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***Salmonella enterica* Causes More Severe Inflammatory Disease in C57/BL6 *Nramp1*^{G169} Mice Than Sv129S6 Mice**

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Abstract

Salmonella enterica serovar Typhimurium (*S. Typhimurium*) causes systemic inflammatory disease in mice by colonizing cells of the mononuclear leukocyte lineage. Mouse strains resistant to *S. Typhimurium*, including Sv129S6, have an intact *Nramp1* (*Slc11a1*) allele and survive acute infection, whereas C57/BL6 mice, homozygous for a mutant *Nramp1* allele, *Nramp1*^{G169}, develop lethal infections. Restoration of *Nramp1* (C57/BL6 *Nramp1*^{G169}) reestablishes resistance to *S. Typhimurium*; mice survive at least 3 to 4 weeks postinfection. Since many transgenic mouse strains are on a C57/BL6 genetic background, C57/BL6 *Nramp1*^{G169} mice provide a model to examine host genetic determinants of resistance to infection. To further evaluate host immune response to *S. Typhimurium*, we performed comparative analyses of Sv129S6 and C57/BL6 *Nramp1*^{G169} mice 3 weeks following oral *S. Typhimurium* infection. C57/BL6 *Nramp1*^{G169} mice developed more severe inflammatory disease with splenic bacterial counts 1000-fold higher than Sv129S6 mice and relatively greater splenomegaly and blood neutrophil and monocyte counts. Infected C57/BL6 *Nramp1*^{G169} mice developed higher proinflammatory serum cytokine and chemokine responses (interferon- γ , tumor necrosis factor- α , interleukin [IL]-1, and IL-2 and monocyte chemotactic protein-1 and chemokine [C-X-C motif] ligand 1, respectively) and marked decreases in anti-inflammatory serum cytokine concentrations (IL-10, IL-4) compared with Sv129S6 mice postinfection. Splenic dendritic cells and macrophages in infected compared with control mice increased to a greater extent in C57/BL6 *Nramp1*^{G169} mice than in Sv129S6 mice. Overall, data show that despite the *Nramp1* gene present in both strains, C57/BL6 *Nramp1*^{G169} mice develop more severe, Th1-skewed, acute inflammatory responses to *S. Typhimurium* infection compared with Sv129S6 mice. Both strains are suitable model systems for studying inflammation in the context of adaptive immunity.

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Declaration of Conflicting Interests

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Keywords

anemia; dendritic cells; inflammation; macrophages; mouse; *Nramp1*; *Salmonella enterica*; typhoid fever

The bacterial species *Salmonella enterica* is divided into typhoidal and nontyphoidal serotypes. Typhoidal serotypes include Typhi and Paratyphi A, B, and C and cause systemic infection in humans with bacterial colonization of the spleen, liver, bone marrow, and, chronically, the gallbladder.²⁹ Nontyphoidal serotypes generally cause self-limiting diarrhea in healthy people and remain a leading cause for gastroenteritis on a global scale.³⁸ Nontyphoidal gastroenteritis is modeled in calves and in mice treated with antibiotics.³⁸ As with many bacterial infections, treatment of both typhoidal and nontyphoidal salmonellae has become increasingly complicated by the emergence of multi-drug-resistant bacteria.²⁷

