survival of rfaH and rfaG mutants (7.6- and 8.6-fold, respectively) was evident (Fig. 4D). A more detailed analysis of the survival early after phagocytosis showed that both WT and rfaG Salmonella were not killed by BM-DC within the first 2 h of intracellular life (Fig. S3C; data for other LPS mutant strains not shown). The increased uptake of rfaG and rfaL strains by BM-DC and the reduced intracellular survival in BM-DC of the rfaG strain could be restored by addition of the plasmid for the expression of rfaL, or rfaG (Fig. 4E).

We next analyzed whether the intracellular fate of Salmonella in DC is affected by opsonization before uptake (Fig. 4F). As expected, the rates of uptake were 3- to 5-fold higher if bacteria were opsonized with mouse normal serum. The intracellular survival was slightly reduced for all strains after opsonization. However, the defect in intracellular survival of the rfaG strain was not affected by opsonization. Although uptake of opsonized and nonopsonized bacteria will result in different form in processing by DC, our data show that the defect of strains lacking the O-Ag remains present.

Infection of murine macrophages with Salmonella induces the formation of spurious phagosomes (SP) (33). A previous study showed that uptake of Salmonella by SP and the maintenance of SP in macrophages were required for intracellular survival of Salmonella (34). Salmonella serovars not adapted to murine hosts as well as attenuated mutant strains showed reduced induction of SP (33, 34). We analyzed whether SP formation was also induced in BM-DC and whether the LPS defect of the rfaG strain affects SP formation (Fig. S4). The infection with Salmonella WT or rfaG strains induced SP formation in BM-derived macrophages, as well as in BM-DC (Fig. S4, A and B). The number of SP per cell was similar for both strains in BM-DC and in the range of stimulation by PMA. In macrophages, higher numbers of SP were detected after infection with the rfaG strains. In accordance with our previous observations, the uptake of the rfaG strain was ~10-fold higher by primary macrophages as well as by BM-DC (Fig. S4C). In macrophages, only low numbers of Salmonella-containing SP were observed per cell, whereas the number of SP containing the rfaG strain was much higher (Fig. S4D). This difference was less pronounced in BM-DC, and at 60 min after infection the number of SP containing the WT or the rfaG strain was similar (Fig. S4D).

The O-Ag structure modulates immunological functions of DC

The response of BM-DC to infection with Salmonella WT and various mutant strains with altered O-Ag was analyzed. As a representative costimulatory molecule, the expression of CD86 was quantified (Fig. 5A). The infection with strains defective in rfbP, rfaG, rfaL, or rfaH resulted in higher levels of CD86 compared with BM-DC infected with the same amount of Salmonella WT. Similar data were obtained for other costimulatory molecules (CD80 and MHC-II; data not shown). As a further parameter for DC activation, the secretion of TNF was analyzed. Infection with any of the four strains with defects in the O-Ag biosynthesis induced higher levels of TNF secretion than infection with the WT (Fig. 5B). Highest TNF release was observed for BM-DC infected with the rfaG strain. To test whether the different activation of WT and mutant-infected BM-DC has consequences for the capacity of DC to internalize Ag, we infected BM-DC with Salmonella-expressing GFP and simultaneously added fluorescently labeled OVA (Fig. 5C). As expected from the results shown in Fig. 4, the internalization of the rfaG strain was higher than that of Salmonella WT. A higher rate of internalization of OVA was observed in cells infected with the rfaG strain, indicating increased activation. To control whether the increased uptake of rfaG Salmonella results in the uptake of OVA by the same phagocytic event, the fluorescence microcopy was performed (Fig. 5D). We found that phagocytosed bacteria and the internalized soluble Ags were located in separate compartments and only occasionally colocalized. This separation was observed in BM-DC infected with WT as well as with rfaG bacteria. This result indicates that the ability of BM-DC to internalize soluble Ags is not altered although the degree of activation is dependent on the structure of O-Ag.
Increased stimulation of DC does not enhance antimicrobial capacity

