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Intracellular Salmonella Inhibit Antigen Presentation by Dendritic Cells

Cédric Cheminay,² Annette Möhlenbrink,² and Michael Hensel³

Dendritic cells (DC) are important APCs linking innate and adaptive immunity. During analysis of the intracellular activities of Salmonella enterica in DC, we observed that viable bacteria suppress Ag-dependent T cell proliferation. This effect was dependent on the induction of inducible NO synthase by DC and on the function of virulence genes in Salmonella pathogenicity island 2 (SPI2). Intracellular activities of Salmonella did not affect the viability, Ag uptake, or maturation of DC, but resulted in reduced presentation of antigenic peptides by MHC class II molecules. Increased resistance to reinfection was observed after vaccination with SPI2-deficient Salmonella compared with mice vaccinated with SPI2-proficient Salmonella, and this correlated with an increased amount of CD4+ as well as CD8+ T cells. Our study is the first example of interference of an intracellular bacterial pathogen with Ag presentation by DC. The subversion of DC functions is a novel strategy deployed by this pathogen to escape immune defense, colonize host organs, and persist in the infected host. The Journal of Immunology, 2005, 174: 2892–2899.

Bacterial pathogens have evolved various mechanisms to evade the immune response of multicellular organisms. During the initial phase of an infection, the invading microbes can avoid various innate immune defenses, but there are also mechanisms to counteract the development of an adaptive immune response (1). As a principal component of the innate immunity able to prime adaptive responses, the dendritic cell (DC) plays a pivotal role in immunity and consequently is a particularly interesting target for pathogens (2).

Disease outcome after infection with different serotypes of the species Salmonella enterica ranges from self-limiting diarrhea and localized gastrointestinal inflammation to typhoid fever, a systemic infection with high lethality caused by Salmonella enterica serovar typhimurium (3). During typhoid fever, the food-borne pathogen penetrates mucosal barriers, spreads via the reticulo-endothelial system, and proliferates massively in organs of the host. Acute as well as asymptomatic infections can lead to a chronic carrier state, in which bacteria are shed with feces. In addition, despite the long contact with infecting bacteria, the host organism is often unable to prime a long term protective immunity to reinfection (4). The infection of susceptible mice with S. enterica serovar typhimurium is a model for the pathogenesis of human typhoid fever. In this model, the eradication even of attenuated strains by T cell-mediated immunity is a slow process compared with that of other pathogens, such as Listeria monocytogenes (for review, see Ref. 5).

S. enterica is a facultative intracellular pathogen, and virulence factors required for intracellular survival and replication are also necessary for the successful colonization of deeper tissues. Intracellular pathogenesis requires repair mechanisms and metabolic flexibility to compensate for damages and nutritional limitations within the host cell, but intracellular Salmonella also directly influence their host cell. A cluster of virulence genes within Salmonella pathogenicity island 2 (SPI2) encodes a type III secretion system (T3SS) (6). By means of this system, Salmonella residing inside a membrane-bound compartment translocate a set of effector proteins into their host cell (for review, see Ref. 7). This pathogenic interference affects normal maturation of bacteria-containing phagosomes as well as innate immune defense mechanisms against intracellular Salmonella within macrophages (8, 9).

After host invasion, Salmonella reside inside DC of the subepithelial dome of Peyer’s patches (10). In vitro observations suggest that DC can serve as an alternative invasion pathway to M cells (11) and as vehicle in the spread of that pathogen (12). We recently observed that virulence genes are expressed within bone marrow-derived DC (BM-DC), and virulence proteins are translocated by the SPI2-encoded T3SS (13). This activity was not required for intracellular survival (14); however, an altered maturation of the Salmonella-containing vacuole depending on the function of SPI2 genes was observed (13). In this study we investigated the consequences of Salmonella infection on the fundamental function of DC, the presentation of Ags.

