

Neutrophils Are a Source of Gamma Interferon during Acute *Salmonella enterica* Serovar Typhimurium Colitis

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Gamma interferon (IFN- γ) is an important driver of intestinal inflammation during colitis caused by *Salmonella enterica* serovar Typhimurium. Here we used the mouse colitis model to investigate the cellular sources of IFN- γ in the cecal mucosa during the acute phase of an *S. Typhimurium* infection. While IFN- γ staining was detected in T cells, NK cells, and inflammatory monocytes at 2 days after infection, the majority of IFN- γ -positive cells in the cecal mucosa were neutrophils. Furthermore, neutrophil depletion blunted mucosal *Ifng* expression and reduced the severity of intestinal lesions during *S. Typhimurium* infection. We conclude that neutrophils are a prominent cellular source of IFN- γ during the innate phase of *S. Typhimurium*-induced colitis.

Individuals with primary immunodeficiencies impairing production of gamma interferon (IFN- γ) or IFN- γ signaling present in childhood with disseminated bacterial infections. The vast majority of these infections are caused by two bacterial pathogens: nontyphoidal *Salmonella* serovars and weakly virulent *Mycobacterium* species (1). Nontyphoidal *Salmonella* serovars, such as *S. enterica* serovar Typhimurium, cause a localized gastroenteritis in immunocompetent individuals, which is characterized by acute intestinal inflammation and diarrhea (reviewed in references 2 and 3). The importance of innate immune responses during gastroenteritis is illustrated by the rapid onset of intestinal inflammation, which gives rise to symptoms within 24 h of ingesting *S. Typhimurium* (4). A histopathological hallmark of *S. Typhimurium*-induced gastroenteritis is a severe acute infiltrate of neutrophils in the ileal and colonic mucosae (5, 6). Studies using a mouse model of *S. Typhimurium*-induced colitis show that in animals lacking IFN- γ , the severity of intestinal inflammation is markedly attenuated (7, 8).

Conventional wisdom holds that IFN- γ is produced during the innate phase of a bacterial infection by natural killer (NK) cells and NKT cells (reviewed in reference 9) while CD4⁺ and CD8⁺ T cells become the principal cellular sources at later stages, which are governed by adaptive immune responses (reviewed in references 10 and 11). Consistent with this idea, splenic NK cells are a prominent cellular source of innate IFN- γ production in models of systemic *S. Typhimurium* infection (12–14). Furthermore, CD1D-restricted NKT cells contribute to innate IFN- γ production in the livers and spleens of mice with disseminated *S. Typhimurium* infection (15, 16). However, the identities of the innate immune cells contributing to early IFN- γ production in the intestinal mucosa have not been fully resolved. The goal of this study was to identify the cellular sources of innate IFN- γ production in the intestinal mucosa during *S. Typhimurium*-induced colitis.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *S. Typhimurium* strain IR715 is a fully virulent, nalidixic acid-resistant derivative of wild-type isolate ATCC 14028 (American Type Culture Collection) (17). Bacteria were cultured overnight aerobically at 37°C in Luria-Bertani (LB) broth or on LB agar plates with 0.05 mg/ml nalidixic acid.

Animal experiments. Mouse experiments were performed according to USDA guidelines and were approved by the Institutional Animal Care

and Use Committee at the University of California at Davis. Female, 8- to 12-week-old C57BL/6 mice (Jackson Laboratory) or mice carrying an internal ribosome entry site (IRES)-enhanced cyan fluorescence protein (eYFP) reporter cassette inserted between the translational stop codon and 3' untranslated region (UTR)/poly(A) tail of the *Ifng* gene (Jackson Laboratory, lab stock number 017581) were used for the mouse colitis model. In brief, mice were inoculated intragastrically with streptomycin (0.1 ml of a 200-mg/ml solution in sterile distilled water), and 24 h later mice were inoculated intragastrically either with sterile LB broth or with bacteria (0.1 ml containing between 1×10^9 and 1×10^{10} CFU/ml). For Ly6G depletion experiments, mice were given 0.5 mg/animal of either anti-Ly6G antibody (clone 1A8, Bio X Cell BE0075) or an isotype control (purified rat IgG2a, clone 2A3, Bio X Cell BE0089) in 500 μ l of sterile Dulbecco phosphate-buffered saline (DPBS) intraperitoneally for 2 days. The first injection was administered concurrently with streptomycin treatment and the second at 24 h after infection. At 2 days after infection, mice were euthanized and samples of the cecum were collected for the isolation of mRNA and histopathological analysis. For bacteriological analysis, colon contents, mesenteric lymph nodes, and the liver and spleen were homogenized, and serial 10-fold dilutions were spread on agar plates containing the appropriate antibiotics. The cecum from each animal was collected to obtain cellular populations for analysis by flow cytometry.