We performed infection experiments of BM-DC with mixtures of Salmonella WT and rfaG or rfaH mutant bacteria (Fig. 6). Either strain was tagged by different antibiotic resistance markers, thus allowing the quantification of the relative uptake and intracellular survival of each strain. The ratios of WT to mutant strain were 1:10, 1:1, and 10:1. As anticipated from the previous results, the internalization of the rfaG and rfaH strains was generally higher, and a 10-fold excess of the WT strains was required to achieve a higher amount of internalized WT Salmonella (Fig. 6A). The activation of infected DC as quantified by the CD86 expression was dependent on the amount of the mutant strains, but not affected by varying amounts of the WT strain (Fig. 6B). The level of activation was closely correlated to the amount of rfaG bacteria, either used for individual infection (Fig. 6B, □) or in mixture with WT Salmonella (Fig. 6B, □). Similar results were observed with mixed infections with the rfaH strain and for the quantification of other activation markers of activation (data not shown). These observations show that LPS with lacking or highly truncated O-Ag is the major factor for the activation of DC.

FIGURE 4. Role of LPS for survival of Salmonella in macrophages and DC. A, Structure of S. enterica LPS and enzymes involved in LPS biosynthesis. The function of RfaG (also referred to as WaaG) is required for the addition of the outer core, whereas RfbP and RfaL (WaaL) catalyze the transfer of the O-Ag to the outer core. RfaH (not depicted) is an antiterminator, which regulates the stability of the long mRNA transcript for O-Ag biosynthesis genes, and rfaH mutants show a highly reduced amount of O-Ag. (Model adapted from Ref. 47.) B, LPS profiles of S. Typhimurium, isogenic mutant strains deficient in rfbP, rfaG, rfaL, or rfaH, as well as complemented rfaG and rfaL mutant strains. A silver-stained SDS-PAGE was prepared, as described for Fig. 3E. Phagocytic uptake (C) and intracellular survival and replication (D) of S. Typhimurium WT and various LPS mutant strains by macrophages or BM-DC were quantified. Phagocytic uptake was determined by quantification of gentamicin-protected bacteria at 1 h after infection, whereas survival and replication were determined, as described in Fig. 1. E, Relative uptake and intracellular survival of mutant strains and complemented mutant strains in BM-DC. F, Effect of opsonization on relative uptake and intracellular survival of mutant strains and complemented mutant strains in BM-DC. Before infection of BM-DC, bacteria were either opsonized by incubation for 30 min at 37°C in buffer containing 20% mouse normal serum (black bars), or mock treated by incubation in serum-free buffer (open bars). Infection by various bacterial strains was performed at a MOI of 10, and uptake and survival of the bacteria were quantified, as described for Fig. 1. Means and SDs for triplicate assays are shown, and the data are representative for the outcome of three independent experiments. Statistical analysis, mutant strains vs S. Typhimurium WT: *, p < 0.05; ***, p < 0.001.
FIGURE 5. LPS with defective O-Ag enhances BM-DC activation and Ag uptake. A, BM-DC were infected with Salmonella WT or mutant strains with defects in LPS biosynthesis. The surface expression of the costimulatory marker CD86 on BM-DC from C57BL/6 mice was analyzed 16 h after infection with S. Typhimurium WT or various mutant strains, as indicated. The histograms show mock-infected DC (gray), and DC infected with equal numbers of WT bacteria (light lines) or bacteria of the mutant strain (bold lines), as indicated. B, After infection with Salmonella WT and various mutant strains for 15 h, the amounts of TNF secreted into cell culture supernatants were quantified. C, Uptake of OVA in presence of WT or rfaG Salmonella. BM-DC were infected with Salmonella strains harboring pFPV25.1 for constitutive expression of GFP and simultaneously stimulated with 100 μg × ml⁻¹ OVA Alexa Fluor 647 conjugate. Cells were analyzed 1 h after infection for fluorescence in FL-1 (GFP fluorescence corresponding to bacterial uptake) and FL-4 (Alexa 647 corresponding to OVA uptake). D, Compartmentation of internalized OVA and Salmonella. BM-DC were infected as in C, fixed 1 h after infection, and analyzed by epifluorescence microscopy. Identical exposure times for the fluorescence channels were selected to allow comparison of the signal intensities. Scale bar, 20 μm. Statistical analysis, mutant strains vs S. Typhimurium WT: *** p < 0.001.