Materials and Methods

Bacterial strains and culture conditions

S. enterica serovar typhimurium NCTC 12023 was used as a wild-type (WT) strain. SPI2-deficient strains HH102 (sscC:apfT) and HH104 (sscC:apfT) and plasmid-complemented strains HH103 and HH105 have been described previously (15). Strain HH104 was the standard SPI2 mutant for in vitro and in vivo experiments. P7B12 (sipC::mTn5) is an SPI1-deficient, noninvasive mutant (16), and P10H1 (purD::mTn5) is an SPI2-proficient, auxotrophic strain (17). The species Salmonella bongori, lacking SPI2 (18), was used as an avirulent control strain. Mutant strains deleted in SPI3 (mp492) or SPI4 (mp493) were constructed using the one-step deletion approach as described previously (9, 19). Bacterial cultures were grown overnight in Luria broth (LB) at 37°C with aeration. Bacteria grown under these conditions are noninvasive and not cytotoxic to...
DC. Carbenicillin, kanamycin (both at 50 μg/ml), and nalidixic acid (100 μg/ml) were added if required for selection of strains. Viable bacteria in the inocula were verified by plating serial dilutions onto LB-agar plates in the absence or the presence of the appropriate antibiotic. The plasmid pFPV25.1 (20) was used to visualize bacteria by constitutive expression of GFP. Killing of bacteria was performed by UV irradiation at 312 nm for 10 min and was controlled by plating onto agar plates.

Preparation and culture of host cells

DC were prepared from bone marrow of 6- to 8-wk-old BALB/c mice for proliferation assays (Charles River Breeder) or C3H/HeN mice (Elevage Janvier) for hen egg lysozyme (HEL) presentation assay essentially as previously described (21). The CD11c+ cell population was enriched using MACSsorting (Miltenyi Biotec) with a purity of ~95%. After lysis of erythrocytes, DC were also directly isolated by MACSsorting from suspension of total splenic cells of BALB/c mice. The cells were allowed to adhere to cell culture plates for at least 6 h before infection. The viability of 5 × 105 sorted DC after infection in low adherence, 24-well plates (Costar) was determined by the Live/Dead assay ( Molecular Probes) at different times by flow cytometry (FACSCalibur; BD Biosciences). The results were verified by the MTT test.

Cells expressing OVA-specific TCR were prepared from cell suspensions of spleens of sex- and age-matched DO11.10 mice (JAX) by magnetic sorting of CD4+ cells (Miltenyi Biotec).

Bacterial infection of DC and T cell proliferation assay

Before infection, bacteria were opsonized in 20% normal mouse serum (Dianova). Bacteria were added at various multiplicities of infection (MOI) and centrifuged onto DC for 5 min at 500 × g to synchronize the infection. After 1 h, noninternalized bacteria were removed by two washes with PBS. To kill remaining extracellular bacteria, infected cells were incubated for 1 h in medium containing 100 μg/ml gentamicin. After washing, the cells were incubated in the presence of 25 μg/ml gentamicin throughout the experiment. The absence of extracellular bacteria was tested by plating supernatants onto LB-agar plates. When infections were performed in low adherence plates, cells were recovered by centrifugation (1300 × g for 5 min), and medium or PBS was carefully removed.

T-cell proliferation assays were performed in 96-well plates with a density of 103 sorted DC/well. DC were gamma-irradiated (3600 rad). Bacterial infections and stimulation with 50 μg/ml OVA (Sigma-Aldrich) were performed in parallel for 2 h. The Ag was removed, and the gentamicin concentration was reduced. Splenic CD4+ T cells isolated from DO11.10 mice were added to a DC/T cell ratio of 1:1 in a final volume of 200 μl of medium containing 25 μg/ml gentamicin. Two days later, the cells were pulsed with 1 μCi of [3H]thymidine (Amersham Biosciences) in 50 μl of medium for an additional 24 h before cell harvesting and quantification of thymidine incorporation (beta-counter). To test the effect of inducible NO synthase (iNOS) on the stimulation of T cells by Ag-pulsed DC, the specific inhibitors L-Nω-monomethylethyl-L-arginine (L-NMMA) and L-Nω-(1-iminoethyl)-lysine were added with the addition of T cells.