Quantitative real-time PCR. For quantitative analysis of mRNA levels, 1 μ g of RNA from each sample was reverse transcribed in a 50- μ l volume (TaqMan reverse transcription reagent; Applied Biosystems), and 4 μ l of cDNA was used for each real-time reaction. Real-time PCR was performed using Sybr green (Applied Biosystems) and an Applied Biosystems ViiA 7 fast real-time PCR system. The data were analyzed using a comparative cycle threshold method (Applied Biosystems). Increases in cytokine expression in infected mice were calculated relative to the average level of the respective cytokine in four control animals from the corresponding time point after inoculation with sterile LB broth. A list of

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TABLE 1 Primers for quantitative real-time PCR

Gene	Primer 1	Primer 2
<i>Ifng</i>	5'-TGATGGCATGTCAGACA GCA-3'	5'-GGCACAAGTCATATAG CCTGACAC-3'
<i>Gapdh</i>	5'-TGTAGACCATGTAGTTGA GGTCA-3'	5'-AGGTCGGTGTGAAC GGATTG-3'

genes analyzed in this study and the respective primers is provided in Table 1.

Histopathology. Tissue samples were fixed in formalin, processed according to standard procedures for paraffin embedding, sectioned at 5 μ m, and stained with hematoxylin and eosin. A veterinary pathologist performed a blinded scoring for inflammatory changes on a rating scale from 0 (not detected) to 5 (severe) for each of the following five histological parameters: neutrophil infiltration, infiltration by mononuclear cells, submucosal edema, epithelial injury, and exudate (18).

Preparation of cecal cell suspensions. The cecum and proximal colon were collected from either C57BL/6 or eYFP-IFN- γ reporter mice (Jackson Laboratory). Fat and connective tissue were removed. Intestinal sections were cut longitudinally from the proximal colon to the tip of the cecum. The cecal content was removed by scraping gently with the flat edge of scissors. The sections were then washed in cold 1 \times Hanks balanced salt solution (HBSS) (Gibco catalog no. 14185) containing 0.015 M HEPES (Gibco catalog no. 15630) a total of six times to remove mucus and remaining fecal matter. Tissue was then transferred into 10 ml of pre-warmed (37°C) 1 \times RPMI medium (Sigma catalog no. R1145) containing 10% fetal bovine serum, penicillin-streptomycin (Gibco catalog no. 15240-062), 0.015 M HEPES, and 2.9 units/ml of collagenase (FALGPA) (Sigma-Aldrich catalog no. C6885). Additionally, for cecal lymphocytes bound for intercellular staining and fluorescence-activated cell sorter (FACS) analysis, 0.15 mg of brefeldin A was added to the disassociation medium. Tissue was then processed using a protocol provided by the manufacturer. In brief, tissue was run twice on program Brain_01 on the

gentleMacs Dissociator. Tissue samples were then rotated slowly at 37°C for 35 min in a hybridization oven. Samples were then disassociated using a high-speed program on the gentleMacs Dissociator as recommended by the manufacturer. The cell suspension was then strained twice through a 70- μ m cell strainer or until a firm pellet was obtained. With the exception of the incubation time at 37°C, samples remained chilled on ice during all steps in tissue processing.