We next investigated whether the different degree of activation of DC can affect the intracellular fate of the intracellular bacteria (Fig. 6C). Surprisingly, the difference in intracellular survival between the WT and the rfaG or rfaH strains was rather constant and not affected by the different amounts of strains with defective O-Ag present in the mixtures. The presence of higher amounts of rfaG or rfaH Salmonella and inherent higher activation of the host cells had no apparent effect on the intracellular survival of WT strains present in the mixture.

The subcellular localization of WT and LPS-deficient strains was analyzed by immunofluorescence microscopy (Fig. 6D). Strains were labeled by the expression of distinct fluorescent proteins. After simultaneous infection of BM-DC with a mixture of WT (GFP) and WT (mCherry), we observed that compartments harboring the bacteria were frequently colocalized. By contrast, the distribution of the WT and the rfaG strain was different, and events of colocalization of compartments harboring WT and rfaG bacteria were very rare. This observation shows that the intracellular fate of Salmonella with intact and defective LPS is different. The simultaneous presence of bacteria with defective LPS had no effect on the fate of WT bacteria present within the same DC and did not affect their survival.

The O-Ag controls degradation of internalized bacteria in DC

Finally, we followed the intracellular fate of S. Typhimurium and E. coli strains after internalization by BM-DC. To investigate whether the internalized bacteria enter the lysosomal degradaton pathway, BM-DC were first pulsed with dextran-Texas Red for 2 h to load lysosomal compartments. Infection with various strains was performed, and after intracellular presence of the bacteria for 8 h, the colocalization of the tracer and the intracellular bacteria was quantified (Fig. 7). There was a clear difference between bacteria with an intact O-Ag and those with truncated or entirely absent O-Ag (Fig. 7, B and C). Approximately 80 and 90% of S. Typhimurium rfaG and E. coli HB101 were closely colocalized with the lysosomal marker, indicating that these bacteria rapidly entered the lysosomal degradation pathway. In contrast, only 20% of S. Typhimurium WT and of the complemented rfaG strain were associated with dextran-Texas Red (Fig. 7, B and C). These observations indicated that intact O-Ag is a crucial component of the bacterial cell envelope that enables Salmonella to control its intracellular fate after uptake by DC. We have previously reported that intracellular Salmonella in epithelial cells controls their fate by the function of the SPI2-encoded T3SS (18). To control whether the association of the rfaG strain with the lysosomal marker is a result of a defective SPI2-T3SS function due to outer membrane defects, we followed the translocation of a representative effector protein. Both the WT and the rfaG strain translocated an SseJ-HA fusion protein into BM-DC, indicating that the T3SS function is not affected by the lack of the O-Ag in the rfaG strains (Fig. S5). Taken together, these data show that in DC the integrity of the LPS, but not the function of the SPI2-T3SS, is essential to control intracellular survival of Salmonella.
Discussion

Persistence of Salmonella in DC is considered as an important prerequisite for the development of systemic infections by Salmonella. DC might act as vehicles for the spread of Salmonella and other pathogenic microbes, and the ability to persist within DC would enable the efficient use of DC as vehicle.

Previous work reported that BM-DC possess only a low capacity of killing and degradation of internalized S. Typhimurium and allow the pathogen to form a static intracellular population (18, 19, 35). Surprisingly, the intracellular persistence of Salmonella in DC was independent from the function of virulence factors known to control the intracellular survival and replication in other types of host cells (18, 19). In this study, we demonstrate that the intracellular survival is even independent from the synthesis of new proteins of intracellular Salmonella as well as of the infected host cell. Because preformed components were required, this prompted us to investigate the role of the bacterial envelope. We noted a marked difference in the intracellular survival of Salmonella and related E. coli strains with defects in the LPS. The in-depth characterization of isogenic Salmonella strains with defined defects in LPS biosynthesis shows that the integrity of the core oligosaccharides and the polymeric O-Ag is important to prevent the activation of DC after phagocytosis of Salmonella and the intracellular survival. A model summarizing these events is shown in Fig. S6.
In addition to the protection against killing of intracellular bacteria, LPS integrity appears important to reduce or delay the immune responses toward a bacterial infection. A recent analysis in a model with intestinal epithelial cells demonstrated a similar role of the O-Ag in delaying the activation of antimicrobial functions of intestinal epithelial cells (C. Duerr, S. Zenk, C. Chassin, J. Pott, D. Gütte, M. Hensel, and M. Hornef, unpublished observations). The work proposed that intact O-Ag delayed the recognition of the lipid A moiety of LPS by TLR4. Such delayed response may be crucial to enable *Salmonella* to breach barriers of the host before an efficient immune response is mounted.