Analysis of presentation of an epitope of HEL associated with MHC class II (MHC-II) molecule

In low adherence, 24-well plates, 5 × 105 sorted BM-DC were stimulated for 2 h with 0.5 or 1 mg/ml HEL as dose-response positive control. Simultaneously with stimulation by 1 mg/ml HEL, the cells were infected with Salmonella WT or SPI2, with or without pFPV25.1 for GFP expression, at an MOI of 25 as described above. Eight hours after infection, we stained the cells for 1 h with the supernatant of the C4H3 hybridoma (22) diluted 1/2 in PBS, 2% BSA, and 2% goat serum, followed by 1-h staining with biotinylated mAb anti-rabbit IgG (BD Biosciences; 1/100). Finally, the cells were stained for 30 min with streptavidin-allophycocyanin (BD Biosciences; 1/200) in parallel to staining with a PE-conjugated rat mAb anti-MHC-II (BD Biosciences; used at the recommended concentration) and were analyzed by flow cytometry. The ratio of the means of fluorescence intensity for C4H3 and MHC-II signals was calculated.

Quantification of iNOS activity in DC

Sorted DC (5 × 105) in 1 ml of medium were seeded onto glass coverslips in 24-well plates and infected with viable or UV-killed WT or SPI2-deficient Salmonella at an MOI of 25. After 24 or 48 h, the supernatants were collected, and the cells were fixed with 3% paraformaldehyde in PBS for 15 min. The Abs were diluted in a blocking solution consisting of 2% goat serum and 2% BSA in PBS. For all Abs, we used an incubation period of 1 h at room temperature, except for the Ab against iNOS. The coverslips were incubated first with hamster mAb anti-CD11c (BD Biosciences; 1/100), which was detected by a goat anti-hamster IgG conjugated to Cy5 (Dianova; 1/400). After overnight incubation in the presence of 1% saponin with rabbit mAb anti-iNOS (BIOMOL; 1/1000) at 4°C, the cells were finally stained with donkey anti-rabbit IgG conjugated to Cy3 (Dianova; 1/100). The coverslips were mounted on fluoroprep (BioMérieux) and sealed with Entellan (Merck). Samples were analyzed using a confocal laser-scanning microscope (Leica; TCS-NT). Epifluorescence images were merged with the phase contrast image to visualize the bacteria. To analyze iNOS activity, we quantified the concentration of nitrite present in the supernatants by the Griess assay.

Infection protocol for vaccination experiment

Groups of 6- to 8-wk-old female C57BL/6 mice (Charles River Breeder) were orally immunized with 300 μl of PBS containing 1011 CFU of Salmonella deficient in SPI2 or PurD, whereas the naive group received only PBS. Three weeks after immunization, mice were challenged with an oral infection of 107 CFU of wild-type Salmonella. Survival was recorded over 7 wk. In parallel, mice of the cohorts were killed on day 4 after challenge infection, and the cellular composition of different organs was analyzed using half the organs. The other half was homogenized, and the numbers of WT and mutant bacteria present in each compartment were quantified by plating serial dilutions in LB-agar plates containing nalidixic acid or kanamycin. The cell suspension of Peyer’s patches, mesenteric lymph nodes, and spleen of infected mice was essentially performed as previously described (17).

Analysis of cell populations present in lymphoid organs by flow cytometry

The cell suspension of Peyer’s patches, mesenteric lymph nodes, and spleen of infected mice was essential performed as previously described (17). For a control, nonimmunized and nonchallenged mice were analyzed.