Flow cytometry. A total of 4×10^6 intestinal cells were resuspended in 2 ml of DPBS and were stained with Aqua Live/Dead cell discriminator (Invitrogen no. L34597) as per the manufacturer's protocol. To distinguish cell populations shown in Fig. 1, cells were surface stained for 20 min in the dark at 4°C with optimized concentrations with anti-CD3 allophycocyanin (APC)/Cy7 (clone 17A2; Biolegend), anti-NK1.1 phycoerythrin (PE) (clone PK136; Biolegend), anti-CD11B PeCy7 (clone M1/70; eBioscience), anti-CD11C Alexa Fluor 700 (clone N418; eBioscience), anti-LY6C Pacific Blue (clone HK1.4; Biolegend), and anti-LY6G peridinin chlorophyll protein (PerCp)/Cy5.5 (clone 1A8; Biolegend). Cells were washed twice with PBS containing 1% bovine serum albumin and 1 mM EDTA (FACS buffer). After staining, cells were fixed and permeabilized using BD Cytofix/Cytoperm (BD 51-2090KZ). Permeabilized cells were then stained with anti-IFN- γ APC (clone XMGI.2; eBioscience) per the manufacturer's protocol. Cells were then washed twice with BD Perm/Wash buffer (BD 554723) and once with FACS buffer. Samples were then resuspended in 300 μ l FACS buffer for analysis. To confirm neutrophil morphology, LY6G⁺ LY6C⁺ cells were sorted using a MoFlo high-speed cell sorter (Dako). Cytospin samples were prepared from the sorted cells using a Shandon cytocentrifuge (Thermo Scientific, MI) and dried prior to staining with a Diff-Quik staining kit (IMEB, Inc., San Marcos, CA).

To determine cellular populations for experiments using eYFP-*Ifng* reporter mice shown in Fig. 2, single-cell suspensions were surface stained with anti-CD3 APC (clone 17A2; Biolegend), anti-NK1.1 PE/Cy7 (clone PK136; Biolegend), anti-CD11B APC/Cy7 (clone M1/70; eBioscience), anti-LY6C Pacific Blue (clone HK1.4; Biolegend), and anti-LY6G PerCp/Cy5.5 (clone 1A8; Biolegend). Samples were then fixed for 1 h using BD

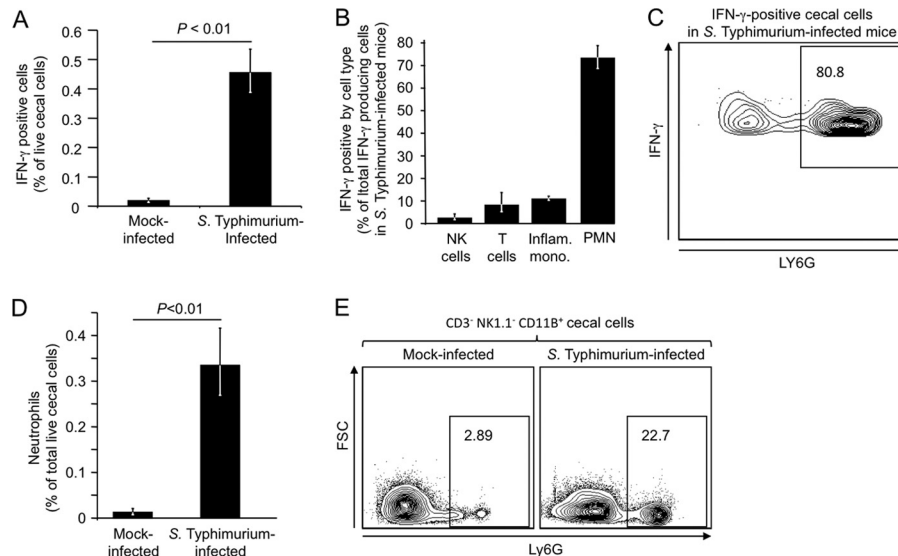


FIG 1 Detection of IFN- γ -positive cells in the cecal mucosa by intracellular cytokine staining. Flow cytometric analysis of cecal cell suspensions generated 2 days after inoculation of streptomycin-pretreated mice (C57BL/6) with *S. Typhimurium* or sterile medium (mock infection) is shown. (A) Numbers of IFN- γ -positive cells in mock-infected or *S. Typhimurium*-infected mice are shown as a percentage of total live cecal cells. (B) Numbers of IFN- γ -positive NK cells, T cells, inflammatory monocytes (Inflam. mono.), and neutrophils (PMN) as a percentage of all live IFN- γ -positive cecal cells. (C) Representative flow cytometry image showing that a large fraction of IFN- γ -positive live cecal cells express the neutrophil marker LY6G. (D) Number of neutrophils as percentage of total live cecal cells. (E) Representative flow cytometry images from a mock-infected mouse (left panel) or an *S. Typhimurium*-infected mouse (right panel), showing the fraction of CD3⁻ NK1.1⁻ CD11B⁺ cells expressing the neutrophil marker LY6G. In panels A, B, and D, each bar represents the geometric mean \pm standard error from flow cytometry measurements performed with 4 different animals.