The mechanisms of killing of internalized microbes by DC are only partially understood. There are several indications that phagosomes in DC and macrophages have distinct compositions. Studies with soluble Ags revealed the presence of a mildly acidified compartment in DC that mediates retention of the Ag (36). More recently, the role of the NADPH oxidase NOX2 in maintaining an alkaline phagosomal environment was reported (37). NOX2 activity limits Ags degradation and allows cross-presentation by MHC II pathways in DC. It is not known to what extent these events also take place in phagosomes harboring particulate Ags, but the different fate of pathogens such as *Salmonella* in macrophages and DC suggests that distinct intracellular environments exist in either cell type. Our results indicate that intact LPS of *S. Typhimurium* either directs the formation of such compartment, or protects the pathogen against the specific antimicrobial effectors present in the phagosome of DC.

The LPS has multiple roles in the adaptation of the bacteria to life in mammalian hosts in mediating protection against antimicrobial peptides, complement, and further antimicrobial molecules of the innate immune system (10). Although the role of LPS for the survival of extracellular bacteria within host environments has been exemplified for various bacterial species, including *Salmonella* (38), the contribution of the LPS to adaptation to an intracellular lifestyle has only been studied partially. The role of LPS with intact O-Ag (smooth LPS) for the intracellular replication of *Brucella* spp. was observed, and bacteria with rough LPS lacking the O-Ag were deficient in replication in monocytes (39) and induced to a higher extent the maturation of DC (40). Increased presentation of Ags expressed by *Salmonella* strains with rough LPS was observed after uptake by macrophages (41), indicating increased degradation. Infection with *Salmonella* mutant strains expressing LPS without O-Ag resulted in increased activation of DC functions, such as expression of costimulatory molecules.

Previous studies indicated that intracellular fate of *S. Typhimurium* in murine DC is different after FcRγ-dependent or -independent uptake (42). Increased presentation of a model Ag expressed by *Salmonella* was observed if opsonized bacteria were internalized by Fcγ-mediated phagocytosis. Microscopic analyses revealed increased targeting of bacteria to lysosomes and increased degradation after Fcγ-mediated uptake (42). Reduced intracellular survival after FcγIII-mediated uptake was reported (43), although the assay conditions did not address the different rates of uptake. Our data indicate increased uptake, but not decreased intracellular survival in DC after opsonization of *Salmonella*. The uptake into SP and the maintenance of SP have been reported to contribute to intracellular survival of *Salmonella* in murine macrophages (33). From our analyses of SP formation, we conclude...
that *Salmonella* is able to induce SP in BM-DC, and that the lack of O-Ag and outer core of the LPS does not affect the induction of SP. The similar number of SP containing WT or rfaG bacteria indicates that SP formation is unlikely to contribute to the reduced survival of the rfaG strain in BM-DC.

We have recently analyzed the influence of the composition of the O-Ag on the function of the T3SS of *Salmonella* and found that variation of the LPS O-Ag of *S. Typhi* and *Salmonella* murium-dependent on the donor species of the DC used for infection controls the intracellular fate of human-adapted *Salmonella*. Molecular studies have to reveal the mechanisms of LPS in controlling the intracellular fate of intracellular *Salmonella*. Further cellular and molecular studies have to reveal the mechanisms of LPS in controlling the intracellular fate of *Salmonella* in DC.

**Acknowledgments**

We thank Daniela Jäckel for excellent technical support and Mathias Hornef for sharing unpublished data.