Statistical analysis

For each condition of in vitro assay, two or three independent preparations of bacteria were used to infect DC in duplicate or triplicate (flow cytometry, n = 4; T cell proliferation, n = 6–8). The results presented in the figures are the mean ± SD. Statistical analysis was conducted using two-tailed Student’s t test. For immunization/challenge experiments, the statistical difference between the different survival curves was tested in a pairwise fashion using the two-tailed, log-rank test. As suggested by Olsen (23), the data from three independent experiments performed under similar conditions (age and sex of mice and bacterial preparation) were cumulated into one graph. The medians were tested by Friedman’s two-way ANOVA by ranks, and only at p < 0.05 did we use Dunn’s post-test to compare each pair of column. Statistical significance was always defined as follows: *, p < 0.05; **, p < 0.01; and ***, p < 0.001. All analyses were performed using the statistical program PRISM-3 (GraphPad).
Results

Intracellular activities of Salmonella reduce the capacity of DC to stimulate T cell proliferation

As a model system for Ag presentation, we investigated the MHC-II-dependent presentation of OVA by murine splenic DC or BM-DC to CD4+ T cells expressing an OVA-specific TCR. Stimulation of BM-DC with OVA resulted in dose-dependent stimulation of OVA-specific T cell proliferation (data not shown), and an Ag concentration of 50 μg/ml was used for further studies. After simultaneous stimulation of murine splenic DC with OVA for 2 h and infection with wild-type S. enterica serovar typhimurium (WT), a strong reduction of T cell proliferation was observed, and this inhibition was dependent on the MOI used (Fig. 1A). The effect of Salmonella WT infection was similar in splenic DC and BM-DC (Fig. 1, A and B). Because BM-DC could be obtained in much higher quantities then splenic DC, BM-DC were used for subsequent studies. Infection with an equal number of Salmonella WT killed by UV irradiation did not result in a reduction of OVA-specific T cell proliferation, indicating that viable bacteria were involved in the inhibition of T cell proliferation. Infection with S. bongori, a species not causing systemic pathogenesis, also did not affect T cell proliferation (Fig. 1B). Under our assay conditions, Salmonella infection was not cytotoxic and did not affect the uptake of OVA (data not shown). Contact with live or dead bacteria induced a similar up-regulation of the DC maturation marker CD86 (data not shown). We questioned whether, in addition to bacterial viability, protein biosynthesis by Salmonella was required for suppression of T cell proliferation. Addition of chloramphenicol to BM-DC infected with Salmonella WT restored T cell proliferation (Fig. 1C), indicating that bacterial protein biosynthesis is necessary for bacterial interference with DC stimulation of T cells.

Infection of DC with viable Salmonella induces iNOS expression that affects T cell proliferation

Even at high MOI, only a subset of BM-DC (~40%) was infected with Salmonella, whereas the majority of DC internalized OVA (data not shown). The strong reduction in T cell proliferation suggests that a soluble factor produced by Salmonella-infected DC had an inhibitory effect on uninfected DC and/or on T cells. It was shown that DC express iNOS in response to bacterial infection (24), and it is known that NO synthesized by iNOS and its reaction products have a potent immunosuppressive activity (for review, see Ref. 25). After verification that BM-DC expressed iNOS in a dose-dependent manner in response to Salmonella infection (Fig. 2, A and B), we investigated a potential role of iNOS in generating NO as immunosuppressive mediator by performing experiments in the presence or the absence of specific iNOS inhibitors. Addition of L-NMMA (Fig. 2C) or L-NAME-(1-iminoethyl)-lysine (data not shown) to BM-DC partially restored T cell proliferation, yet the levels of T cell proliferation were lower than those in assays with UV-killed Salmonella. This observation indicated the presence of a second factor, either synthesized by intracellular Salmonella or induced in the host cell by Salmonella infection, that affects stimulation of T cell proliferation.