Laboratory mice infected via the natural oral route with *S. enterica* serovar Typhimurium (*S. Typhimurium*) develop systemic infections and are widely used to model typhoid fever.³⁸ The bacteria reside within macrophages and colonize the lymph nodes that drain the gastrointestinal tract and also colonize the spleen, liver, and meninges.^{21,24,43} One mouse genetic locus that contributes to *S. Typhimurium* resistance and enables animals to survive acute infection is *Nramp1* (*Slc11a1*).^{8,9,16,22,39,40,45} This gene encodes a cation transporter that removes divalent cations, including iron and manganese from phagosomes, thereby conferring resistance to intracellular pathogens that reside within vesicles.⁸ Mouse strains that encode wild-type *Nramp1* include Sv129S6, C3H/HeN, CBA, A/J, and DBA/2 (LD₅₀ >10⁵).³¹ *Nramp1*-deficient mice include BALB/c, B10.D2, and DBA/1 mice as well as the commonly used C57/BL6 strain, and these mice succumb to infection within 5 days of inoculation.^{11,38} A transgenic C57/BL6 strain expressing a functional allele of *Nramp1* is resistant to *S. Typhimurium* in that it survives for at least 2 weeks following intravenous inoculation with 10⁴ bacteria and nearly 3 weeks after intraperitoneal inoculation with 10⁵ bacteria.^{11,16} C57/BL6 *Nramp1*^{G169} mice are of particular interest because many transgenic mouse lines have a C57/BL6 background; use of the C57/BL6 *Nramp1*^{G169} strain to study acute and persistent responses to infection allows for the application of host genetics without extensive backcrossing.

The objective of this study was to carry out a direct comparison of hematopathology resulting from oral *S. Typhimurium* infection of Sv129S6 and C57/BL6 *Nramp1*^{G169} mice. Previous examination of Sv129S6 mice demonstrated that oral inoculation with 10⁹ bacteria results in a mild to severe acute infection that resolves into a chronic systemic infection of tissue macrophages²¹ that is most severe at 3 weeks postinfection.^{3,24} Sv129S6 mice develop hematological manifestations of disease, including microcytic anemia, neutrophilia and monocytosis, bone marrow myeloid hyperplasia and hemophagocytosis, and splenomegaly secondary to macrophage infiltration and extramedullary hematopoiesis (EMH). Loss of splenic iron staining was also observed in these studies.³ We report herein that oral inoculation with *S. Typhimurium* results in inflammatory disease manifestations that are significantly more severe at 3 weeks postinfection in C57/BL6 *Nramp1*^{G169} than in Sv129S6 mice.

Materials and Methods

Bacterial Cultures

Freshly struck colonies of virulent *S. Typhimurium* strain SL1344 (StrR),³³ inoculated into Luria-Bertani broth with streptomycin (200 mg/ml), were grown at 37°C with aeration

overnight. Bacteria were pelleted and resuspended in sterile phosphate-buffered saline (PBS) prior to infection of mice.

Animals

Research protocols were in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and approved by the University of Colorado and University of Washington Institutional Biosafety and Animal Care and Use Committees. Six- to 8-week-old Sv129S6/SvEv-Tac (Sv129S6) mice (Taconic Farms, Hudson, NY) and C57/BL6 *Nramp1*^{G169} mice homozygous for the wild-type copy *Nramp1* allele derived from Sv129 mice^{9,11} (University of Washington, Seattle, WA) were bred in-house under Modified Specific Pathogen Free facilities at the University of Washington, Seattle, or the University of Colorado, Boulder. Mice were fasted for 2 to 4 hours prior to infection via oral gavage with 1×10^9 colony-forming units (CFU) of *S. Typhimurium* in 100 μ l PBS, as determined by plating. Control mice were mock-infected with 100 μ l PBS and housed separately. Mice were evaluated at 3 weeks postinfection (4 repeated experiments) since most severe pathology occurs at this time point.³ Numbers of mice per sex and strain are as indicated in the figure legends. Across experiments, 4 C57/BL6 *Nramp1*^{G169} mice, not included in the analyses, died prior to the study end point of 3 weeks, and all had signs of neurological disease (previously described);^{3,43} no early deaths occurred in the Sv129S6 mice.

Hematopathology

Blood and tissue collection and processing—Blood was collected by cardiocentesis into K₃EDTA and serum tubes immediately following carbon dioxide inhalation. Fresh blood smears were Wright stained and reviewed for morphology, and manual leukocyte differential counts were correlated to automated analyzer results (Advia120; Bayer, Tarrytown, NY). Following euthanasia, whole spleen was weighed; a section of each was weighed, homogenized, and plated for bacterial CFU. No CFU were found in any mock-infected animals. Remaining spleen and 1 femur were collected into 10% neutral buffered formalin. Bone marrow brush preparations, made immediately postmortem from the second femur, were Wright stained for cytological examination, including 200-cell counts and calculated myeloid to erythroid (M:E) ratios. Tissues were processed by routine histological methods; paraffin sections were stained with hematoxylin and eosin (HE) and Perl's Prussian blue for ferric iron (hemosiderin). Intensity of iron tissue staining was subjectively scored on a scale of 0 to 4+ by light microscopy.

