Title

Salmonella Infection Enhances Erythropoietin Production by the Kidney and Liver, Which Correlates with Elevated Bacterial Burdens

Permalink

https://escholarship.org/uc/item/7hn8x4dk

Journal

Infection and Immunity, 84(10)

ISSN

0019-9567

Authors

Li, Lin-Xi
Benoun, Joseph M
Weiskopf, Kipp
et al.

Publication Date

2016-10-01

DOI

10.1128/iai.00337-16

Peer reviewed

CrossMark
click for updates

Salmonella Infection Enhances Erythropoietin Production by the Kidney and Liver, Which Correlates with Elevated Bacterial Burdens

Lin-Xi Li,[a]* Joseph M. Benoun,[a] Kipp Weiskopf,[b,c,d] K. Christopher Garcia,[b,c,d] Stephen J. McSorley[a]

Center for Comparative Medicine, Department of Anatomy, Physiology and Cell Biology, School of Veterinary Medicine, University of California, Davis, Davis, California, USA[a]; Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, California, USA[b]; Department of Molecular and Cellular Physiology and Department of Structural Biology, Stanford University School of Medicine, Stanford, California, USA[c]; Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, California, USA[d]

Salmonella infection profoundly affects host erythroid development, but the mechanisms responsible for this effect remain poorly understood. We monitored the impact of *Salmonella* infection on erythroid development and found that systemic infection induced anemia, splenomegaly, elevated erythropoietin (EPO) levels, and extramedullary erythropoiesis in a process independent of *Salmonella* pathogenicity island 2 (SPI2) or flagellin. The circulating EPO level was also constitutively higher in mice lacking the expression of signal-regulatory protein α (SIRPα). The expression level of EPO mRNA was elevated in the kidney and liver but not increased in the spleens of infected mice despite the presence of extramedullary erythropoiesis in this tissue. In contrast to data from a previous report, mice lacking EPO receptor (EPOR) expression on nonerythroid cells (EPOR rescued) had bacterial loads similar to those of wild-type mice following *Salmonella* infection. Indeed, treatment to reduce splenic erythroblasts and mature red blood cells correlated with elevated bacterial burdens, implying that extramedullary erythropoiesis benefits the host. Together, these findings emphasize the profound effect of *Salmonella* infection on erythroid development and suggest that the modulation of erythroid development has both positive and negative consequences for host immunity.

In the United States, *Salmonella enterica* infections are an important cause of foodborne infection, where they are responsible for periodic outbreaks of gastroenteritis that can be traced to a variety of sources (1–3). Other *Salmonella enterica* serovars impact developing nations, where they can be transmitted from human to human and cause a systemic infection called typhoid fever (4–6). Although there are two licensed vaccines for typhoid, neither of these vaccines provides effective immunity to young children or is widely used in areas of endemicity (7, 8). An additional problem is that nontyphoidal *Salmonella* serovars are now a major cause of bacteremia in sub-Saharan Africa, where they are associated with HIV, poor nutrition, or malaria coinfection (9, 10). Thus, the development of new vaccines for typhoid and nontyphoidal salmonellosis (NTS) is an important public health priority. However, the generation of effective vaccines will require a greater understanding of how the innate and adaptive immune responses recognize and combat *Salmonella* infections (11).

Some *Salmonella* serovars cause disease in a single species, while other serovars infect a broad range of animals, causing local or disseminated infection depending on host susceptibility. Although *Salmonella enterica* serovar Typhimurium causes gastroenteritis in humans, it causes a disseminated infection in mice that displays some similarities to human typhoid and NTS (12, 13). This murine infection has been widely used as a model to understand *Salmonella* pathogenesis and the host immune response to infection (11, 14). *Salmonella* immunity in mice requires the coordinated activation of innate and adaptive immune compartments, leading to a wide variety of immune effector mechanisms that eliminate bacteria and establish protective immunity to secondary challenge (11, 15). Both CD4 and CD8 T cells contribute to the resolution of primary *Salmonella* infection, while CD4 T cells play a dominant role in providing secondary protective immunity (16–18). The role of B cells is more complex, since this cell population is not required for primary clearance but contributes

to secondary protective immunity via antibody-dependent and antibody-independent mechanisms (19–22). In order to survive within an immunocompetent host, *Salmonella* has evolved multiple mechanisms to combat innate and adaptive immune responses (23–26). Some of these evasion strategies rely on the expression of *Salmonella* pathogenicity island (SPI) genes that encode a molecular syringe to deliver effector proteins to infected cells and disrupt cellular signaling or vesicle trafficking (27–29). *Salmonella* can also shut down the synthesis of flagellin soon after infecting host tissues (30), most likely because this antigen serves as a target for innate and adaptive immune responses (31, 32).

In addition to these inhibitory effects of *Salmonella* on host immune responses, recent studies demonstrate that *Salmonella* infection disrupts homeostatic erythroid development (33, 34), although the consequence of this for the host and pathogen is poorly understood. Our laboratory reported previously that *Salmonella*-induced splenomegaly is largely a consequence of extramedullary erythropoiesis in the spleen (33). Approximately 10 to 30% of the spleen of a healthy naive mouse is composed of

erythroid cells, but this proportion rises to 80 to 85% at the peak of *Salmonella*-induced splenomegaly (33). This striking effect of *Salmonella* on erythroid development has been largely ignored despite the fact that several studies over the last 40 years have noted that bacterial endotoxin can affect erythropoiesis (35–38).