SPI2-dependent reduction of MHC-II-dependent Ag presentation by DC

Our previous studies showed that Salmonella in BM-DC are capable of altering maturation of the Salmonella-containing vacuole in an SPI2-dependent manner (13). In this study we compared the stimulation of OVA-dependent T cell proliferation by BM-DC infected with Salmonella WT and a strain deficient in SPI2-encoded T3SS. T cell proliferation was lower if BM-DC were infected with WT compared with the SPI2 mutant strain at a low MOI (Fig. 3A). However, the difference was not always significant at an MOI of 25 (Fig. 3, A and B). Because induction of iNOS was pronounced at high MOI, we considered that an SPI2 phenotype in BM-DC might be masked by iNOS activity. In the presence of L-NMMA, a strong reduction of T cell proliferation was observed only after DC infection with WT, but not SPI2-deficient Salmonella (Fig. 3B). These data indicate that the intracellular activity of the SPI2 system in combination with the induction of iNOS dramatically affects the ability of DC to stimulate T cell proliferation. To test the specificity of our observation, various Salmonella strains defective in SPI1, SPI3, or SPI4 were used for infection at an MOI of 10 and did not show any difference in their ability to inhibit T cell proliferation compared with WT. Furthermore, a purD-deficient strain (PurD) with an attenuation of virulence similar to an SPI2 mutant strain in vivo, but expressing a functional SPI2-encoded T3SS, was equivalent to WT in inhibition of OVA-dependent T cell proliferation (Fig. 3C). The higher T cell proliferation observed after infection with SPI2 mutant strains deficient in sseB

FIGURE 1. Intracellular activities of Salmonella reduce the capacity of DC to stimulate T cell proliferation. Gamma-irradiated CD11c+ sorted spleen DC (A) or BM-DC (B and C) were stimulated for 2 h with 50 μg/ml OVA and simultaneously infected with opsonized Salmonella. After 1 h, extracellular bacteria were killed by addition of 100 μg/ml gentamicin. After washing, CD4+ sorted spleen T cells from DO11.10 transgenic mice were added at a 1:1 ratio of DC and T cells. After incubation for 48 h in the presence of 25 μg/ml gentamicin, the cells were pulsed with 1 μCi of [3H]thymidine for an additional 24 h. T cell proliferation was quantified by thymidine incorporation (expressed as cpm). A, Spleen DC were infected with S. typhimurium (S.T.) at an MOI of 25, 10, or 5. In the following experiment we used an MOI of 25. B, Stimulation of T cell proliferation was compared after uptake of WT S. typhimurium, WT bacteria killed by UV irradiation (UVK), and viable or dead S. bongori (S.B.). C, The protein synthesis of S. typhimurium was blocked using different concentrations of chloramphenicol (Cm) ranging from 25 to 6.25 μg/ml. Statistical analyses were conducted using two-tailed unpaired Student’s t test. Statistical significance was defined as follows: *, p < 0.05; **, p < 0.01; ***, p < 0.001 (n = 6–8).
SPI2 inhibits DC capacity to process and/or present Ags by MHC-II pathway

We investigated the mode of interference of intracellular Salmonella with T cell stimulation by DC. There was no difference between DC infected with WT Salmonella or the SPI2 mutant in DC viability (Fig. 4A), OVA uptake (Fig. 4B), iNOS activity (Fig. 4D), or up-regulation of MHC-II and costimulatory molecules (Fig. 4C). To determine whether intracellular Salmonella interfere with Ag processing and/or presentation by BM-DC, peptide loading on MHC-II was analyzed. The C4H3 Ab (22) specific for MHC-II loaded with a peptide of the model Ag HEL was used as previously described (26), but with flow cytometry. A lesser number of MHC-II molecules loaded with the HEL epitope were observed after infection with Salmonella WT compared with SPI2 (Fig. 4E).

These observations indicate that BM-DC harboring viable Salmonella WT were reduced in their capacity to process and/or present Ags by the MHC-II pathway and the involvement of SPI2-encoded T3SS in this interference.