Photomicrographs were taken (Olympus BX50; Olympus Corporation, Center Valley, PA) using QImaging MicroPublisher RTV 5.0 (JH Technologies, Fremont, CA).

Serum Cytokines and Chemokines

Serum cytokine and chemokine concentrations were analyzed using kits from MesoScale Discovery (Gaithersburg, MD), including Mouse TH1/TH2 9-Plex and Mouse MCP-1 Ultra-Sensitive, per the manufacturer's instructions. Standard curves, run in parallel and in duplicate, were used to interpolate the concentration (pg/ml) of each analyte. Plates for all kits were incubated on the IKA MTS 2/4 Digital Plate Shaker, washed on a Thermo Electron Corporation (Bedford, OH) Wellwash 4 Mk2 Microplate Strip Washer, and scanned on a Sector Imager 2400 (MesoScale Discovery), and data were analyzed using Discovery Workbench (3.0) software (MesoScale Discovery). The lower level of detection was calculated as 2.5 standard deviations above the signal for the zero calibrator; the upper level was set to the highest calibrator (10,000 pg/ml). An extended standard curve on a subsequent run was used to detect levels of cytokines above the original high calibrator (up to 40,000 pg/ml).

Iron Analyses

At 3 weeks postinfection, spleens were collected from infected and control mice to measure total non-heme iron per gram of spleen. A portion of spleen was weighed and homogenized. Tissue iron concentrations were determined by acid digestion of fresh tissue samples³⁶ followed by quantification using a spectrophotometric iron assay kit per the manufacturer's instructions (BioVision, Mountain View, CA). A standard curve was routinely established (0–10 nmol of iron), and calculations were then performed to determine the iron concentration of splenic tissue for each mouse (nmol iron/g spleen).

Flow Cytometry

Weighed portions of spleens were mechanically dispersed and passed through a 70- μ m pore-size cell strainer. Cells were counted on a hemocytometer to estimate the number of cells per spleen. Aliquots were lysed in PBS with 0.2% NP-40 and plated for bacterial CFU. Cells were washed in RPMI, passed through a 40- μ m pore-size cell strainer, and resuspended in staining buffer (PBS + 1% fetal bovine serum [FBS] and 0.02% azide) containing anti-mouse CD16/32 (eBioscience, San Diego, CA) to block Fc receptors.²⁰ Cells were then permeabilized in 0.1% saponin and stained with phycoerythrin-conjugated anti-mouse CD68 (FA11; AbD Serotec, Raleigh, NC), allophycocyanin-conjugated anti-mouse CD11c (eBioscience), and phycoerythrin-Cy7-conjugated anti-mouse Gr1 (Ly-6C/G, RB6-8C5; eBioscience), fixed in 1% paraformaldehyde/1% sucrose and stained for DNA in 10 μ g/ml DAPI in buffer containing 0.1% saponin. Fluorescently labeled cells were quantified using a CyAn ADP flow cytometer (Beckman Coulter, Brea, CA) and analyzed using FlowJo software (Tree Star, Ashland, OR). Isotype controls used were rat IgG2a-RPE (for CD68; AbD Serotec), Armenian Hamster IgG (for CD11c; eBioscience), and rat IgG2b-K-PE-Cy7 (for Gr1; eBioscience). The numbers of neutrophils, inflammatory monocytes, macrophages, dendritic cells, and plasmacytoid dendritic cells per spleen were calculated as follows, for example: [(number of neutrophils per sample)/(total cells per sample)] $\times$ (number of cells per spleen). The number of total nucleated cells per flow sample was established based on cell granularity (side scatter) and DAPI content.

