Microbiota restricts trafficking of bacteria to mesenteric lymph nodes by CX3CR1hi cells

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The intestinal microbiota has a critical role in immune system and metabolic homeostasis, but it must be tolerated by the host to avoid inflammatory responses that can damage the epithelial barrier separating the host from the luminal contents1–4. Breakdown of this regulation and the resulting inappropriate immune response to commensals are thought to lead to the development of inflammatory bowel diseases such as Crohn’s disease and ulcerative colitis5. We proposed that the intestinal immune system is instructed by the microbiota to limit responses to luminal antigens. Here we demonstrate in mice that, at steady state, the microbiota inhibits the transport of both commensal and pathogenic bacteria from the lumen to a key immune inductive site, the mesenteric lymph nodes (MLNs). However, in the absence of Myd88 or under conditions of antibiotic-induced dysbiosis, non-invasive bacteria were trafficked to the MLNs in a CCR7-dependent manner, and induced both T-cell responses and IgA production. Trafficking was carried out by CX3CR1hi mononuclear phagocytes, an intestinal-cell population previously reported to be non-migratory6. These findings define a central role for commensals in regulating the migration to the MLNs of CX3CR1hi mononuclear phagocytes endowed with the ability to capture luminal bacteria, thereby compartmentalizing the intestinal immune response to avoid inflammation.

How the intestinal immune system discriminates between commensal and pathogenic microorganisms remains an enigma. Commensals have the same immunostimulatory molecules as pathogenic bacteria and have been shown to trigger inflammation and disease if they penetrate the intestinal epithelial barrier. Host sensing of commensals has been shown to be important for the proper development and functionality of the immune system, as germ-free mice have an altered immune system organization and reduced cellularity, especially in the small-intestinal lamina propria, compared to mice harbouring a complex microbiota (reviewed in ref. 9). This feature of host immune system dependence on the microbiota led us to examine whether commensal bacteria have a role in modulating mucosal immune responses to specific microbes. To this end, we investigated the consequences of antibiotic-mediated depletion of the intestinal microbiota6 (Supplementary Fig. 1a) on the host response to a non-invasive strain of Salmonella enterica serovar Typhimurium. We used this strain because, although it has limited CD18-dependent access to the blood and spleen, it cannot cross the epithelium overlying intestinal lymphoid tissues, and hence does not reach the Peyer’s patches10,11. We were thus able to investigate a potential role for commensals in regulating access of this non-invasive strain of Salmonella to the lamina-propria-draining lymphatics that traffic luminal contents to the MLNs, sites of immune system induction.

Oral infection with non-invasive Salmonella normally induces an IgG but not an IgA response10 (Fig. 1a). Unexpectedly, after treatment with antibiotics, infected mice mounted a strong Salmonella-specific faecal IgA response in addition to an IgG response (Fig. 1a). In untreated mice, Salmonella-specific T cells were limited to the spleen and small-intestinal lamina propria, but antibiotic-treated mice additionally had antigen-specific T cells in the MLNs (Fig. 1b and Supplementary Fig. 1b). This was a Salmonella-specific response, as there was no difference in interferon-γ (IFN-γ)-producing T cells after anti-CD3 stimulation and there was little IFN-γ production from MLN T cells isolated from mice that did not receive antigenic stimulation or that were Salmonella-naive (Supplementary Fig. 1b and data not shown). This indicates that immune responses specific for non-invasive Salmonella are only observed in the lamina-propria-draining MLNs if commensal bacteria are depleted before infection.

We proposed that commensals could influence the amount of antigen (in this case, Salmonella) that reaches the MLNs. In untreated mice, infection with both pathogenic and non-pathogenic strains of Salmonella was associated with a reduction in MLN T cell counts compared to those in untreated antibiotic-treated animals (Supplementary Table 1). This, in turn, suggested that the MLN T cells could be trafficking to the MLNs. To test this, mice were treated with antibiotics for 4 weeks, infected with Salmonella enterica serovar Typhimurium, and killed 2 days later. Because the MLNs are a strategic location to capture luminal bacteria, we examined whether commensals could influence the amount of antigen (in this case, Salmonella) that reaches the MLNs. In untreated mice, Salmonella-specific T cells were limited to the spleen and small-intestinal lamina propria and were not present in the MLNs. In contrast, in antibiotic-treated mice, Salmonella-specific T cells were present in the MLNs, indicating that the MLNs were being trafficked to the MLNs. However, in the absence of Myd88 or under conditions of antibiotic-induced dysbiosis, non-invasive bacteria were trafficked to the MLNs in a CCR7-dependent manner, and induced both T-cell responses and IgA production. Trafficking was carried out by CX3CR1hi mononuclear phagocytes, an intestinal-cell population previously reported to be non-migratory6. These findings define a central role for commensals in regulating the migration to the MLNs of CX3CR1hi mononuclear phagocytes endowed with the ability to capture luminal bacteria, thereby compartmentalizing the intestinal immune response to avoid inflammation.