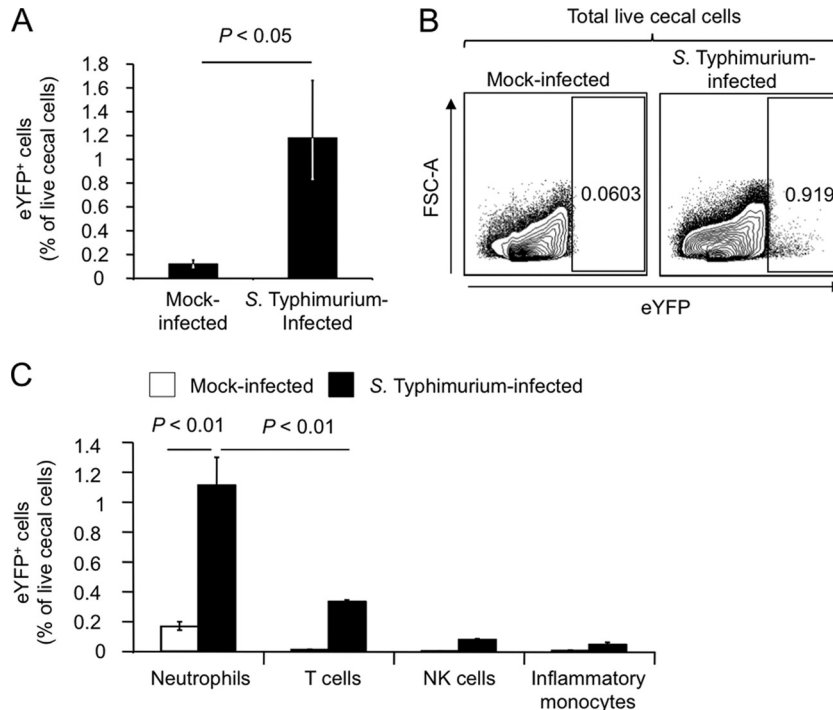


FIG 2 Detection of *Ifng* expression in the cecal mucosa using an eYFP reporter. Streptomycin-pretreated mice expressing eYFP under the control of the *Ifng* promoter were inoculated with *S. Typhimurium* or sterile medium (mock infection) and cecal cell suspensions generated 2 days later. (A) Numbers of eYFP⁺ cells in mock-infected or *S. Typhimurium*-infected mice are shown as percentage of total live cecal cells. (B) Representative flow cytometry images from a mock-infected mouse (left panel) or an *S. Typhimurium*-infected mouse (right panel), showing eYFP⁺ cells among total live cecal cells. (C) Numbers of eYFP⁺ NK cells, eYFP⁺ T cells, eYFP⁺ inflammatory monocytes, and eYFP⁺ neutrophils in mock-infected or *S. Typhimurium*-infected mice are shown as percentage of total live cecal cells. In panels A and C, each bar represents the geometric mean \pm standard error from flow cytometry measurements performed with 4 different animals.

Cytofix (BD 554714), washed twice in FACS buffer, and resuspended for FACS analysis.

For neutrophil depletion experiments, cell suspensions were surface stained for 20 min in the dark with anti-CD3 PE (clone 17A2; Biolegend), anti-NK1.1 PE (clone PK136; Biolegend), anti-B220 PE (clone RA3-6B2; Biolegend), anti-CD11B APC/Cy7 (clone M1/70; Biolegend), and anti-GR-1 APC (clone RB6-8C5).

Cells were washed twice, resuspended in FACS buffer, and analyzed using an LSR II (Beckman-Coulter, San Jose, CA) flow cytometer. The resulting data were analyzed using FlowJo software (TreeStar, Inc., Ashland, OR). All gates were based on fluorescence-minus-one controls.

Statistical analysis. To determine statistical significance between treatment groups in the animal experiments, an unpaired Student *t* test was used. A *P* value of less than 0.05 was considered to be significant.

RESULTS

Neutrophils are a source of IFN- γ during *S. Typhimurium* colitis.