Erythrocytes develop from hematopoietic stem cells via intermediate stages, before they shed nuclei and enter the circulation (39, 40). CD71 and Ter119 define late stages in the process of erythroid development (41, 42), and *Salmonella* infection is known to induce a large expansion of these immature Ter119$^+$ CD71$^+$ reticulocytes while decreasing mature erythrocytes (33). This expansion of immature reticulocytes correlates with a large increase in the level of serum erythropoietin (EPO) that can be inhibited by EPO neutralization (33), demonstrating that dysregulated EPO production is responsible for this effect. However, the signals that drive EPO production and the location of EPO secretion during *Salmonella* infection are not well defined. With regard to EPO responsiveness, it has recently been appreciated that EPO receptor (EPOR) can be expressed by nonerythroid cells (43). A recent study using EPOR-rescued mice lacking EPOR on nonerythroid cells found that *Salmonella* bacteria were rapidly eliminated, suggesting that EPO signaling in nonerythroid cells encourages bacterial persistence (34). This effect correlated with EPO-mediated inhibition of inflammatory cytokine and nitric oxide production by activated macrophages (34), suggesting that EPO might represent a critical mediator of host immune evasion. Signal-regulatory protein α (SIRPα) is a transmembrane glycoprotein expressed by macrophages that interacts with CD47 on erythroid cells, inhibiting phagocytosis and allowing red blood cells (RBCs) to remain in the circulation (44, 45). We recently reported that SIRPα-deficient mice display basal splenomegaly and have a higher number of splenic erythroid cells, including reticulocytes (46). Furthermore, this abnormal basal erythroid development correlated with the increased susceptibility of these mice to *Salmonella* infection (46), thus strengthening the connection between erythroid dysfunction and *Salmonella* persistence.

Here, we further examine the interaction of *Salmonella* infection with erythroid development and show that *Salmonella* and lipopolysaccharide (LPS) induce extramedullary erythropoiesis and splenomegaly. While erythropoiesis was induced prominently in the spleen, increased EPO production derives from the kidney and liver of infected mice. Furthermore, we show that mice lacking EPOR expression on nonerythroid cells do not display any heightened susceptibility to infection and that treatment to reduce numbers of circulating erythrocytes actually increases EPO production and encourages bacterial persistence. Thus, the modulation of erythroid development can have both positive and negative consequences for host immunity to *Salmonella* infection.

MATERIALS AND METHODS

Mice. C57BL/6 mice were purchased from the National Cancer Institute (Frederick, MD) and The Jackson Laboratory (Bar Harbor, ME). Congenic SIRPα-deficient (*Sirpa*$^{-/-}$) mice (B6.129P2-Sirpa⟨tm1Nog⟩/Rbrc) and EPOR$^{-/-}$$_{rescued}$ mice (EpoR$^{-/-}$::HG1-EpoR) were provided by RIKEN BRC, Japan. Mice used for experiments were 6 to 12 weeks old, unless otherwise noted. All of the animal experiments were approved by the University of California, Davis, Institutional Animal Care and Use Committee.

***Salmonella* infection and LPS treatment.** *Salmonella enterica* serovar Typhimurium strain SL1344 and a variety of mutants, including BRD509 (*aroA* mutant) (47), BRD509-SPI2 (*aroA aroD spiB* mutant) (29), and BRD509-Flagellin (*aroA aroD fliC fljB* mutant) (18), were grown overnight in LB broth without shaking before bacterial concentrations were estimated by using a spectrophotometer (optical density at 600 nm). All mice were infected intravenously (i.v.) in the lateral tail vein with 5×10^5 CFU of bacteria diluted in 200 μl phosphate-buffered saline (PBS). For intraperitoneal (i.p.) infection, mice were administered 500 CFU of SL1344 diluted in 500 μl PBS. The actual *Salmonella* bacterial dose administered was confirmed by plating serial dilutions of the bacterial culture onto MacConkey agar plates. To determine *Salmonella* bacterial loads *in vivo*, spleens and livers were removed from infected mice at various time points. Serial dilutions of organ homogenates were plated onto MacConkey agar plates, and bacterial counts per organ were calculated. For LPS treatment, C57BL/6 mice were injected i.v. with 1 to 10 μg of LPS [from *Escherichia coli* serotype EH100 (Ra); Enzo Life Sciences, NY] diluted in 200 μl PBS in the lateral tail vein daily for 7 consecutive days.

Measurement of packed cell volume. C57BL/6 mice were injected intravenously with 5×10^5 CFU of BRD509. Whole blood was collected retroorbitally into heparinized capillary tubes every week after infection. Blood was transferred to BD heparinized Microtainers, briefly vortexed, and placed on ice. All samples were transported to the UC Davis Comparative Pathology Laboratory for packed cell volume (PCV) analysis.

Flow cytometry. Spleens were harvested from naive, immunized, or infected mice, and single-cell preparations were generated in PBS supplemented with 2% fetal bovine serum (FBS). Aliquots of each single-cell suspension were stained with CD71-fluorescein isothiocyanate (FITC) (clone R17217) and Ter119-allophycocyanin (APC) (clone Ter119; eBioscience, San Diego, CA) and analyzed by using an LSRFortessa flow cytometer (BD Biosciences, San Jose, CA). Flow cytometric data were analyzed by using FlowJo software (TreeStar, Ashland, OR).

Determination of EPO levels by ELISAs. Mice were bled retroorbitally at various time points postinfection or postimmunization, and serum was collected. Serum was analyzed for EPO protein levels by an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (R&D Systems, MN). Briefly, serial dilutions of serum samples were added to 96-well ELISA plates (Costar, Corning, NY) precoated with purified anti-EPO antibody. Bound EPO was detected by using biotinylated anti-EPO antibody, followed by the ExtrAvidin peroxidase substrate (Sigma-Aldrich, St. Louis, MO). Color change was analyzed by using a spectrophotometer (SpectraMax M5; Molecular Devices, Sunnyvale, CA), and EPO concentrations were calculated according to a standard curve.