Figure 1 | Induction of immune response against non-invasive Salmonella after antibiotic treatment. a–c. Mice were left untreated (– ABX) or treated with antibiotic (+ ABX) for 4 weeks. a. Mice were orally infected with non-invasive, non-pathogenic Salmonella (invA/aroA), and Salmonella-specific IgG in the blood and IgA in the faeces were measured by ELISA. Bars represent the average from five mice from one of three independent experiments. ND, not detected. ***P < 0.001, unpaired t-test. Error bars represent standard error of the mean (s.e.m.). b. T cells from spleen, mesenteric lymph nodes (MLNs), Peyer’s patches (PPs) and small-intestinal lamina propria (SI) of mice infected with non-invasive Salmonella were cultured with irradiated splenocytes and boilded Salmonella antigen, and IFN-γ was measured by ELISA. Bars represent the average from three mice per treatment group and are representative of two independent experiments. *P < 0.05, unpaired t-test. Error bars represent s.e.m. c. Bacterial titres in the spleen and MLNs were determined for mice infected with non-invasive Salmonella (invA), e.t.u., colony-forming units. Data points represent organs from a single mouse; data were pooled from six experiments. ***P < 0.001, Mann–Whitney Test. Error bars represent the geometric mean.

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non-invasive *Salmonella* resulted in bacteria reaching the spleen, but very few bacteria were observed in the MLNs (Fig. 1c and Supplementary Fig. 1c). Importantly, after antibiotic treatment, there were high titres of the bacteria in the MLNs, as previously reported, in addition to the spleen (Fig. 1c and Supplementary Fig. 1c). As expected, no bacteria were found in the Peyer’s patches in either condition (data not shown). The increase in bacteria reaching the MLNs was unlikely to be the result of increased epithelial permeability after treatment with antibiotics, as we did not observe an increase in bacteria reaching the spleen. This was confirmed by using a direct test for intestinal permeability with fluorescein isothiocyanate (FITC)-conjugated dextran (FITC-dextran) gavage (Supplementary Fig. 1d). In addition, the increased *Salmonella* in the MLNs was not a consequence of impaired colonization resistance on depletion of the microbiota, as there was equivalent expansion of *Salmonella* in the presence of any individual antibiotic, yet only vancomycin or ampicillin treatment allowed for its trafficking to the MLNs and induction of the bacterium-specific IgA (Supplementary Fig. 2).

Our results raised the question of whether commensal bacteria could gain access to the MLNs in the same way as non-invasive *Salmonella*, allowing for the induction of mucosal-specific immune responses. Antibiotic-treated and untreated mice were infected by oral gavage with a non-invasive, non-pathogenic bacterium, *Escherichia coli* K-12. In the control mice, the bacteria could not be found in the MLNs, nor was there induction of *E. coli*-specific IgA (Supplementary Fig. 3a, b). By contrast, in antibiotic-treated mice *E. coli* was detected in the MLNs and there was induction of a specific IgA response (Supplementary Fig. 3a, b). These results indicate that the microbiota functions to limit the colonization of the MLNs by both commensal and pathogenic bacteria, thereby constraining the induction of intestinal immune responses against both.

To investigate whether a specific microbial recognition system is involved in microbiota-dependent restraint of *Salmonella* trafficking to the MLNs, we examined mice bearing mutations in diverse signalling pathways. As observed in antibiotic-treated mice, Myd88<sup>−/−</sup> mice infected with non-invasive *Salmonella* had increased bacterial counts in the MLNs compared to Myd88<sup>−/−</sup>-littermate controls (Fig. 2a). Consistent with our observations in commensal-depleted animals, there was also induction of *Salmonella*-specific IgA and antigen-induced IFN-γ-producing T cells in the MLNs in Myd88<sup>−/−</sup> animals but not in littermate controls (Fig. 2b, c). In contrast, there was no increase in bacteria reaching the MLNs or induction of IgA in NOD2- or NALP3-deficient (NALP3 also known as NLRP3) animals, suggesting that MyD88 specifically relays a microbial signal via a Toll-like receptor (TLR) (Supplementary Fig. 4a). This indicates that detection of commensals in a MyD88-dependent manner functions to reduce bacterial colonization of the MLNs and thereby reduce intestinal immune responses.

