Supporting Information

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SI Materials and Methods

Experimental Animals. SAMP1/YitFc (SAMP) mice were originally propagated at the University of Virginia and then at Case Western Reserve University, with founders provided by S. Matsumoto (Yakult Central Institute for Microbiological Research, Tokyo, Japan). AKR were purchased from The Jackson Laboratory. SAMP and WT AKR were maintained under specific pathogen-free conditions, fed standard laboratory chow (Harlan Teklad), and kept on 12-h light/dark cycles. All procedures were approved by Case Western Reserve University's Institutional Animal Care and Use Committee and Association for Assessment and Accreditation of Laboratory Animal Care guidelines.

Cells Isolation and Culture. Mesenteric lymph nodes (MLNs) and spleens were removed aseptically and gently pressed against a 100-μm cell strainer to obtain single-cell suspensions. Resulting cells were cultured (1×10^6/mL) in RPMI 1640 with 10% FBS and 1% penicillin/streptomycin. BM macrophages precursors were harvested from femurs of mice and cultured for 7 d in DMEM containing 10% FBS, 25 mM Hepes buffer, 1 mM sodium pyruvate, 5×10^{-5} 2-ME, antibiotic, and 25% of LADMAC cell conditioned medium as a source of M-CSF; fresh media was changed on day 5. After day 7, cells were plated in DMEM/F12-10 supplemented with 10% (vol/vol) FBS, 10 mM L-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin.

ELISA. Bone marrow-derived macrophages (BMDMs) were stimulated for 24 h with muramyl dipeptide (MDP) (1, 10, 100, 200 μg/mL); BMDMs were also stimulated with LPS (10 ng/mL) or the combination of MDP (10 μg/mL) and LPS (10 ng/mL). Cell-free supernatants were harvested from cultured cells and secreted cytokines measured by ELISA (QUANSYS; eBioscience). All ELISAs were performed according to manufacturer's instructions.

Western Blot Analysis. Cells were stimulated with MDP and then washed twice with PBS and harvested by scraping. Cells were centrifuged at $500 \times g$, rinsed again with PBS, and centrifuged at $500 \times g$. The pellet was then resuspended in lysis buffer and allowed to sit on ice for 10 min. The suspension was centrifuged at $16{,}000 \times g$ for 10 min, and the pellet was discarded. Protein concentrations were standardized through the Bradford assay (Bio-Rad). Western blot was then completed on nitrocellulose membranes (Invitrogen) as described previously (1). Membranes were blotted with antibodies as follow: anti-P105, anti-phospho-IκBα, total-IκBα, and anti-actin (Cell Signaling).

Histology. Colons and ilea from experimental mice were removed, flushed of fecal contents, opened longitudinally, and placed in Bouin's fixative. Tissues were embedded in paraffin and stained with hematoxylin and eosin. Inflammation was evaluated by a trained pathologist in a blinded fashion using an established scoring system previously described (2). The colon total inflammatory index represented the sum of four individual indices: active inflammation, chronic inflammation, percentage reepithelialization, and percentage of ulceration. The total inflammatory index for ileal specimens represented the sum of three individual indices: active inflammation, chronic inflammation, and villous distortion.

Images Acquisition. Images were obtained on an Olympus BX41 microscope (magnification, 100× and 200×; objective, 10×; eyepiece, 10×). The photo was obtained by Spot Imaging Solution System provided by Spot Diagnostic Instruments.

Induction of Colitis and MDP Administration. Induction of acute colitis was achieved in AKR, SAMP, and bone marrow (BM) chimeric mice by exposing them to 3% dextran sodium sulfate (DSS) in their drinking water for 7 d. Treated mice were monitored daily for body weight. Mice were administered MDP (100 μg, i.p.) or PBS for 3 consecutive days (days 0, 1, and 2 of DSS colitis induction).

Colonoscopic Investigation. Colonoscopy was performed using a flexible digital ureteroscope (URF-V, Olympus America), which has an 8.5Fr (2.8 mm) tapered-tip design, and motion range is 180° in up angle and 275° in a down angle. The endoscope system included a video system center (Olympus America), a xenon light source (Olympus America), and a video recorder (MediCapture). The video images and pictures were recorded to USB flash memory in MPEG-2 format and in JPG, TIFF, PNG, and DICOM formats with maximum 1,280 × 1,024 pixels, respectively. The colonoscopy was performed on the day 7 of DSS treatment, and the inflammation was evaluated for thickening, vascular pattern, fibrin visible, granularity, and stool. Mice were anesthetized by isoflurane, USP (Webster Veterinary), and any other preparations such as fasting or laxatives were not required.