Determination of *Epo* mRNA levels by real-time qPCR. C57BL/6 mice were injected intravenously with (i) 5×10^5 CFU of BRD509 on day 0, (ii) 1 μg LPS daily for 7 days, or (iii) PBS daily for 7 days. Spleen, liver, kidney, and thymus were harvested on day 7, and total RNA was isolated by using an RNeasy kit (Qiagen, Valencia, CA). SYBR green-based real-time quantitative PCR (qPCR) for *Epo* mRNA was carried out by using the following primers: *Epo* forward primer 5′-CAGAGACCCTTCAGCTTC ATATAG-3′, *Epo* reverse primer 5′-TCTGGAGGCGACATCAATTC-3′, *Hprt* forward primer 5′-CTTTCCCTGGTTAAGCAGTACA-3′, and *Hprt* reverse primer 5′-GCCTGTATCCAACACTTCGA-3′. Data were acquired by using a ViiA 7 real-time PCR system (Applied Biosystems) and analyzed by the comparative threshold cycle (C_T) method. Expression levels of *Epo* mRNA were normalized to the amounts of *Hprt* mRNA in each sample.

CV1-hIgG4 treatment. The high-affinity SIPRα variant (CV1-hIgG4, referred as CV1 below) was described previously (48). To deplete mature RBCs, *Salmonella*-infected mice were injected i.p. with 200 μg CV1 in 500 μl PBS on day 3 and day 6 postinfection. Mice were sacrificed on day 7 for analysis.

Statistical analysis. Statistical analysis was performed by using an unpaired *t* test for normally distributed continuous-variable comparisons and a Mann-Whitney U test for nonparametric comparisons (Prism; GraphPad Software, Inc.).
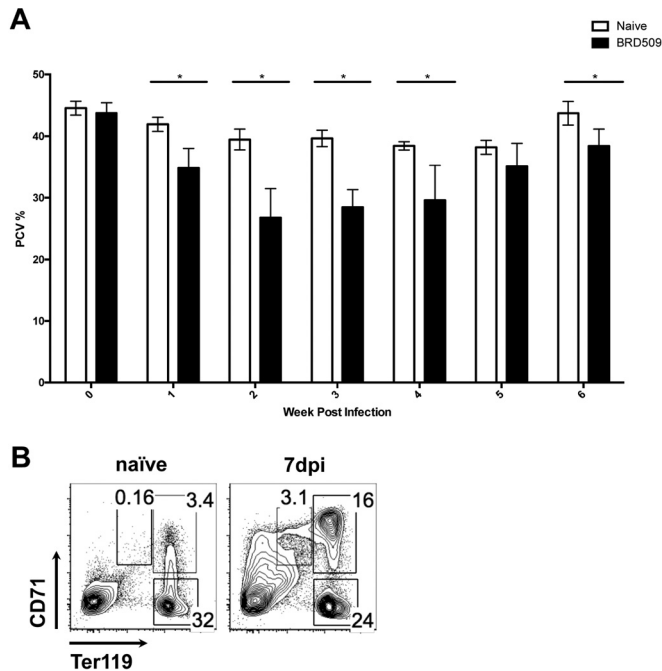
FIG 1 Systemic *Salmonella* infection induces anemia and extramedullary erythropoiesis. C57BL/6 mice were infected i.v. with 5×10^5 CFU of an attenuated *Salmonella* strain (BRD509). (A) The percentages peripheral blood packed cell volume (PCV%) were measured weekly after *Salmonella* infection. Statistical significance was established via multiple *t* tests. (B) Percentages of RBCs (Ter119$^+$ CD71$^-$), reticulocytes (Ter119$^+$ CD71$^+$), and proerythroblasts (Ter119^{med} CD71$^+$) in the peripheral blood of naive and *Salmonella*-infected mice were measured by flow cytometry. dpi, days postinfection; *, $P < 0.05$. Comparisons were made between infected and uninfected (week 0) groups.

RESULTS

***Salmonella* infection induces anemia and extramedullary erythropoiesis.** In order to examine the effect of infection on erythroid development, C57BL/6 mice were injected intravenously with 5×10^5 CFU of an attenuated strain of *Salmonella* (BRD509), and erythrocytes were subsequently examined in the blood and spleen. The hematocrit of whole blood was significantly reduced following *Salmonella* infection (Fig. 1A), while the corresponding weight of the spleen and proportion of splenic reticulocytes and their progenitors (proerythrocytes) were increased (Fig. 1B). These data are in broad agreement with results of previous reports describing *Salmonella* infection-induced anemia and splenic erythroid expansion and splenomegaly (33, 38, 49). Next, we examined whether LPS alone was sufficient to initiate erythroid dysfunction by regular i.v. injection of 5 or 10 μg of LPS into C57BL/6 mice. One week later, the overall size and weight of spleens of LPS-injected mice had increased substantially (Fig. 2A). This splenomegaly also correlated with markedly increased splenic cellularity and a 10-fold expansion in the percentage of CD71$^+$ Ter119$^+$ erythroblasts in the spleens of LPS-injected mice (Fig. 2A and B). The extramedullary splenic erythropoiesis detected in *Salmonella*-infected mice correlated with a significant rise in serum erythropoietin levels (Fig. 2C). *In vivo* survival of *Salmonella* is controlled by the expression of *Salmonella* pathogenicity island 2 (SPI2) genes and the downregulation of flagellin (50). In order to determine whether either of these virulence fac-

tors affected the development of anemia and splenomegaly, we infected mice with *Salmonella* strains lacking the expression of SPI2 or flagellin. Since SPI2 mutants display low levels of bacterial growth *in vivo*, we also infected some mice with higher doses of this mutant strain. The high doses of both the SPI2- and flagellin-deficient bacteria induced splenomegaly and anemia in C57BL/6 mice (Fig. 2D). Thus, *Salmonella* infection or repeated exposure to LPS can induce EPO production, splenomegaly, and extramedullary erythropoiesis in a process that does not depend on the SPI2 type III secretion system (T3SS) or flagellin.