To investigate cellular sources of IFN- γ during gastroenteritis, streptomycin-pretreated mice (C57BL/6) were inoculated with *S. Typhimurium* or sterile medium (mock infection). In this model, mice inoculated with *S. Typhimurium* develop acute cecal inflammation, which recapitulates aspects of human gastroenteritis (19). We used flow cytometry to identify the innate cell types in the cecal mucosa that contributed to IFN- γ production during *S. Typhimurium* infection using intracellular cytokine staining. To detect cells producing IFN- γ during infection, single-cell suspensions from the cecum were generated 48 h after infection in the presence of brefeldin A, which blocks the release of IFN- γ by inhibiting protein transport from the endoplasmic reticulum to the Golgi apparatus. The 48-h time point was chosen since this is the

time required for developing overt cecal inflammation after streptomycin-pretreated mice are infected with derivatives of *S. Typhimurium* strain ATCC 14028 (18).

Intracellular cytokine staining revealed that the fraction of cecal cells positive for IFN- γ was significantly ($P < 0.01$) increased in *S. Typhimurium*-infected mice compared to mock-infected mice (Fig. 1A). The populations of IFN- γ -positive cells in *S. Typhimurium*-infected mice were further differentiated using antibodies against CD3 (a T cell marker), NK1.1 (an NK cell marker), CD11B (a subunit of complement receptor [CR] 3), CD11C (a subunit of CR4), LY6C (a marker expressed by inflammatory monocytes and neutrophils), and LY6G (a neutrophil marker) (see Fig. S1 in the supplemental material). The LY6G/LY6C markers are superior to GR-1 (granulocyte differentiation antigen 1) and the mouse macrophage marker F4/80 in identifying neutrophils/monocytes (20). Several cell types, including T cells (IFN- γ ⁺ CD3⁺ cells), NK cells (IFN- γ ⁺ CD3⁻ NK1.1⁺ cells), and inflammatory monocytes (IFN- γ ⁺ CD3⁻ NK1.1⁻ CD11B⁺ LY6C⁺ LY6G⁻ cells) were identified as minor contributors to IFN- γ production, with each accounting for 10% or less of the total number of IFN- γ -positive cells in the cecal mucosa (Fig. 1B; see Fig. S1 in the supplemental material). Remarkably, the vast majority of all IFN- γ -positive cells in the cecal mucosa of *S. Typhimurium*-infected mice were neutrophils (IFN- γ ⁺ CD3⁻ NK1.1⁻ CD11B⁺ LY6C⁺ LY6G⁺ cells), which accounted for more than 70% of all IFN- γ -positive cells (Fig. 1B and C). CD3⁻ B220⁻ NK1.1⁻ CD11B⁺ LY6C⁺ LY6G⁺ cells were analyzed by microscopy, which confirmed their neutrophil morphology (data not shown).