Statistics

A Student's *t*-test (Fig. 1a) and 1-way analyses of variance (ANOVAs) with Tukey post hoc tests (Fig. 1b and c, Fig. 2, Fig. 8, Fig. 9) were performed and considered significant at $P < .05$. JMP Pro 9.0.2 (SAS, Cary, NC).

Results

C57/BL6 *Nramp1*^{G169} mice have greater tissue bacterial colonization and develop more severe hematopathology than Sv129S6 mice at 3 weeks postinfection

To directly compare the response to infection of the C57/BL6 *Nramp1*^{G169} and Sv129S6 strains, mice were inoculated by oral gavage with approximately 1×10^9 CFU of virulent *S. Typhimurium*. At 3 weeks postinfection, there were 1000-fold higher splenic bacterial counts in the C57/BL6 *Nramp1*^{G169} mice (Fig. 1a). In addition, approximately 3-fold greater splenomegaly (Fig. 1b) occurred as a result of hematopoietic response to anemia (Figs. 2–5), EMH (Figs. 6), and increased phagocytic cell infiltrates. EMH is the generation of hematopoietic cells outside of the bone marrow. In the mouse, the spleen remains an active site of hematopoiesis through adulthood, and expanded splenic EMH can occur secondary to inflammatory disease and anemia.^{3,28,34} Blood neutrophil counts increased approximately 3-fold more upon infection in C57/BL6 *Nramp1*^{G169} mice than in Sv129S6 mice (Fig. 2), consistent with increased acute inflammatory responses. Both strains also developed increased peripheral blood monocyte counts, statistically significant only in the C57/BL6

Nramp1^{G169} mice (Fig. 2). Splenic architecture was more extensively disrupted in C57/BL6 *Nramp1*^{G169} mice, characterized by subcapsular splenic abscesses and loss of white pulp (splenic lymphoid follicles) (Fig. 6). Reduced white pulp correlated with the more significant peripheral blood lymphopenia (Fig. 2) than in Sv129S6 mice. Expansion of red pulp secondary to EMH and mononuclear cell infiltrates occurred in both strains (Fig. 6). The more severe splenic pathology observed in C57/BL6 *Nramp1*^{G169} mice compared with Sv129S6 mice is consistent with the higher bacterial tissue loads observed in the former.

Bone marrow myeloid hyperplasia was observed in both strains (Figs. 4, 5), consistent with increased production of myelocytic cells reflected in peripheral blood counts (ie, neutrophilia and monocytosis) (Fig. 2). More severe bone marrow erythroid hypoplasia was present in C57/BL6 *Nramp1*^{G169} mice, with 6% to 22% erythroid cells (% of total nucleated cells), compared with 15% to 34% in Sv129S6 mice. Control mice had approximately 35% to 45% erythroid cells per total nucleated cells in bone marrow. Decreased erythrocyte production in the bone marrow paralleled expansion of the red pulp in the spleen secondary to EMH³ and inflammatory cell infiltrates (Fig. 6). As a result of the combined myeloid hyperplasia and erythroid hypoplasia, C57/BL6 *Nramp1*^{G169} mice had more than 2-fold higher bone marrow M:E ratios than Sv129S6 mice (Fig. 2). Microcytic anemia, demonstrated by decreased mean cell volume (MCV) and hemoglobin compared with controls, was more severe in the C57/BL6 *Nramp1*^{G169} mice (Fig. 2). These observations are further indicative of more severe inflammatory disease in C57/BL6 *Nramp1*^{G169} mice relative to Sv129S6 mice.