Adaptive immune response to Salmonella is affected by SPI2 function

Being cautious of using an artificial model of exogenous Ag presentation in which DC are heavily infected, we questioned whether SPI2-mediated suppression of Ag presentation would also affect development of the adaptive immune response induced by live bacteria in infected hosts. Few tools are currently available to follow the CD4⁺ T cell-specific immunity induced by live bacteria during Salmonella infection. For example, T cell clones specific for FliC or SipC are known (27, 28), but analyses of responses to these Ags did not appear useful, because expression is down-regulated after host invasion (29, 30). We also excluded the use of OVA-expressing Salmonella as a reporter system, because expression of a foreign Ag can affect the virulence of the bacteria (31).
Furthermore, killing of bacteria in the gastrointestinal tract might bias T cell specificity to released foreign Ag.

We immunized mice with SPI2 or PurD strains and analyzed the immune response to challenge with a lethal dose of Salmonella WT 3 wk after vaccination. After oral application, both SPI2 and PurD strains persisted at low numbers in various organs, and equal numbers of the bacteria were recovered from immunized animals at least 4 wk after vaccination (data not shown). We assumed that equal quantities of Salmonella Ags were liberated and that differences in the immune response induced by both strains corresponded to their different activities within colonized organs. Compared with nonimmunized mice, both vaccinations resulted in partial protection against the lethal infection, but survival in the SPI2-vaccinated group was significantly higher than that in the PurD-vaccinated group (Fig. 5A). The survival correlated to the bacterial organ burden of WT bacteria (data not shown). Similar numbers of PurD and SPI2 bacteria were recovered from the organs (Fig. 5). We next analyzed the size and composition of the T and B cell compartments in spleens (Fig. 5, C–F), mesenteric lymph nodes, and Peyer’s patches (data not shown). After challenge infection, reduced numbers of T cells (Fig. 5C), CD4+ T cells (Fig. 5D), and CD8+ T cells (Fig. 5E) as well as B cells (Fig. 5F) were detected in naive mice, as previously described (32), but also in PurD-vaccinated mice. In contrast, a significantly greater number of T cells of both CD4+ and CD8+ subpopulations were primed in SPI2-vaccinated mice (Fig. 5, C–E) even after challenge with Salmonella WT that induced a strong NO-mediated immunosuppression (33). These results correlate well with the previous observation that T cells are essential for sterile immunity after infection with an SPI2-deficient strain (34).

A majority of Salmonella reside with DC in mesenteric lymph nodes 1 day after oral infection

To investigate whether the SPI2-mediated inhibition of T cell priming correlates to the interaction of Salmonella with DC in vivo, we determined in which cells the bacteria were residing. After oral infection even with very high inocula of 1011 CFU of SPI2- or PurD-deficient strains, the low amount of bacteria in the different organs (data not shown) (17) prevented detection by histology. One day after infection with the same dose of Salmonella WT, we observed by confocal microscopy single Salmonella inside DC of the subepithelial dome of Peyer’s patches (Fig. 6A) as previously described (10), whereas some clusters of bacteria probably resulting from intracellular replication did not colocalize with CD11c staining (Fig. 6A). As expected from our previous observations (12), Salmonella were detectable in the interfollicular region of the mesenteric lymph nodes 1 day after oral infection (Fig. 6B). Using three-dimensional image reconstitution, we determined that 51.1% of the bacteria (n = 300) were residing inside the DC of the mesenteric lymph nodes, with equal contributions of the CD11b- and CD8α-positive subsets (Fig. 6, C and D). In addition, 16% of Salmonella were colocalizing with DC, but were not counted as being within DC because the bacteria were not fully surrounded by CD11c staining in all optical planes. These observations indicate that early after infection, DC are frequently interacting with invading Salmonella and may represent a specific target for this pathogen.