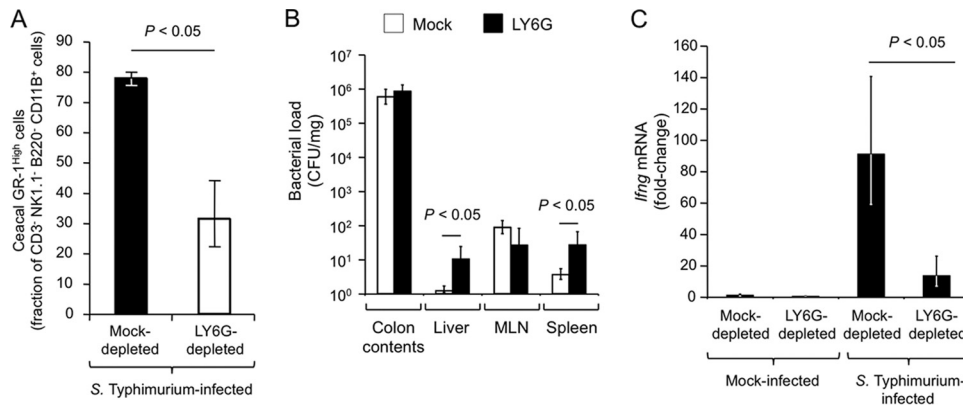


FIG 3 Neutrophil depletion blunts cecal *Ifng* expression during *S. Typhimurium* colitis. Streptomycin-pretreated mice were injected with an anti-LY6G antibody (LY6G depleted) or with an isotype control antibody (mock-depleted) and inoculated with *S. Typhimurium* or sterile medium (mock infection). Two days after infection, organs were collected for analysis. Each bar represents the geometric mean \pm standard error from measurements performed with 4 different animals. (A) Number of neutrophils (GR-1^{High} cells) as a percentage of the total number of phagocytes (CD3⁻ NK1.1⁻ B220⁻ CD11B⁺ cells) present in live cecal cell populations. (B) Bacterial numbers recovered from cecal contents, livers, mesenteric lymph nodes (MLN), and spleens of mice. (C) *Ifng* expression was determined by quantitative real-time PCR. Data are expressed as fold change over transcript levels detected in mice that were mock infected and mock depleted.

We also quantified neutrophil recruitment into the cecal mucosa. This analysis suggested that *S. Typhimurium* infection was associated with a significant ($P < 0.01$) increase in the fraction of neutrophils (CD3⁻ B220⁻ NK1.1⁻ CD11B⁺ LY6G⁺ LY6G⁺ cells) present in the live cecal cell population (Fig. 1D and E).

We next wanted to use an independent approach to test whether neutrophils are a cellular source of IFN- γ production during *S. Typhimurium* colitis. To this end, mice expressing an enhanced cyan fluorescence protein (eYFP) reporter cassette under the control of the *Ifng* promoter (21) were pretreated with streptomycin and inoculated with sterile medium (mock infection) or with *S. Typhimurium*. Single-cell suspensions from the cecum were generated 48 h after infection and analyzed by flow cytometry. The fraction of eYFP⁺ cells present in the cecal mucosa increased significantly ($P < 0.05$) in *S. Typhimurium*-infected mice compared to mock-infected mice (Fig. 2A and B). The population of eYFP⁺ cells was further differentiated using antibodies against relevant surface markers (CD3, NK1.1, CD11B, LY6C, and LY6G). Strikingly, the majority of eYFP⁺ cells in the cecal mucosa of *S. Typhimurium*-infected mice were neutrophils (eYFP⁺ CD3⁻ NK1.1⁻ CD11B⁺ LY6C⁺ LY6G⁺ cells) (Fig. 2C). Collectively, these data suggested that neutrophils recruited into the cecal mucosa during *S. Typhimurium* infection are a prominent early cellular source of IFN- γ .

LY6G depletion blunts early *Ifng* expression during *S. Typhimurium* colitis. The hypothesis that neutrophils are a major cellular source of IFN- γ suggests that depletion of this cell type might result in a blunted mucosal expression of *Ifng*, the gene encoding IFN- γ , during *S. Typhimurium* infection. To test this prediction, LY6G-positive cells were depleted using intraperitoneal injection of an anti-LY6G antibody or an isotype control antibody (mock depletion) prior to pretreatment with streptomycin and inoculation with *S. Typhimurium* or sterile medium (mock infection). Depletion of neutrophils was confirmed using a panel of surface markers that were compatible with the anti-LY6G antibody used for depletion (CD3, NK1.1, B220, CD11B, and GR-1) (see Fig. S2 in the supplemental material). Analysis of cecal cell suspensions by flow cytometry revealed that treatment with anti-LY6G antibody significantly ($P < 0.05$) reduced the number of neutrophils

(CD3⁻ NK1.1⁻ B220⁻ CD11B⁺ GR-1^{High} cells) recruited into the cecal mucosa at 48 h after *S. Typhimurium* infection (Fig. 3A). Compared to that in mock-depleted animals, LY6G depletion resulted in a significant ($P < 0.05$) increase in the bacterial load in the liver and spleen (Fig. 3B). Importantly, LY6G depletion significantly ($P < 0.05$) blunted *Ifng* expression in the cecal mucosa during *S. Typhimurium* infection (Fig. 3C). These data further supported the idea that neutrophils contribute to mucosal IFN- γ production during *S. Typhimurium* colitis.