***Salmonella* infection and LPS injection elevate EPO production in the kidney and liver.** Previous studies demonstrated that elevated EPO production is essential for extramedullary erythropoiesis driven by *Salmonella* infection (33). Under homeostatic conditions, EPO is produced in the kidney (51), but the source of elevated EPO levels during *Salmonella* infection is currently unclear. In order to explore the source of EPO in more detail, we used ELISAs to detect EPO in organ lysates following *Salmonella* infection but found that this methodology gave inconsistent results. Therefore, we examined EPO mRNA expression in several different organs after C57BL/6 mice were infected with *Salmonella* or injected with LPS. The level of EPO mRNA was unaltered in the thymus and spleen of *Salmonella*-infected or LPS-injected mice (Fig. 3), indicating that splenic erythropoiesis is most likely not induced by local EPO production. In marked contrast, LPS and *Salmonella* infection resulted in elevated EPO mRNA levels in both the kidney and liver (Fig. 3). At baseline, the relative expression level of EPO mRNA was higher in the kidney than in the liver (Fig. 3), suggesting that this organ is the major site of EPO production.

EPOR expression on nonerythroid cells does not affect bacterial persistence. It was reported previously that mice lacking EPOR expression on nonerythroid cells (EPOR$^{-/-}$$_{rescue}$) display an enhanced ability to clear *Salmonella* infection (34). This finding suggested a novel mechanism of bacterial persistence where the induction of elevated EPO levels is able to suppress bacterial killing by macrophages and other EPO-responsive nonerythroid cells (52). We reexamined this issue by infecting EPOR$^{-/-}$$_{rescue}$ and littermate control mice with an attenuated *Salmonella* strain and monitored bacterial counts at weekly intervals. Surprisingly, there was no statistically significant difference detected in bacterial loads in the spleen or liver of EPOR$^{-/-}$$_{rescue}$ and littermate control mice at any time point postinfection (Fig. 4A). Since it was possible that any effect of EPO on persistence was masked by the slow growth of the attenuated bacteria used in our experiment, we challenged EPOR$^{-/-}$$_{rescue}$ and littermate control mice with a virulent strain, SL1344. Again, there was no significant difference in bacterial loads in the liver or spleen between EPOR$^{-/-}$$_{rescue}$ and littermate control mice (Fig. 4B). Thus, the expression of EPOR on nonerythroid cells does not appear to adversely affect the growth or survival of attenuated or virulent *Salmonella in vivo*.

Depletion of erythroid cells increases EPO production and bacterial growth. Previous studies have shown that SIRPα-deficient (*Sirpα*$^{-/-}$) mice exhibit enhanced basal erythropoiesis and display enhanced susceptibility to *Salmonella* infection (46). Given the correlation between EPO levels and susceptibility in *Salmonella*-infected wild-type mice, we examined EPO levels in SIRPα-deficient mice. EPO levels were markedly elevated in the sera of *Salmonella*-infected SIRPα-deficient mice (Fig. 5), thus providing further evidence to link circulating EPO levels with en-
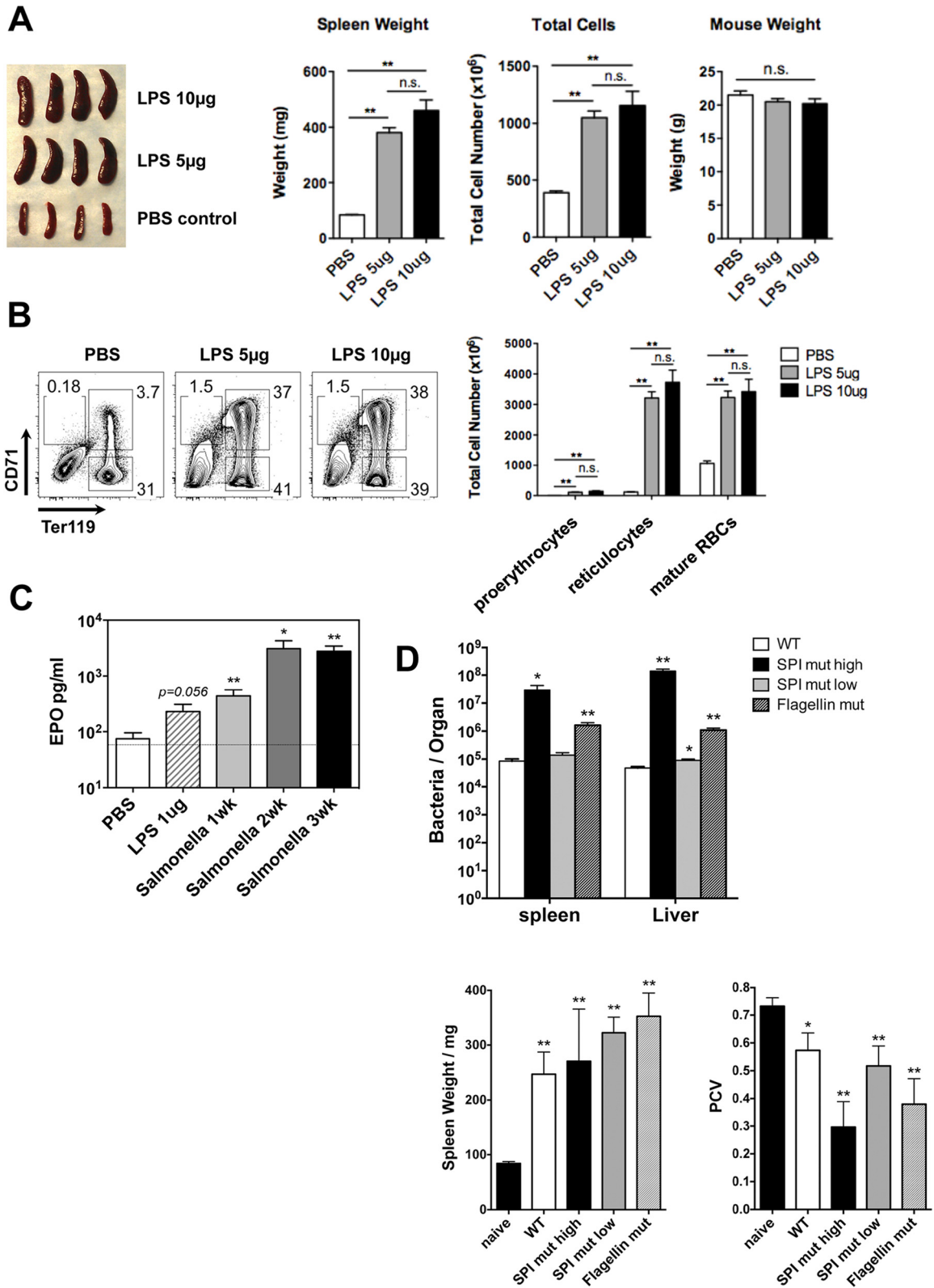
FIG 2 LPS induces erythropoiesis and EPO production. C57BL/6 mice were injected with 5 µg or 10 µg LPS daily for 7 consecutive days or infected i.v. with 5×10^5 CFU of attenuated *Salmonella* strains (BRD509 or mutant strains). (A) Sizes, weights, and total cell numbers of mouse spleens 7 days after LPS injection. (B) Total RBCs and RBC precursors recovered from the spleens of mice injected with LPS. (C) Blood EPO levels 7 days after LPS injection or *Salmonella* infection. (D) Bacterial counts per organ, spleen weights, and PCV in mice infected with either BRD509 or mutant strains. For the SPI2 mutant, both a low dose (5×10^5 CFU) and a high dose (5×10^7 CFU) were used for i.v. infection. Error bars represent the means ± standard errors of the means. *, $P < 0.05$; **, $P < 0.01$; n.s., not significant (by an unpaired *t* test). WT, wild type.