The spleen has reduced concentrations of non-heme iron in both mouse strains following infection

The spleen is the primary site for iron storage in the mouse.^{6,28} We previously demonstrated markedly decreased Prussian blue iron staining in tissues of Sv129S6 mice infected for 1, 3, and 6 weeks with *S. Typhimurium*.³ Prussian blue staining for ferric iron (hemosiderin) and biochemical measurement of non-heme iron were used to further evaluate splenic iron concentrations at 3 weeks postinfection in C57/BL6 *Nramp1*^{G169} and Sv129S6 mice. Noteworthy in the current studies was the lower measured concentration of splenic non-heme iron in control C57BL/6 *Nramp1*^{G169} mice compared with control SV129S6 mice (Fig. 1c). Like Sv129S6 mice, C57/BL6 *Nramp1*^{G169} mice then had similar loss of iron from splenic reticuloendothelial cells, as demonstrated by markedly decreased splenic Prussian blue staining at 3 weeks postinfection (Fig. 7) and decreased measurable nonheme splenic iron postinfection (Fig. 1c). In addition, erythrocyte microcytosis, poikilocytosis, particularly fragmentation (schistocytes and keratocytes), and echinocytosis, were seen (Fig. 3), findings previously reported in the Sv129S6 mice along with a variable magnitude of anemia.³ Erythrocyte microcytosis and fragmentation are features consistent with rodent iron-deficiency anemia.⁴ Splenomegaly and decreased splenic iron are consistent with utilization of iron to meet increased hematopoietic demand and EMH in response to anemia and inflammatory disease in infected mice.³

Significantly more dendritic cells and macrophages infiltrate the spleen of C57/BL6 *Nramp1*^{G169} mice

The observations of differential splenomegaly and peripheral blood neutrophilia and monocytosis suggested that C57/BL6 *Nramp1*^{G169} mice may have increased infiltration of professional phagocytes into the spleen compared with Sv129S6 mice. To establish the cellular nature of the inflammatory infiltrate in the spleen, the accumulation of cell types was examined at 3 weeks postinfection by flow cytometry. Dendritic cells were identified as CD11c⁺ and plasmacytoid dendritic cells were additionally Gr1⁺.⁷ Macrophages were identified as CD68⁺, CD11c⁻, and Gr1^{low/-};³⁰ inflammatory monocytes as CD68⁺ and

Gr1^{int},^{15,35} and neutrophils as CD68⁺ and Gr1^{high} (Fig. 8).^{12,13} There were 10-fold more splenic dendritic cells in infected C57/BL6 *Nramp1*^{G169} mice than in control mice, whereas infected Sv129S6 mice displayed only a 3- to 4-fold increase relative to control Sv129S6 mice postinfection (Fig. 9). Splenic plasmacytoid dendritic cells were increased in both mouse strains. Macrophages were nearly 7 times as abundant in infected vs control C57/BL6 *Nramp1*^{G169} spleens but were not significantly increased in Sv129S6-infected mouse spleens at 3 weeks (Fig. 9). There were also no significant differences in the enrichment of splenic neutrophils or inflammatory monocytes between the 2 strains. Overall, the data show that infected C57/BL6 *Nramp1*^{G169} mice accumulate more splenic dendritic cells and macrophages than infected Sv129S6 mice, relative to their respective controls.

C57/BL6 *Nramp1*^{G169} mice develop more robust inflammatory serum cytokine responses to infection

Serum cytokine concentrations reflect the intensity of acute inflammation. To establish whether C57/BL6 *Nramp1*^{G169} mice have significantly higher proinflammatory serum cytokine concentrations than Sv129S6 mice at 3 weeks postinfection, multiplex immunoassays were performed. Multiple proinflammatory, classical activation cytokines—interferon- γ (IFN- γ), tumor necrosis factor- α (TNF- α), interleukin (IL)-1, IL-2, and IL-12—were increased 3 weeks postinfection in both strains. Four of these cytokines—IFN- γ , TNF- α , IL-1, and IL-2—climbed to significantly higher levels in C57/BL6 *Nramp1*^{G169} mice (Fig. 10). Chemokine (C-X-C motif) ligand 1 (CXCL-1) (KC-Gro) and monocyte chemoattractant protein-1 (MCP-1) are chemoattractants that recruit neutrophils or monocytes, respectively.⁴² There was significantly more of these serum proteins in *S. Typhimurium*-infected C57/BL6 *Nramp1*^{G169} mice than in Sv129S6 mice (Fig. 10). In control animals, serum concentrations of anti-inflammatory cytokines IL-10 and IL-4 were higher in C57/BL6 *Nramp1*^{G169} mice compared with Sv129S6 mice. Infection resulted in a significant decrease in serum IL-4 and a declining trend in serum IL-10 and IL-5 concentrations only in C57/BL6 *Nramp1*^{G169} mice. Overall, the data suggest that C57/BL6 *Nramp1*^{G169} mice respond to *S. Typhimurium* infection with decreased anti-inflammatory and significantly greater proinflammatory serum cytokine and chemokine responses than Sv129S6 mice and suggest, at the time point investigated, a predominant Th1 response underlying the more severe acute infection.