**Disclosures**

The authors have no financial conflict of interest.

**References**


**Supplementary Material**

**Supplementary Tables**

Table S1. Oligonucleotides used in this study

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Legends Supplementary Figures

**Fig. S1.** Dose-dependent effects of tetracycline on the intracellular fate of *S. Typhimurium* in the macrophage-like cell line RAW264.7 and murine BM-DC. (A) Schematic representation of the experimental setup. Host cells were infected with *S. Typhimurium* WT at MOI of 10 (macrophages) or 25 (BM-DC). At 2 h after infection, various concentrations of tetracycline (Tet) as indicated were applied. After incubation for 1 h, tetracycline was removed by washing and incubation was continued to 15 h post infection. The amount of viable intracellular bacteria at 2 h and 15 h after infection was determined by plating of cell lysates onto agar plates as described in **Fig. 1A**. The relative intracellular replication in RAW264.7 macrophages (B) or survival in BM-DC (C) without and with increasing inhibitor concentrations was quantified as described in **Fig. 1**. Means and standard deviations of three assays are shown. Statistical analysis, antibiotic-treated cells vs. mock-treated cells: n.s., not significant; ***, P < 0.001.

**Fig. S2.** Dose-dependent effects of tetracycline on *S. Typhimurium* replication and protein translocation in macrophages. RAW264.7 cells were infected with *S. Typhimurium* WT harboring plasmid p2777 for the constitutive expression of the GFP and expression of HA-tagged effector protein SseJ. SseJ-HA is only expressed in intracellular bacteria and translocated into host cells by the SPI2-encoded T3SS. The infection was performed basically as described for and tetracycline was added in various concentrations and for various periods of time as indicated. At 15 h after infection, cells are fixed and immuno-staining for *Salmonella* (rabbit α-O 4,5, α-rabbit Cy5, blue) and the HA tag (rat α-HA, α-rat Cy3, red) was performed. The fluorescence intensities in the green and blue channels correlate to the amount of intracellular *Salmonella*, intensity in the red channel correlates to the synthesis of the effector protein SseJ by intracellular *Salmonella* and the translocation by the SPI2-T3SS.
Note that prolonged application for tetracycline for 2 to 15 h p.i. or high concentrations (80 µg × ml⁻¹) of the antibiotic abrogate SseJ translocation, inhibit *Salmonella* proliferation, and also suppress GFP expression. However LPS signals can occasionally still be detected. Scale bar, 20 µm.

**Fig. S3.** Increased phagocytosis of *Salmonella* mutant strains with truncated LPS.

Quantification of the uptake of various *S. Typhimurium* and *E. coli* strains by RAW264.7 macrophages (MOI 10) and BM-DC (MOI 25). All bacteria harbored pFPV25.1 for the constitutive expression of GFP. (A) Infected cells were analyzed by flow cytometry for GFP fluorescence 1 h after infection. The mean fluorescence intensities (MFI) in the GFP (FL-1) channel for the eukaryotic cell population were determined and means and standard deviations are shown for two independent assays. (B) Representative fields of view are shown for BM-DC infected with wild-type *Salmonella* and various LPS mutant strains. Scale bar, 20 µm. (C) The intracellular survival of *Salmonella* WT and the *rfaG* mutant strain in the early phase of infection was determined by gentamicin protection assays as described in Fig. 1. BM-DC were infected with WT and *rfaG* *Salmonella* and lysed immediately (0 h), 1 h or 2 h after infection for determination of the number of viable intracellular bacteria. Statistical analysis, *E. coli* or *S. Typhimurium* mutants strains vs. *S. Typhimurium* WT: n.s., not significant; *, P < 0.05; ***, P < 0.001.