Kidney

Liver
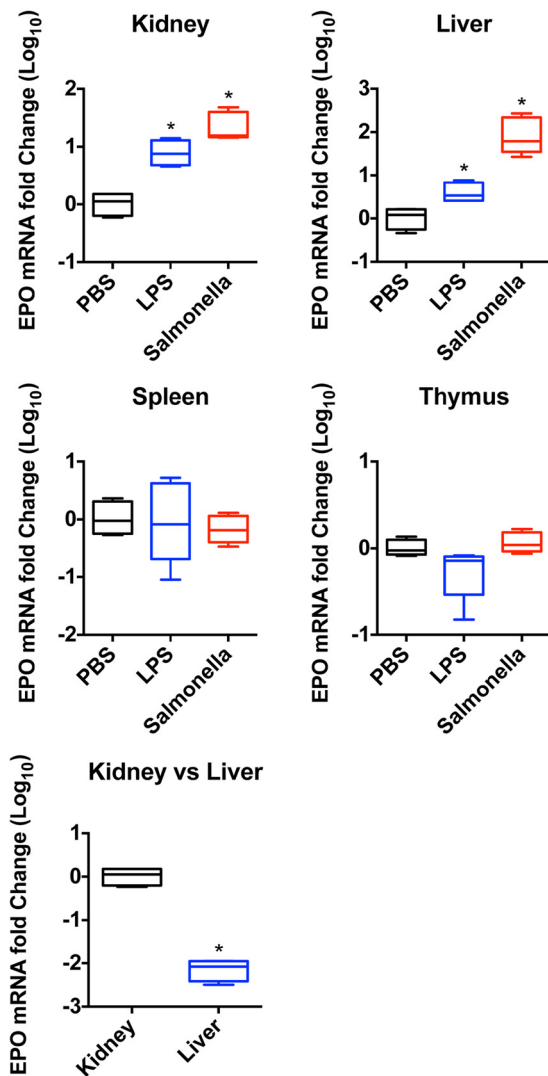

Spleen

Thymus

Kidney vs Liver

FIG 3 EPO mRNA levels are upregulated in kidney and liver during extramedullary erythropoiesis. C57BL/6 mice were injected with 1 μg LPS daily for 7 consecutive days or infected i.v. with 5×10^5 CFU of an attenuated *Salmonella* strain (BRD509). Spleen, liver, kidney, and thymus were harvested on day 7, and total mRNA was isolated as described in Materials and Methods. Graphs show the fold changes (log$_{10}$) of EPO mRNA levels in mice receiving LPS or *Salmonella* compared with mice injected with PBS as controls. *, $P <$ 0.05 (by a Mann-Whitney U test).

hanced *Salmonella* susceptibility. As noted above, our laboratory and others previously demonstrated that neutralization of EPO reduces bacterial growth (33, 52). Since the experiments using EPOR$^{-/-}$$_{\text{rescue}}$ mice described above suggested that any effect of EPO most likely involves erythroid responsiveness to EPO, we decided to examine whether depletion of erythroid cells could enhance bacterial clearance.

A high-affinity SIRPα variant (CV1) has been developed and used as a therapeutic to enhance tumor-specific monoclonal antibodies (48). CV1 antagonizes the "do not eat me" signal delivered by CD47 and thus has the effect of enhancing macrophage phagocytosis of CD47-expressing tumor cells. Since this same CD47 signal regulates erythroid homeostasis in the spleen, we injected *Salmonella*-infected C57BL/6 mice with CV1 and exam-

ined the effect on erythroid expansion. While *Salmonella*-infected mice displayed a marked expansion of erythroblasts (CD71$^+$ Ter119$^+$) and mature red blood cells (CD71$^-$ Ter119$^+$) in the spleen, this expansion was curtailed in *Salmonella*-infected mice administered CV1 (Fig. 6A). Thus, CV1 effectively prevented infection-induced splenic erythroid expansion by antagonizing CD47. Since EPO production and erythroid expansion correlate with enhanced bacterial persistence (33, 52), our expectation was that this CV1-mediated reduction in erythroid expansion would limit bacterial growth. However, mice injected with CV1 actually displayed significantly higher bacterial burdens in the spleen despite the fact that extramedullary erythropoiesis was effectively curtailed (Fig. 6B). A possible explanation for this increased bacterial growth was that CV1-injected mice displayed elevated levels of EPO in the circulation (Fig. 6C). Thus, directly blocking erythroid expansion or the availability of EPO itself has differential effects on bacterial growth *in vivo*.