**Discussion**

In this study we determined that intracellular activities of *S. enterica* affect the capacity of DC to present Ags and their ability to stimulate T cell proliferation. This pathogenic interference requires bacterial viability and protein biosynthesis. By an unknown mechanism, viable intracellular *Salmonella* induce iNOS expression and production of NO by DC (Fig. 2), resulting in suppression of T cell proliferation after Ag presentation, but, in contrast with the recent report by Eriksson et al. (35), without influencing bacterial viability. In addition, we observed that intracellular activities of SPI2 result in a reduction of Ag presentation on MHC-II molecules. Although the mechanism of the SPI2-dependent effect is not fully understood, we speculate that the interference with intracellular...
transport processes by SPI2-encoded T3SS might affect Ag processing and surface presentation of peptide-loaded MHC-II. It has been reported that SPI2-encoded T3SS plays roles in the inhibition of intracellular vesicle transport (36) and modification of the actin as well as the microtubule cytoskeleton (37, 38). These observations could explain the protection of intracellular Salmonella against reactive oxygen and nitrogen radicals in macrophages (8, 9) as well as the delayed maturation of phagosomes harboring Salmonella WT in DC (13) and the inhibition of the Ag presentation capacity of these cells.

FIGURE 6. Localization of Salmonella in infected organs 1 day after oral infection. One day after oral infection with 10^{11} CFU of Salmonella WT, sections of Peyer’s patches (A) and mesenteric lymph nodes (B) were immunostained for Salmonella LPS (green), CD11c (blue), and CD11b (red). Bacteria were localized in the subepithelial dome of Peyer’s patches situated between the epithelial surface and the B cell follicle, delimited by the dotted line (A). A. The unmarked arrows indicate single bacteria within CD11c^{+} DC (blue), and the marked arrow marks a cluster of replicating bacteria in a CD11c^{-} cell. B, Salmonella, as indicated by arrows, were present in the interfollicular region of mesenteric lymph nodes between the B cells follicles delimited by the dotted lines. C and D, Representative images of Salmonella colocalization within DC of infected mesenteric lymph nodes. The sections were immunostained for Salmonella (green), CD11c (blue), and CD11b (C, red) or CD8a (D; red).
Our observations indicate that the activity of the SPI2 system is required to alter MHC-II-dependent presentation of Ags. During infection, this interference is likely to result in a reduced presentation of Ags derived from intracellular Salmonella, thereby preventing the development of Salmonella-specific CD4+ and probably also CD8+ T cell subpopulations. Supporting this last observation, cross-presentation between the MHC-I and -II pathways has been demonstrated recently (39). This ablation of the development of an adaptive immune response to live Salmonella present in infected organs can explain the massive systemic proliferation of bacteria during a long period after infection and might also be a requirement for the development of the carrier state, one of the major problems regarding both the transmission and spread of Salmonella infections.

DC have been shown to be the principal cells interacting with Salmonella after passage through M cells (10), and the CD18 present in infected organs can explain the massive systemic proliferation of bacteria. Based on these in vivo observations and effects on Ag presentation, we propose that DC are perfect Trojan horses in which Salmonella can survive, move to deeper tissue using the intrinsic migration capacity of DC, and actively suppress the adaptive immune response by means of SPI2 functions. Additional work will reveal the contribution of DC to tissue invasion and spread of Salmonella in vivo.

Our observation will have important implications in the development of novel live vaccines against Salmonella infections, but also as carrier strain for heterologous Ags. Obviously, rational design of a live vaccine has to involve mutations that will prevent the suppression of Ag presentation and T cell proliferation by the carrier (41).

In this study, we demonstrate the first example of an intracellular bacterial pathogen that affects the MHC-II-dependent Ag presentation of DC. Our findings are in line with a recent report by Tobar et al. (42), who observed interference of WT Salmonella with MHC-I and MHC-II presentation by DC. The direct inhibition of the Ag processing and/or presentation capacities of these cells by intracellular Salmonella via a defined virulence system represents a novel strategy of immune escape for this pathogen and until now has been characterized only during viral infections of DC. Future work is needed to reveal the molecular mechanisms of Salmonella interference with DC functions resulting in the induction of iNOS and the SPI2-dependent reduction of presentation of peptide-loaded MHC-II molecules.

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Disclosures

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