BM Chimeric Mice. BM chimeric mice experiments were conducted as previously described (3). Mice receiving BM transfer were irradiated (900 radiation absorbed dose) immediately before transplantation. BM was harvested from femurs and tibias of 4-wk-old SAMP or AKR mice with RPMI medium (10% FCS), and cell suspensions were washed and diluted to a concentration of 30×10^6 cells per mL in HBSS. A total of 7.5×10^6 cells per 250 μL was injected i.v. into the lateral tail veins of recipient mice. BM transfer mice were placed on antibiotic water (0.7 mM neomycin sulfate, 80 mM sulfamethoxazole, and 0.37 mM trimethoprim) for 2 wk after irradiation, and then given autoclaved water for 4 wk to reconstitute normal gut flora.

Myeloperoxidase Assay Activity. Colon samples were assayed for myeloperoxidase (MPO) activity as previously described (4, 5). Specimens were collected, weighed, diluted 20-fold in 0.5% hexadecyl-trimethil-ammonium bromide buffer, homogenized with a Polytron PT 10–35 tissue homogenizer (Pro Scientific), and centrifuged to pellet debris. Samples (7 μL) were loaded in triplicate into a 96-well plate and exposed to substrate solution (200 μL; 5 mM potassium phosphate buffer containing 0.0005% H_2O_2 and 0.167 mg/mL *O*-dianisidine). The average rate of absorbance change at 450 nm over two sequential 30-s intervals was used to calculate MPO activity.

***Salmonella* Infection Assays.** An overnight culture of *Salmonella enterica* serovar Typhimurium SL1344 was diluted 1:7 and grown at 30 °C for 1 h, and then added to BMDMs at a multiplicity of infection of 10. For immunofluorescent confocal microscopy, BMDMs were plated on glass coverslips and infected for 90 min in the presence or absence of MDP (10 μg/mL; L18-MDP, InVivoGen). BMDMs were then fixed in 4% paraformaldehyde/PBS, permeabilized in 0.4% Triton X-100/PBS and blocked in 2% FBS/0.4% Triton X-100/PBS. Coverslips were stained in 2% FBS/0.4% Triton X-100/PBS with rabbit anti-*Salmonella* antibody (1:1,000; PA1-7244, Fisher), followed by goat anti-rabbit–Alexa 568 secondary antibody (1:1,000; Life Technologies), and mounted on slides in Vectashield with DAPI (Vector Labs). Samples were visualized by confocal microscopy using a 40× objective lens on a Leica TCS-SP5 II confocal/multiphoton high-speed upright microscope equipped with a Q-Imaging Retiga EXi cooled CCD

camera and Image ProPlus Capture and Analysis software (Media Cybernetics). *Salmonella* per cell were scored in *z*-stack overlays from six separate fields in two distinct experiments and quantitated in an automated fashion using a customized visual basic Image-Pro Plus macro (ImageQ). For quantification of viable intracellular bacteria, BMDMs were infected for 30 min, then washed twice with PBS, and cultured in DMEM supplemented with 10% FBS and 10 μg/mL gentamicin (Sigma) for 1 h. Cells were lysed in 50 μL of lysis buffer (PBS/0.1% Triton X-100), and dilutions were plated in duplicate onto Luria broth plates. After growth overnight at 30 °C, colonies recovered were counted, and colony-forming units (CFU) per well were calculated (6).

***Salmonella* Infection in Vivo.** SAMP and AKR control mice (4–6 wk) were housed individually or in groups of up to four animals. Water and food were withdrawn 4 h before per os (p.o.) treatment with 20 mg of streptomycin. Afterward, animals were supplied with water and food ad libitum. At 20 h after streptomycin treatment, water and food were withdrawn again for 4 h before the mice were infected with 10^9 CFU of *Salmonella enterica* serovar Typhimurium SL1344 (100-μL suspension in PBS, p.o.) or treated with sterile PBS (control). Thereafter, drinking water ad libitum was offered immediately and food 2 h postinfection. Forty-eight hours later, mice were euthanized by CO_2 asphyxiation, and tissue samples from the intestinal tracts, MLNs, spleens, and livers were removed for bacterial counts.