DISCUSSION

Salmonella infection of inbred mice causes profound enlargement and increased cellularity of the spleen, which is sometimes ascribed to the proliferation of innate and adaptive immune cells responding to infection. Although the numbers of splenic macrophages, NK cells, dendritic cells, T cells, and B cells increase during *Salmonella* infection, the largest increase is actually found among erythroid cells, which expand to account for 80 to 85% of the spleen at the peak of infection (33). This extramedullary erythropoiesis causes a large expansion of the red pulp, which disrupts the splenic architecture and has the potential to influence innate and adaptive immunity to infection (53). The relationship between *Salmonella* infection, extramedullary erythropoiesis, and host immunity therefore represents an important but poorly understood component of the host-pathogen dynamic.

Previous studies have shown that Toll-like receptor (TLR) recognition of *Salmonella* increases EPO production and red blood cell expansion and that EPO neutralization alone can decrease bacterial growth (33, 52). Our current data confirm that extramedullary erythropoiesis is a consequence of *Salmonella* infection and further show that this expansion is accompanied by a reduction in circulating red cells. Furthermore, these erythroid alterations in the spleen are not a direct consequence of the type III secretion system encoded by SPI2 or bacterial expression of flagellin. Thus, erythroid dysregulation is induced in the spleen by virulent or highly attenuated *Salmonella* and is likely a direct consequence of host recognition of bacterial pathogen-associated molecular patterns (PAMPs) (35, 36). In support of this idea, injection of mice with LPS alone also induced splenomegaly and extramedullary erythropoiesis. This finding conflicts somewhat with previously reported data showing that LPS or the cytokines gamma interferon (IFN-γ) and interleukin-1 (IL-1) can inhibit circulating EPO and EPO mRNA production (54–56). Conversely, our data show that the levels of EPO mRNA in the kidney and liver were markedly elevated during *Salmonella* infection, suggesting that these two organs are the primary sources of elevated EPO levels. Indeed, both of these organs were previously shown to account for elevated EPO levels in response to reduced oxygen tension (52, 57). As expected, the EPO mRNA level was not increased in primary or secondary lymphoid tissues during infection. It is not yet clear whether kidney and liver cells are responding to hypoxia during *Salmonella* infection or whether
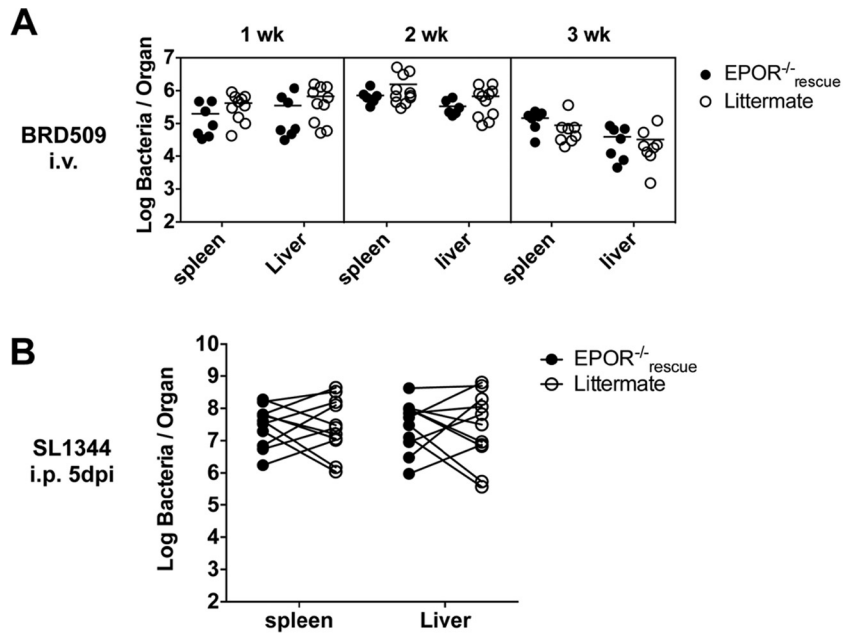
FIG 4 EPO does not appear to act on nonerythroid cell lineages to modulate host protective immunity against *Salmonella*. EpoR$^{-/-\text{rescue}}$ mice and littermate controls were infected with either 5×10^5 CFU of an attenuated *Salmonella* strain (BRD509) i.v. (A) or 500 CFU of a virulent *Salmonella* strain (SL1344) i.p. (B). Spleens and livers were recovered at 1, 2, and 3 weeks post-BRD509 infection or at 5 days post-SL1344 infection. The bacterial counts per organ were determined by serial dilution and plating. No statistical significance was determined.

EPO is enhanced by the detection of PAMPs or inflammatory cytokines. The fact that EPO production is highly dependent on TLR signaling (33) suggests that the latter pathway may be more important than hypoxia. Whatever the stimulus, the erythroid dysfunction detected in the spleen during *Salmonella* infection is likely to be driven by elevated EPO production from the kidney and liver.