Discussion

Variability between mouse genetic backgrounds can contribute to different immunological outcomes in response to infection or disease.^{19,32} We previously reported a hematopathological disorder in Sv129S6 mice that is most severe 3 weeks after natural oral route of infection with *S. Typhimurium*.³ To establish whether commonly used *S. Typhimurium*-resistant mouse strains with different genetic backgrounds develop differential inflammatory responses to oral infection with *Salmonella*, a second mouse strain also replete with *Nramp1* but on a C57/BL6 background (C57/BL6 *Nramp1*^{G169}) was compared with Sv129S6 mice. We found C57/BL6 *Nramp1*^{G169} mice, infected concurrently, were less resistant to infection than Sv129S6 mice, as demonstrated by decreased survival postinfection, and developed more severe acute infection, including greater tissue bacterial colonization, splenomegaly, microcytic anemia, and peripheral blood neutrophil and monocyte counts. C57/BL6 *Nramp1*^{G169} mice also developed more robust inflammatory serum cytokine responses to infection, including IFN- γ , TNF- α , and IL-1, and decreases in anti-inflammatory serum cytokine concentrations (IL-10, IL-4) compared with Sv129S6 mice. The increased concentrations of serum chemokines, CXCL-1 and MCP-1, in C57/BL6 *Nramp1*^{G169} compared with Sv129S6 mice are reflected by increased blood neutrophil and monocyte counts, as well as tissue phagocyte infiltration. Finally, the

number of splenic dendritic cells and macrophages in infected compared with control mice increased significantly more in C57/BL6 *Nramp1*^{G169} mice than in Sv129S6 mice. Interestingly, C57/BL6 (*Nramp1*^{-/-}) mice have been shown to have larger numbers of splenic dendritic cells compared with Sv129 mice,¹ further highlighting differences between mouse strains and immune responses.

We and others^{3,4,10,14} have reported splenomegaly and proliferative EMH with iron deficiency and/or inflammatory disease in rodents, which has also been shown to vary in degree between mouse strains¹⁰ with multiple gene polymorphisms implicated.⁴⁴ Loss of splenic iron is consistent with utilization of iron to meet increased hematopoietic demand and EMH in response to anemia and inflammatory disease in infected mice. Demonstrated here and in previous studies with *S. Typhimurium*-infected mice³ are decreased splenic iron, erythrocyte microcytosis and fragmentation, all morphological features of iron deficiency. Iron deficiency has been associated with a systemic proinflammatory state in mice,²⁵ and anemia and reactive erythropoiesis are known to override the induction of hepcidin secondary to inflammation.^{23,26} Sparing of splenic macrophages from iron overload has been shown in mice deficient for hepcidin.¹⁸ Therefore, despite the coexistence of inflammatory disease in these mice, iron is not sequestered in macrophages as occurs in anemia of chronic disease⁴¹ but rather used for reactive hematopoiesis and thus systemically depleted as demonstrated in these and prior studies.³ The findings of lower splenic iron in C57/BL6 *Nramp1*^{G169} control mice and postinfection in both strains may increase the hematological impact of infection.