**Fig. S4.** Effect of LPS integrity on formation of spacious phagosomes after infection of macrophages or DC. Bone marrow-derived macrophages (left panels) or (DC right panels) were infected with WT *S. Typhimurium* (black bars) or the *rfaG* strain (white bars) at MOI of 10 or 5, respectively. Each strain harbored plasmid pFPV25.1 for constitutive expression of GFP (A, green). As control, non-infected cells were stimulated for 1 h with PMA at final concentrations of 60 or 120 ng x ml⁻¹ (gray bars). Phagocytosis of the bacteria was allowed
for 1 h and 10, 30 or 60 min after infection, the cells were fixed by addition of 3 % PFA. In
addition, cells were stained with anti CD11c and Cy5-labeled secondary antibody for
detection of DC (A, blue). The formation of SP was scored microscopically used differential
interference contrast and SP were defined a phagosomes with distinct membrane appearance,
central transparency and a diameter of 1.5 μm or larger. Representative micrographs of
infected or PMA-stimulated macrophages or DC are shown in panel A. The location of SP is
indicated by red arrows head. Scale bar, 20 μm. The number of SP per cell was scored (B),
the number of internalized Salmonella per cells (C) and the number of SP containing
Salmonella (D). Statistical analysis, S. Typhimurium WT strain vs. S. Typhimurium rfaG:
n.s., not significant; *, P < 0.05; **, P < 0.01, ***, P < 0.001.

Fig. S5. The SPI2-encoded T3SS is functional in Salmonella rfaG mutant strain inside
macrophages and BM-DC. RAW264.7 macrophages and BM-DC were infected with
Salmonella WT, the rfaG strain or strains P2D6 defective in the SPI2-T3SS. Each strain
harbored plasmid p2777 for the intracellular induced expression of HA-epitope tagged SPI2-
effector SseJ and constitutive expression of GFP (green). Infected cells were fixed 16 h and
immune-stained for SseJ-HA (red) and CD11c (blue). Representative infected cells are
shown. Scale bar, 5 μm.

Fig. S6. Model for the intracellular fate of Salmonella in DC and the contribution of the O-
antigen. LPS truncation leads to an increased uptake of mutant bacteria (B) compared to S.
Typhimurium WT (A) and enhanced immunological DC stimulation, shown by (i) increased
antigen co-uptake (B), (ii) increased expression of co-stimulatory molecules (CD80, CD 86,
MHC-II, D) and (iii) increased cytokine release (TNF, D). On the other hand, Salmonella
strain with truncated LPS are more susceptible to host defense mechanisms, leading to
bacterial degradation (D), whereas WT *Salmonella* form a static, non-dividing bacterial population within DC (C). Compared to WT *Salmonella*, mutant strains lacking O-antigen co-localize with late endosomal marker proteins indicating to be processed according to the regular antigen degradation pathway. However, *Salmonella* WT may either be able to partially block this vesicular maturation process in DC, to escape the degradation pathway, to protect itself from antimicrobial peptides, or to reside in a different subcellular compartment.
Zenk et al., Suppl. Fig. 1
Zenk et. al., Suppl. Fig. 2

RAW 264.7 MΦ

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Zenk et al., Suppl. Fig. 3

A

**MΦ**

S. Typhimurium  
E. coli

MFI

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**DC**

S. Typhimurium  
E. coli

MFI

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B

**Salmonella**

WT  
rfbP  
rfaG  
rfaL  
rfaH

**Salmonella**  
+ phase

C

**rel. uptake**

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*  
***  
n.s.
Zenk et al., Suppl. Fig. 4

B

MΦ

DC

WT

rfαG

60 ng/ml PMA

120 ng/ml PMA

SP per cell

WT

rfαG

10 30 60

time after infection (min)

Salmonella

per cell

MΦ

DC

Salmonella-containing SP per cell

WT

rfαG

10 30 60

time after infection (min)

n.s. n.s.

* ** ***

n.s. n.s. n.s.

* 

n.s. n.s.

Zenk et al., Suppl. Fig. 4
Zenk et al., Suppl. Fig. 5

Mφ

DC

*rfαG*  *WT*  *ssαV*

**CD11c**  **SseJ-HA**  **Salmonella**
Zenk et al., Suppl. Fig. 6

WT
unknown receptor TLR4 MyD88

LPS mutant
TLR4 unknown receptor MyD88

smooth LPS Salmonella
rough LPS Salmonella
DC
opsonizing antibodies
TLR4, MyD88
unknown DC receptor
fluid tracer
endocytotic vesicle
CD80, CD86
MHC II
TNF