It is clear from previous studies that TLR-dependent EPO production drives splenic erythropoiesis and that this correlates with enhanced bacterial growth (33, 52). However, the actual mechanism accounting for enhanced bacterial growth is substantially less clear. One possibility comes from the understanding that EPO has pleiotropic effects on a broad range of cell types *in vivo* (52).
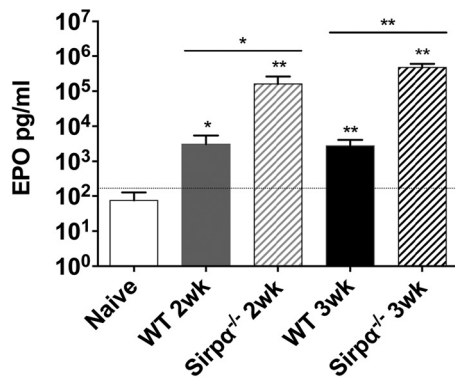

FIG 5 The EPO level is highly elevated in SIRPα-deficient mice after *Salmonella* infection. Blood EPO levels in wild-type and *Sirpα*$^{-/-}$ mice 2 and 3 weeks after infection with an attenuated *Salmonella* strain (BRD509) were measured by an ELISA. Error bars represent the means ± standard errors of the means. *, $P < 0.05$; **, $P < 0.01$ (by an unpaired t test).

Indeed, various effects of EPO and/or EPOR expression on T cells, B cells, neutrophils, dendritic cells, and macrophages have been reported (52, 58). Most importantly, EPO was found to have direct inhibitory effects on macrophage killing of *Salmonella in vitro* (34). Furthermore, mice that lacked EPOR expression on nonerythroid cells were able to control bacterial growth more efficiently (34). Indeed, some of our data add some support to this mechanism since CV1 treatment effectively blocked splenic erythrocyte expansion while maintaining high levels of circulating EPO, and the consequence was enhanced bacterial growth. Similarly, our data demonstrate that circulating EPO levels are elevated in SIRPα-deficient mice, which also display enhanced susceptibility to *Salmonella* (46). Together, these findings reinforce the idea that EPO, rather than extramedullary erythropoiesis, is the critical variable for inhibiting bacterial clearance. Importantly, however, we were unable to detect any difference in the bacterial burdens in wild-type and EPOR$^{-/-}$ mice, which lack the expression of EPO on nonerythroid cells. This negative result was confirmed by using both attenuated and virulent bacteria, neither of which displayed enhanced clearance in EPOR$^{-/-}$ mice. Thus, this data set conflicts with a model where EPOR expression by nonerythroid cells would be responsible for encouraging *Salmonella* growth *in vivo*. Although our mice were obtained from the same source as the mice used in a previous study, it remains possible that minor differences in bacterial strains, infection doses, or host microbiota could account for the divergent results. Indeed, it should also be noted that EPO was previously reported to have proinflammatory and anti-inflammatory effects on cell signaling and cytokine production, and thus, small alterations in the experimental setup between these two studies could have accentuated these effects.

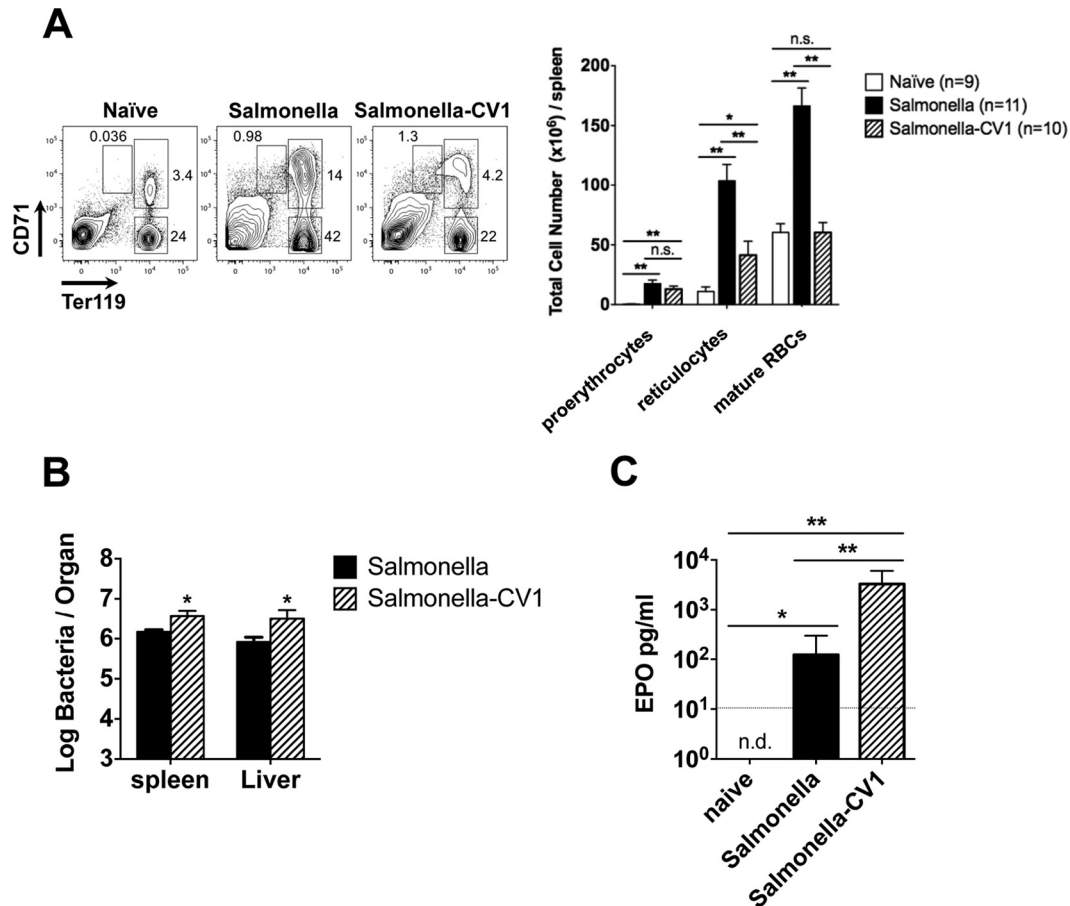Overall, our data are consistent with a model where (i) in-