IFN- γ is an important driver of inflammation during *S. Typhimurium* colitis (7, 8). Depletion of a cell type contributing to mucosal IFN- γ production would thus be expected to reduce the severity of histopathological lesions induced during *S. Typhimurium* infection. To test this prediction, a veterinary pathologist performed blinded scoring of histopathological lesions in sections of the cecum collected from streptomycin-pretreated mock-depleted or LY6G-depleted mice at 48 h after inoculation with *S. Typhimurium* or sterile medium (mock infection). LY6G depletion significantly reduced the numbers of neutrophils detected in histological sections (Fig. 4A), which was consistent with results obtained by flow cytometry (Fig. 3A). Histopathological evaluation revealed epithelial erosion, inflammatory infiltrates in the mucosa, and edema in the submucosa of mock-depleted mice infected with *S. Typhimurium*. These pathological changes were significantly ($P < 0.01$) reduced in *S. Typhimurium*-infected LY6G-depleted mice (Fig. 4B and C).

DISCUSSION

Previous studies on *S. Typhimurium*-induced colitis have implicated NK cells and several subsets of T cells, including CD1D-restricted NKT cells, CD8⁺ T cells, and CD4⁺ T cells, in contributing to mucosal IFN- γ production (15, 18, 22, 23). Consistent with these reports, we detected intracellular IFN- γ staining in NK cells and in T cells isolated from the cecal mucosa. However, neutrophils comprised more than 70% of IFN- γ positive live cecal cells isolated 2 days after oral *S. Typhimurium* infection (Fig. 1B). Furthermore, neutrophil depletion resulted in a significant blunting of mucosal *Ifng* expression in the cecal mucosa 2 days after *S. Typhimurium* infection.

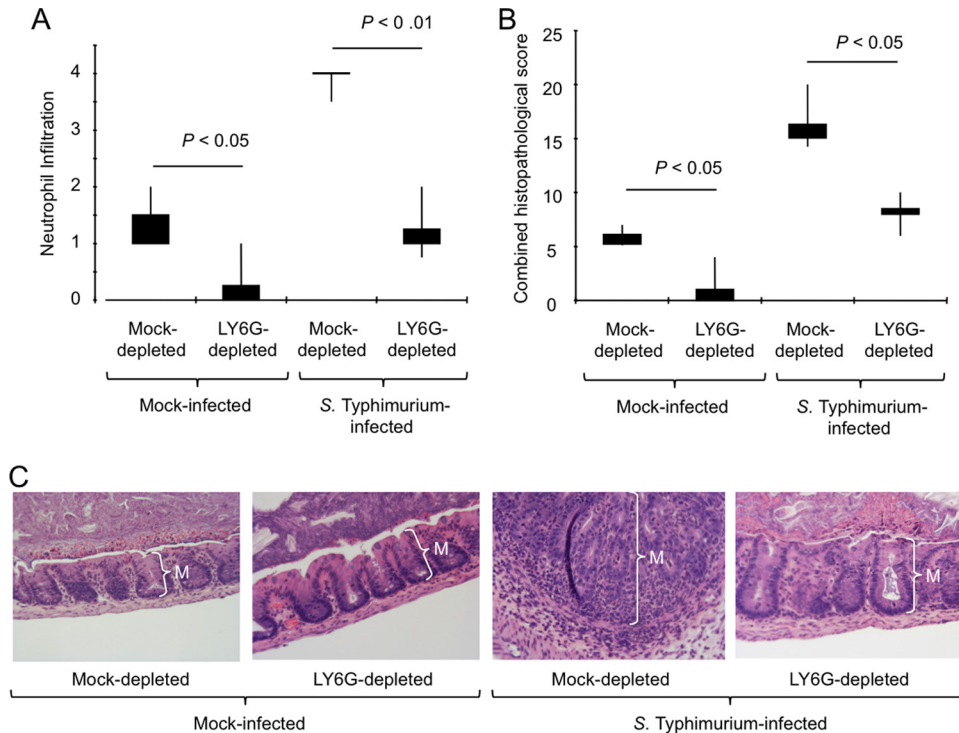


FIG 4 Neutrophil depletion reduces cecal pathology during *S. Typhimurium* colitis. Streptomycin-pretreated mice were injected with an anti-LY6G antibody (LY6G depleted) or with an isotype control antibody (mock depleted) and inoculated with *S. Typhimurium* or sterile medium (mock infection). (A and B) Two days after infection, organs were collected for analysis, and a veterinary pathologist performed blinded scoring of hematoxylin/eosin-stained sections from the cecal mucosa for neutrophil infiltration (A) and histopathological changes (B). Neutrophil infiltration was scored on the following scale: 0, 0 to 5 neutrophils per field; 1, 6 to 20 neutrophils per field; 2, 21 to 60 neutrophils per field; 3, 61 to 100 neutrophils per field; 4, >100 neutrophils per field. Boxes in whisker plots represent the second and third quartiles of scores, while lines indicate the first and fourth quartiles. (C) Representative images of histological sections from the ceca of mice taken at the same magnification. Note the marked thickening of the mucosa (M) due to neutrophil influx, which is observed in the section of an *S. Typhimurium*-infected, mock-depleted mouse.