We next sought to identify microbial products that could prevent trafficking of non-invasive *Salmonella* to the MLNs in antibiotic-treated mice. Re-colonizing antibiotic-treated mice with the caecal contents of unmanipulated mice reduced the amount of *Salmonella* that reached the MLNs (Supplementary Fig. 4b), as did short pre-treatment with caecal contents (2 days before infection with non-invasive *Salmonella*) or with heat-killed *E. coli* or *Salmonella* (Fig. 2d). These results indicate that complete re-colonization was not required to abrogate trafficking of the bacteria. Pre-feeding mice with heat-killed bacteria before infection did not reduce the load of *Salmonella* in the intestine, even though the amount of bacteria reaching the MLNs was reduced (Fig. 2d and Supplementary Fig. 4c). This indicates that recognition of bacterial products can function to reduce colonization of internal organs with a non-invasive bacterium without affecting the intestinal load of that bacterium. As expected, pre-treating MyD88-deficient animals with heat-killed *Salmonella* had no effect (Supplementary Fig. 4d). Pre-treating mice with lipopolysaccharide (LPS), bacterial DNA or zymosan (Fig. 2d and data not shown) also failed to limit MLN titres of *Salmonella*, despite the ability of LPS to induce upregulation of the antimicrobial protein Reg-3γ (Supplementary Fig. 4e). Thus, commensal-dependent intestinal epithelial responses may not be sufficient to prevent trafficking of non-invasive *Salmonella* to the MLNs.

Antigens and pathogens that can penetrate the mucous layer and cross the intestinal epithelium to enter the lamina propria are thought to be trafficked by dendritic cells to the MLNs, where they can prime immune responses<sup>13,14</sup>. This conclusion was based on experiments with invasive bacteria that colonized Peyer’s patches<sup>14,15</sup>. Although lamina propria CD11c<sup>−</sup> cells were also shown to be required for non-invasive *Salmonella* colonization of the lamina propria and the MLNs, it was concluded that the dendritic cells were required only for bacterial translocation across the epithelium<sup>12,16</sup>. We wanted to determine whether non-invasive bacteria, which cannot colonize Peyer’s patches, would also be transported to the MLNs by lamina propria dendritic cells. Our observation that *Salmonella* trafficked to the MLNs only after dysbiosis induced by antibiotics, in the absence of barrier disruption, suggested a specific mechanism that restricts access of bacteria to intestinal immune-priming sites. We therefore examined whether dendritic cells or other lamina propria myeloid cells were responsible for transporting *Salmonella* to the MLNs and initiating immune responses to it. We first used CD11c<sup>+</sup>-diphtheria toxin receptor (DTR) mice, in which injection of diphtheria toxin results in
selective depletion of CD11c+ cells17,18. CD11c-DTR bone marrow chimeraic mice were treated with antibiotics and then injected with diphtheria toxin to deplete dendritic cells (Supplementary Fig. 5) before oral infection with non-invasive Salmonella. There was no decrease in splenic titres of non-invasive Salmonella in diphtheria-toxin-treated versus untreated mice (Fig. 3a), arguing against a role for dendritic cells in bacterial entry to the tissue. However, bacteria titres in the MLNs were significantly reduced after ablation of CD11c cells (Fig. 3a). Thus, in microbiota-depleted mice, CD11c+ cells are at least partially responsible for transporting non-invasive Salmonella to the MLNs. As dendritic cell migration to the MLNs requires their expression of CCR7 (refs 19, 20), we treated CCR7+/− or CCR7−/− mice with antibiotics to deplete commensal bacteria before infecting mice orally with non-invasive Salmonella. There was a substantial reduction in Salmonella in the MLNs of commensal-depleted CCR7−/− mice compared to wild-type mice (Fig. 3b), indicating that this chemokine receptor is required for transport of the bacteria to the MLN.