On the basis of these data, we hypothesize that the increased severity of inflammatory disease in the C57/BL6 *Nramp1*^{G169} mice compared with Sv129S6 mice is driven by a more pronounced Th1 response that is dominated by dendritic cells and macrophages at the time point studied. This is reminiscent of observed Th1/Th2 skewing known to occur in response to other intracellular pathogens in inbred mouse strains.³² A pronounced Th1 response is reflected in the markedly increased serum IFN- γ , TNF- α , IL-1 β , and IL-2. IL-2 promotes T-cell duplication and natural killer cell activation, whereas IL-1 β is a potent proinflammatory cytokine associated with acute and chronic disease.⁵ Decreased serum IL-4 production observed in the third week postinfection also supports a heightened Th1 response in C57/BL6 *Nramp1*^{G169} mice. The greater severity of inflammatory disease in C57/BL6 *Nramp1*^{G169} mice occurred despite higher serum concentrations of the anti-inflammatory cytokines IL-10 and IL-4 in control mice. Thus, 2 things that could contribute to more severe inflammatory disease in infected C57/BL6 *Nramp1*^{G169} mice relative to Sv129S6 mice include (1) the more pronounced production of proinflammatory cytokines and (2) the differential change in the concentration of anti-inflammatory cytokines 3 weeks following infection.

Overall, the data show that C57/BL6 *Nramp1*^{G169} mice develop more severe, Th1-skewed acute inflammation in response to *S. Typhimurium* infection compared with Sv129S6 mice. Both strains prove to be good model systems for studying inflammation in the context of adaptive immunity. C57/BL6 *Nramp1*^{G169} mice can be used to examine host genetics; host genetic difference(s) in addition to *Nramp1* appear to contribute to differences in *Salmonella*-induced hematopathology and resistance to disease observed in C57/BL6 *Nramp1*^{G169} and Sv129S6 mice. In addition, environmental factors such as the host microbiota can potentially influence response to pathogens.^{17,37} With these experiments, we have determined that C57/BL6 *Nramp1*^{G169} mice can serve as a model for host innate and adaptive immune response to *S. Typhimurium* and that host responses to infection vary between mouse strains despite the presence of *Nramp1*.

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Figure 1.

Comparison of spleens from C57/BL6 *Nramp1*^{G169} and Sv129S6 mice at 3 weeks postinfection with *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*). (a) C57/BL6 *Nramp1*^{G169} mice have greater splenic bacterial loads (colony-forming units, CFU) of *S. Typhimurium* per gram of spleen compared with Sv129S6 mice. No bacteria were recovered from control spleens. (b) Infected C57/BL6 *Nramp1*^{G169} mice have more pronounced splenomegaly, based on spleen weight as a percentage of body weight, and (c) decreased total non-heme iron per gram of spleen occurs in both mouse strains post-infection. Data shown are from 5 controls (both strains), 10 *S. Typhimurium*-infected Sv129S6 (Sv129) mice (white bars), and 9 C57/BL6 *Nramp1*^{G169} (B6-N1R) (black bars). C, control; Inf, infected.

Figure 2.

C57/BL6 *Nramp1*^{G169} mice develop greater peripheral blood neutrophilia, monocytois, and lymphopenia 3 weeks following infection with *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) and more severe myeloid hyperplasia compared with Sv129S6 mice. Mean myeloid to erythroid (M:E) ratios for Sv129S6 and C57/BL6 *Nramp1*^{G169} are 2.8:1 and 7:1, respectively, compared with control mice at 1.6:1. Anemia is more severe in C57/BL6 *Nramp1*^{G169} mice 3 weeks postinfection. Data shown are from 5 controls (both strains), 10 *S. Typhimurium*-infected Sv129S6 (Sv129) mice (white bars), and 9 C57/BL6 *Nramp1*^{G169} (B6-N1R) (black bars). C, control; Inf, infected; MCV, mean cell volume.

Figure 3.

Erythrocyte microcytosis and poikilocytosis, including schistocytes, keratocytes (fragmentation; arrows) and echinocytes (arrowheads), are present in peripheral blood of anemic C57/BL6 *Nramp1*^{G169} mice. (a) Control mouse and (b) infected anemic C57/BL6 *Nramp1*^{G169} mouse 3 weeks following infection with *Salmonella enterica* serovar Typhimurium. Wright-Giemsa.