Consistent with the important role of IFN- γ in driving inflammatory changes in tissue (7, 8), the severity of histopathological lesions in the cecal mucosa was significantly reduced in *S. Typhimurium*-infected neutrophil-depleted mice. A marked reduction in the severity of histopathological changes in response to *S. Typhimurium* infection is also observed in ligated ileal loops of calves with CD18 deficiency, a primary immunodeficiency resulting in an inability to recruit neutrophils into the intestinal mucosa because CD18/CD11B (CR3) is required for neutrophil extravasation (24). However, neutrophil deficiency affects many aspects of host-microbe interaction that have to be considered when interpreting these data. While reduced levels of IFN- γ are one possible reason for diminishing the severity of intestinal lesions, it is also possible that neutrophils directly contribute to tissue damage, for example, by releasing reactive oxygen and nitrogen species. Similarly, although it is conceivable that blunted IFN- γ production contributes to an increased bacterial load in the spleens of neutrophil-depleted mice (Fig. 3B), an alternative explanation is that the bactericidal activity of neutrophils is required to prevent spread of bacteria beyond the mesenteric lymph node.

A common approach to detect cytokine expression by flow cytometry is to stimulate cell suspensions *ex vivo* with the protein kinase C agonist phorbol-12-myristate-13-acetate (PMA) in the presence of the ionophore ionomycin. PMA is a potent activator of the T cell antigen receptor on T cells. When cell suspensions from the cecum collected 48 h after *S. Typhimurium* infection are

stimulated *ex vivo* with PMA-ionomycin prior to intracellular cytokine staining, approximately 50% of IFN- γ -positive cells are T cells (18). However, when we performed the same experiment in the absence of *ex vivo* PMA-ionomycin stimulation, T cells accounted for fewer than 10% of IFN- γ -positive cells (Fig. 1B). The latter result likely provides a more realistic view of cell types contributing to IFN- γ production *in vivo*, because the only stimulus provided was an *in vivo* infection with *S. Typhimurium* for 48 h. Importantly, PMA induces neutrophil cell death within 3 to 5 h after stimulation (25–27), thereby depleting this cell type prior to analysis of cytokine staining in live cells by flow cytometry. Thus, the widespread use of *ex vivo* PMA-ionomycin stimulation might be one of the reasons why neutrophils are not commonly detected as a major cellular source of IFN- γ .

While NK cells and NKT cells are commonly thought of as the primary sources of IFN- γ during the innate phase of a bacterial infection (reviewed in reference 9), there is mounting evidence that neutrophils are another important early source of this cytokine. For example, neutrophils produce IFN- γ during acute necrotizing pneumonia caused by *Nocardia asteroides* infection of mice (28). Neutrophils are an early source of IFN- γ after intravenous infection of mice with *Listeria monocytogenes* (29). Neutrophils are a cellular source of IFN- γ in splenocyte populations collected from mice 3 days after oral *S. Typhimurium* infection (30). Furthermore, neutrophils collected from the peritoneal cavities of mice 5 days after intraperitoneal *S. Typhimurium* infection produce

IFN- γ (31). Our results suggest that neutrophils are also a prominent cellular source of mucosal IFN- γ production during *S. Typhimurium*-induced colitis. Induction of *Ifn γ* expression in the cecal mucosa at 2 days after oral *S. Typhimurium* infection is MYD88 dependent (18). Similarly, IFN- γ production elicited by neutrophils recovered from the peritoneal cavities of mice 5 days after intraperitoneal infection with *Toxoplasma gondii* is MYD88 dependent (31). Collectively, these data suggest that neutrophils are an important cellular source of IFN- γ during the innate phase of a bacterial infection.

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We do not declare conflicts of interest.

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