The CD11c+ mononuclear phagocytes within the lamina propria have been subdivided into two major populations: CX3CR1+ and CD103+ cells (Supplementary Fig. 6a and reviewed in ref. 21). CX3CR1+ cells can be further divided into cells expressing high and intermediate levels of the chemokine receptor. CX3CR1hi cells, which also express CD14 and low levels of CD103 (Supplementary Fig. 6a),21,22, have been described to have both dendritic cell- and macrophage-like characteristics. They differentiate from monocyte precursors15,16,22, are thought to be non-migratory in response to in vivo TLR activation17, and are poorly immunostimulatory in vitro. CX3CR1int cells, which also differentiate from monocyte precursors, have been described to have dendritic cell-like characteristics23. CD103+ cells, which do not express CX3CR1, are thought to be conventional myeloid dendritic cells, as they develop from a classical dendritic cell precursor15,16, express CCR7, can activate naive T cells in vitro, and migrate to the MLNs on TLR stimulation21,22,23. In contrast with previous reports that found CCR7 only expressed on CD103+ cells15,16,19, we observed upregulation of CCR7 on both CX3CR1+ and CD103+ cells after in vitro stimulation with LPS (Supplementary Fig. 6b), suggesting that both have migratory potential.

To assess whether intestinal CD103+ or CX3CR1+ cells were involved in transporting Salmonella to the MLNs in the absence of commensals, we next examined mice in which a loxP-flanked stop cassette upstream of the DTR-coding region was knocked into the Cx3cr1 locus (Supplementary Fig. 7a). Expression of CD11c-Cre excises the stop cassette, allowing for DTR expression and selective depletion of CD11c+ CX3CR1hi cells on administration of diphtheria toxin, with no effect on CD11c+ CX3CR1int or CD11c− CD103− cells (Supplementary Fig. 7b, c). Because monocytes have been reported to take up wild-type Salmonella24, we determined that they were not depleted in the diphtheria-toxin-treated animals, but were actually increased in number (Supplementary Fig. 8). When mice were orally infected with non-invasive Salmonella after diphtheria-toxin-mediated depletion of the CX3CR1hi mononuclear cells, there was a marked reduction in the number of bacteria reaching the MLNs (Fig. 3c). This result, which was unexpected in the light of recent studies showing that only the CD103+ cells migrate from the lamina propria to the MLNs after TLR activation, suggested that CX3CR1hi cells are responsible for trafficking of the non-invasive bacteria to the MLNs on disturbance of the microbiota.

To evaluate further the hypothesis that CX3CR1hi cells are responsible for trafficking of non-invasive Salmonella to the MLNs, we orally infected antibiotic-treated CX3CR1−green fluorescent protein (GFP) mice25 with the bacterium and analysed the cell populations in the MLNs. There was an increase in dendritic cells of all subsets after antibiotic treatment (Supplementary Fig. 9a), but after infection with non-invasive Salmonella there was a further increase only in CX3CR1hi cells (Fig. 3d and Supplementary Fig. 9a), and this was not observed if mice were pre-treated with heat-killed Salmonella before infection (Supplementary Fig. 9b), consistent with the limited number of bacteria reaching the MLNs in these animals (Fig. 2d). Furthermore, the co-stimulatory molecule CD80 was upregulated on CX3CR1hi cells, but not on CD103+ dendritic cells, after infection of antibiotic-treated mice with non-invasive Salmonella (Fig. 3e), implicating the CX3CR1hi cells in the process of transport.

**Figure 3** Colonization of MLNs by non-invasive Salmonella requires CCR7-dependent trafficking of CX3CR1hi cells. a–c. Antibiotic-treated mice of the indicated genotype and littermate controls were orally infected with non-invasive Salmonella, and bacterial titres in the spleen and MLNs were determined. a. Mice were treated with diphtheria toxin for two consecutive days before infection. a, ***, p < 0.0001; b, ***, p < 0.005; c, *** p = 0.0006; all Mann–Whitney test. Error bars represent the geometric mean. d. Analysis of dendritic cell subsets in MLNs of untreated or antibiotic-treated Cx3cr1LoxP/+ mice that were mock infected or infected with non-invasive Salmonella. MLN cells were isolated at 48 h, gated on the MHCII+ CD11c+ population, and analysed for expression of CX3CR1 and CD103. Percentages are shown in each gate. Absolute numbers are shown in Supplementary Fig. 9a. e. Expression of CD80 on intestinal myeloid cell subsets. Cells were gated on the indicated cell populations as shown in d. For data in a–c, points represent individual mice pooled from independent experiments. Panels in d represent individual mice from one of five independent experiments.
cells in priming the anti-Salmonella T-cell response. To confirm that CX3CR1hi cells had taken up non-invasive Salmonella, we sorted CX3CR1hi and CD103+ cells from the MLNs of infected antibiotic-treated mice (Supplementary Fig. 9c) and assayed for cells containing colony-forming units. Most of the bacteria were recovered from the CX3CR1hi cells rather than the CD103+ cells, and no bacteria could be detected from cells derived from untreated infected mice (Fig. 4a). These results suggest that commensal-derived signals prevent CX3CR1hi cells from entering bacteria to the MLNs, although we cannot rule out additional mechanisms, for example, reduced killing of internalized bacteria under these conditions.