Figure 4–7.

Hematopathology 3 weeks after oral *Salmonella enterica serovar Typhimurium* (*S. Typhimurium*) infection in C57/BL6 *Nramp1*^{G169} and Sv129S6 mice. Figures 4 and 5. Bone marrow myeloid hyperplasia is more marked as indicated by increased myeloid to erythroid (M:E) ratios in C57/BL6 *Nramp1*^{G169} mice compared with Sv129S6 mice. Figure 4. Bone marrow cytology of (a) Sv129S6 control, M:E ratio 1.5:1; (b) Sv129S6 infected, M:E ratio 3.0:1; (c) C57/BL6 *Nramp1*^{G169} control, M:E ratio 1.8:1; and (d) C57/BL6 *Nramp1*^{G169} infected, M:E ratio 7:1; white arrows indicate erythroid precursor cells. Wright-Giemsa. Figure 5. Bone marrow histology of representative mid-femoral sections demonstrating increased bone marrow cellularity (reduced adipose) and myeloid hyperplasia (arrows) in infected mice (b, d) compared with controls (a, c). Hematoxylin and eosin (HE). Figure 6. Disruption of normal splenic architecture with loss of splenic lymphoid follicles and increased extramedullary hematopoiesis (EMH) (arrows) following infection (b, d) compared to controls (a, c). Megakaryocytes and erythroid precursors are shown (insets)

within areas of expanded EMH in infected mice (b, d). HE. Figure 7. Markedly decreased iron staining (blue) of splenic reticuloendothelial cells in *Salmonella*-infected Sv129S6 and C57/BL6 *Nramp1*^{G169} mice (b, d) compared to controls (a, c). Prussian blue. For all rows: (a) Sv129S6 control, (b) Sv129S6 infected, (c) C57/BL6 *Nramp1*^{G169} control, and (d) C57/BL6 *Nramp1*^{G169} infected.

Figure 8.

Density plots demonstrating a flow cytometric gating procedure for individual animal splenic leukocyte populations. Data shown are from a representative *Salmonella enterica* serovar Typhimurium-infected C57BL6 *Nramp1*^{G169} mouse, including splenic neutrophils (CD68⁻, Gr1^{high}), inflammatory monocytes (CD68⁺, Gr1^{int}), macrophages (CD68⁺, CD11c⁻, Gr1^{low/-}), dendritic cells (CD11c⁺), and plasmacytoid dendritic cells (CD11c⁺, Gr1⁺). Isotype controls were rat IgG2a-RPE (CD68), Armenian Hamster IgG (CD11c), and rat IgG2b-K-PE-Cy7 (Gr1).

Figure 9.

Splenic macrophage and dendritic cell populations are significantly greater in C57BL6 *Nramp1*^{G169} than in Sv129S6 mice at 3 weeks postinfection with *Salmonella enterica* serovar Typhimurium. Mean and SEM of splenic leukocyte cell numbers from control (C; $n = 3$ for each strain) and infected (Inf) mice. Sv129S6 (Sv129) mice (white bars; $n = 4$ infected mice). C57BL6 *Nramp1*^{G169} (B6-N1R) mice (black bars; $n = 5$ infected mice).

Figure 10.

C57/BL6 *Nramp1*^{G169} mice have significantly greater increases in proinflammatory serum cytokine concentrations and decreases in anti-inflammatory serum cytokine concentrations than Sv129S6 mice 3 weeks following *Salmonella enterica* serovar Typhimurium infection. Concentration of serum cytokines as indicated above each graph. Data shown are from 5 controls (both strains), 10 *S. Typhimurium*-infected Sv129S6 (Sv129) mice (white bars), and 9 C57/BL6 *Nramp1*^{G169} (B6-N1R) (black bars). C, control; CXCL-1, chemokine (C-X-C motif) ligand 1; IFN-, interferon-; IL, interleukin; Inf, infected; MCP-1, monocyte chemotactic protein-1; TNF-, tumor necrosis factor-.