To analyze the colonization, MLNs, cecum, fecal pellets, spleens, and livers were removed aseptically and homogenized in cold PBS. The numbers of CFU were determined by plating appropriate dilutions on Mac-Conkey agar plates supplemented with streptomycin (50 μg/mL).

Statistical Analysis. Experiments were conducted at least in duplicates. Univariate and multivariate analyses were conducted using the collective data from replicated experiments. Comparisons of continuous data across experimental groups were conducted using Student *t* tests, one-way or two-way ANOVAs, or linear regression with appropriate post hoc tests when the data fulfilled the assumptions for parametric statistics. Alternative nonparametric tests were used for data with unfulfilled assumptions. Repeated-measure analysis was conducted as described (7). Hazard ratios between treatment groups in survival experiments were estimated and tested with log-rank statistics. The 95% confidence intervals were reported when appropriate. Significance at $P < 0.05$ and SEMs are shown in figures. Other *P* values were reported when deemed appropriate. All analyses were conducted with STATA and GraphPad software.

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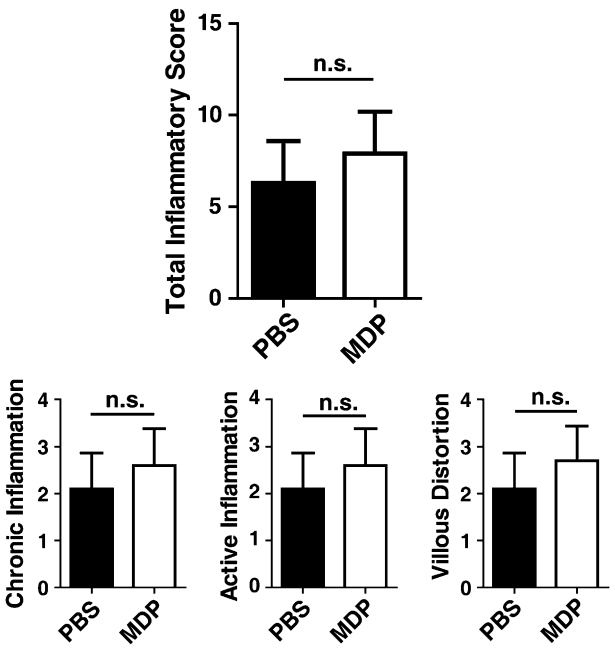

Fig. S1. Effect of MDP on severity of SAMP ileitis. SAMP mice were administered PBS (control) or MDP (100 μg per mouse) twice weekly for 6 wk ($n \geq 5$ per group). Ilea were analyzed for total histological assessment of inflammation determined by the sum of three indices: active inflammation, chronic inflammation, and villous distortion. No significant (n.s.) differences (Mann–Whitney, $P > 0.65$). Data are represented as mean ± SEM. Results represent two independent experiments.

Table S1. Cytokine production from AKR and SAMP BMDMs following stimulation with MDP

BMDMs	AKR-PBS	AKR-MDP	SAMP-PBS	SAMP-MDP
IL-1α	5.633 ± 0.83	15.23 ± 3.43*	8.177 ± 1.52	5.458 ± 1.50
IL-6	26.54 ± 3.8	39.66 ± 5.28*	24.93 ± 4.53	30.69 ± 6.65
IL-10	24.93 ± 4.5	30.69 ± 6.65*	14.29 ± 2.73	20.69 ± 3.90
IL-12p70	10.70 ± 3.9	31.57 ± 7.58*	37.38 ± 7.32	14.07 ± 5.85
TNF-α	33.72 ± 10.1	75.86 ± 17.4*	37.79 ± 10.30	43.93 ± 11.71

BMDMs isolated from preinflamed SAMP (4 wk old) and age-matched AKR control mice were incubated with MDP (10 μg/mL) or medium (control) for 24 h ($n \geq 6$). Cell-free supernatants were analyzed by ELISA for production of IL-1α, IL-6, IL-10, IL-12p70, and TNF-α (in picograms per milliliter). Results are shown as mean ± SEM. *$P < 0.05$, Student t test. Results are representative of at least three independent experiments.