To examine more directly whether CX3CR1hi cells migrate from the small intestine to the MLNs, we assayed the afferent intestinal lymphocytes (Fig. 3d) from the MLNs were isolated from antibiotic-treated, non-invasive Salmonella-infected mice. The numbers of bacteria per 10⁴ cells were determined by plating. No bacteria were observed from cells isolated from uninfected or infected but antibiotic-untreated mice. DCs, dendritic cells. Bars represent pooled data from 15 individual mice from 4 independent experiments. ***p < 0.0001, one-way ANOVA with Bonferroni correction. Error bars represent s.e.m.

b, Cells were gated on the MHCII⁺ CD11c⁺ population and analysed for expression of CX3CR1–GFP and CD103. Data are representative of three independent experiments.

c, Quantification of MHCII⁺ CD11c⁺ CX3CR1–GFP+ cells in the afferent lymph of the indicated mice. **p < 0.01, one-way ANOVA with Bonferroni correction. Error bars represent s.e.m.

d, CD80 expression on CX3CR1–GFP+ cells from lymph of antibiotic-treated mice with and without Salmonella infection.

methods summary

Mice. C57BL/6 mice were purchased from Taconic Farms or Jackson Laboratories. For inducible CX3CR1–DTR mice, we introduced a loxP/loxP cassette followed by DTR into the Cx3cr1 locus. Mice were subsequently crossed to CD11c-Cre mice (Jackson Laboratories).

Depletion of gut commensal microbiota. Animals were treated with antibiotics as described3. Mice were switched to water without antibiotics for 2 days before infecting them as described later.

Infection with Salmonella Typhimurium and E. coli. Mice were infected orally with 1 × 10⁶ Salmonella AinvA c.f.u. (stationary phase) or 2 × 10⁶ Aaro/AinvA c.f.u. Organ titres were determined 2 days after infection. For T-cell analysis, mice were left uninfected or were infected with non-invasive Salmonella. At 48 h, cells in the intestinal lymph were isolated and analysed by flow cytometry.
were analysed 10 days after infection. For antibody titres, mice were infected on day 0 and day 15.

Measurement of T-cell and antibody responses. Analyses of serum and fecal proteins for 
Salmonella-specific IgG and IgA by ELISA were performed as described
[30]. For T-cell responses, T cells from the isolated organ were stimulated in vitro for 72h. IFN-γ in the supernatant was measured by ELISA (BD Biosciences).

Cell isolation. Small-intestinal lamina propria cells were isolated as previously described
[38].

Lymph collection. Afferent lymph was isolated as described
[38].

Antibodies, cell staining and flow cytometry. Flow cytometric analysis was performed on a LSR II (BD Biosciences) instrument and analysed using FlowJo software (Tree Star). All antibodies were from BD Pharmingen or eBiosciences. Heat-killed 
Salmonella and E. coli. 
Salmonella or E. coli were heated to 85 °C for 60 min. Mice were administered 1 × 10⁶ heat-killed bacteria orally daily for 2 days before infecting with non-invasive 
Salmonella.

Statistical analysis. One-way ANOVA with Bonferroni’s post-test or unpaired t-test was performed using a 95% confidence interval. All analyses were performed using GraphPad Prism version 4.0. Differences were considered significant at P values of <0.05.

Full Methods and any associated references are available in the online version of the paper.

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Supplementary Information is available in the online version of the paper.

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METHODS

Mice. C57BL/6 mice were purchased from Taconic Farms or Jackson Laboratories. Ccr7- and Nod2-mutant mice were purchased from Jackson Laboratories. CX3CR1–GFP and CD11c–DTR mice were as previously described. MyD88- and Nalp3-mutant mice were from R. Medzhitov and J. Tschopp, respectively. For inducible CX3CR1–DTR mice, we introduced a loxP-loxP stop cassette followed by DTR into the Cxcr1 locus. Mice were subsequently crossed to CD11c–Cre mice (Jackson Laboratories), allowing deletion of the stop cassette in CD11c+ cells and induction of DTR in CX3CR1+ cells. All animal experiments were performed in accordance with approved protocols for the NYU Institutional Animal Care and Use Committee.

Depletion of gut commensal microbiota. Animals were given ampicillin (A; 1 g l−1; Sigma), vancomycin (V; 500 mg l−1; Amresco), neomycin sulphate (N; 1 g l−1; Sigma), and metronidazole (M; 1 g l−1; Sigma) in drinking water for 4 weeks as described. Mice were switched to water without antibiotics for 3 days before infecting them as described later.

Infection with Salmonella Typhimurium and E. coli. Mice were infected orally with 1 × 10⁶ Salmonella AttVA c.f.u. or 2 × 10⁶ AaroA/AinvA c.f.u. in 100 μl of PBS. For titres, organs were homogenized 2 days after infection, diluted in phosphate-buffered saline (PBS) and plated on LB agar with streptomycin (strep) (50 μg ml−1). Colony counts were expressed as c.f.u. per organ. For antibody titres, mice were infected on day 0 and day 15. Blood and faeces were collected 2 weeks after the second infection.

Generation of CD11c–DTR bone marrow chimaeras. Six-week old Ly5.1 congenic mice (Jackson Labs) were lethally irradiated and reconstituted with bone marrow from wild-type or CD11c–DTR transgenic mice (Ly5.2). After 8–10 weeks, recipients were examined for immune reconstitution by analysing blood for Ly5.2+ haematopoietic cells before treating with antibiotics.

Cell isolation. Small-intestinal lamina propria cells were isolated as previously described. Spleen and MLN T cells were isolated 2 h later. During cell isolation and extracellular staining, all solutions contained Brefeldin (GolgiPlug, BD Biosciences). Cells were stained for cytokines as per the manufacturer’s instructions (BD Bioscience).

RNA extraction and real-time RT–PCR. The terminal ileum was disrupted in Trizol (Ambion) using a TissueLyserII (Qiagen). Real-time PCR for Reg-3y was performed as described.

Intestinal permeability. Intestinal permeability was assessed by oral administration of FITC-dextran (Sigma–Aldrich) at 400 mg kg−1 as described. Serum FITC-dextran concentrations were determined using an Envision 2104 Multispecies Reader (Perkin Elmer).

Quantification of faecal bacterial DNA. Faecal pellets were weighed and resuspended in 200 mM Tris pH 8, 20 mM NaCl, 20 mM EDTA with 3% SDS. Bacteria were lysed by mechanical disruption with silica beads using a Retsch TissueLyser. DNA was extracted using phenol-chloroform. qPCR was performed on a Lightcycler 480 (Roche Applied Science) using SYBR green (Qiagen). 16S rRNA was amplified using forward primer 5′-ACTCCTAGGGGACGACAGT-3′ and reverse primer 5′-ATTACGGCGGCTGCTGGC-3′. A standard curve was generated using limiting dilutions of bacterial DNA to allow conversion of 16S rRNA amplification to nanograms of bacterial DNA. The data were normalized to faecal pellet weight and expressed as ng bacterial DNA/mg of faecal pellet.

Sorting Salmonella-infected cells. Cx3cr1GFP/ - mice were treated with antibiotics and infected with non-invasive Salmonella. Two days later MLN cells were isolated and stained. Cell sorting was performed on an Aria cytometer (BD Biosciences). TCR-β+ CD19+ or TCR-β+ CD19- CD103+ or CX3CR1+ CD103- cell populations. CD19+ or TCR-β+ cells were plated as non-dendritic cells. Sorted cells were plated on LB-strep plates.

Heat-killed Salmonella and E. coli. Salmonella or E. coli were heated to 85 °C for 60 min. Bacteria were plated and allowed to grow overnight to confirm death. Mice were administered 1 × 10⁶ heat-killed bacteria orally daily for 2 days before infecting with non-invasive Salmonella.

Statistical analysis. One-way ANOVA with Bonferroni’s post-test or unpaired t-test was performed using a 95% confidence interval. All analyses were performed using GraphPad Prism version 4.0. Differences were considered to be significant at P values of <0.05.

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