FIG 6 Targeted depletion of erythrocytes from the spleen increases host susceptibility to *Salmonella*. C57BL/6 mice were infected i.v. with 5×10^5 CFU of an attenuated *Salmonella* strain (BRD509). Cohorts of mice were treated with high-affinity SIRPα variants (CV1) on day 3 and day 6 after infection. (A) Percentages and total cell numbers of RBCs (Ter119$^+$ CD71$^-$), reticulocytes (Ter119$^+$ CD71$^+$), and proerythroblasts (Ter119^{med} CD71$^+$) in the spleens of naive, *Salmonella*-infected, and *Salmonella*-infected and CV1-treated mice as measured by flow cytometry. (B) Bacterial counts in the spleens and livers of *Salmonella*-infected mice at 7 days postinfection with or without CV1 treatment. (C) Blood EPO levels in naive, *Salmonella*-infected, and *Salmonella*-infected and CV1-treated mice as measured by an ELISA. An unpaired *t* test was used to calculate statistical significance for panels A to C. Error bars represent the means ± standard errors of the means. *, $P < 0.05$; **, $P < 0.01$; n.d., not detected.

creased EPO production is responsible for encouraging *Salmonella* persistence and (ii) the primary effect of EPO is on cells of an erythroid lineage but that (iii) erythroid cell expansion in the spleen is not absolutely required to encourage bacterial growth. A potential model that focuses more on erythroid cell responsiveness to EPO can be generated from the understanding that extramedullary erythropoiesis encourages splenic hemophagocytosis, a pathological process whereby local macrophages engulf red blood cells and lymphocytes (59, 60). The process of hemophagocytosis can be induced by injection of mice with heat-killed *Brucella* or a variety of TLR ligands (61–63). Indeed, a recent report showed that stimulation of macrophages with heat-killed *Salmonella* or LPS induces hemophagocytosis (64).

Hemophagocytic macrophages display an anti-inflammatory phenotype and appear to be permissive for *Salmonella* growth in a mouse model of chronic *Salmonella* infection (65). Thus, any increase in the number of hemophagocytes would be expected to enhance bacterial persistence *in vivo*. While conceptually appealing, some of our data indicate that this model may not fully explain the effect of EPO and erythropoiesis on bacterial growth. In particular, our use of the high-affinity SIRPα variant (CV1) demonstrates that splenomegaly and erythroblast formation can be blocked in the spleen during *Salmonella* infection and that this correlates with enhanced bacterial growth. However, it seems possible that CV1 would enhance erythrocyte uptake by macrophages and therefore increase the number of hemophagocytes in the spleen. Since hemophagocytes have an anti-inflammatory phenotype (65), any expansion in the number of these cells should provide a niche for enhanced bacterial growth. Mice infected with *Salmonella* also show a loss of iron from the spleen and high levels of expression of ferroportin-1 on macrophages (66), which should limit the availability of iron for intracellular bacterial growth. The enhancement of bacterial growth after CV1 treatment could therefore reflect an increased access of *Salmonella* to iron as a consequence of hemophagocytosis (67).

In addition to the contribution of hemophagocytes to bacterial growth, EPO is also known to have effects on erythroid cells other than driving erythroid expansion. In particular, EPO can affect iron homeostasis as a consequence of modulating transferrin receptor expression in erythroid cells. In human and murine erythroid cell lines, EPO was shown to increase transferrin receptor expression on the cell surface and consequently to increase iron

uptake into cells (68). Such iron sequestration by erythroid cells could deliver more iron to hemophagocytes and thus compensate for reduced erythroid expansion during CV1 treatment. Overall, we suggest that the critical variables for understanding the effect of EPO on *Salmonella* growth might relate to the ability of EPO to increase hemophagocytosis and the availability of iron for subsequent bacterial growth.

Together, our data show that both *Salmonella* and LPS induce extramedullary erythropoiesis and splenomegaly. Furthermore, we show that mice lacking EPOR expression on nonerythroid cells do not display heightened susceptibility to infection but that directly reducing circulating erythrocytes increased EPO production and encouraged bacterial persistence. Thus, interventions that modulate erythroid development can have both positive and negative consequences for host immunity to *Salmonella* infection.

ACKNOWLEDGMENTS

K.W. and K.C.G. declare U.S. patent applications pertaining to CD47-blocking therapies for human disease, including "High affinity SIRP alpha reagents." K.W. declares consulting and/or equity ownership in Forty Seven Inc. and Alexo Therapeutics, Inc. K.C.G. declares consulting and/or equity ownership in Alexo Therapeutics, Inc.

REFERENCES

1. **Anonymous.** 2007. Multistate outbreaks of Salmonella infections associated with raw tomatoes eaten in restaurants—United States, 2005-2006. MMWR Morb Mortal Wkly Rep **56:**909–911.
2. **Anonymous.** 2008. Outbreak of Salmonella serotype Saintpaul infections associated with multiple raw produce items—United States, 2008. MMWR Morb Mortal Wkly Rep **57:**929–934.
3. **Anonymous.** 2009. Multistate outbreak of Salmonella infections associated with peanut butter and peanut butter-containing products—United States, 2008-2009. MMWR Morb Mortal Wkly Rep **58:**85–90.
4. **Jones BD, Falkow S.** 1996. Salmonellosis: host immune responses and bacterial virulence determinants. Annu Rev Immunol **14:**533–561. http://dx.doi.org/10.1146/annurev.immunol.14.1.533.
5. **Crump JA, Mintz ED.** 2010. Global trends in typhoid and paratyphoid fever. Clin Infect Dis **50:**241–246. http://dx.doi.org/10.1086/649541.
6. **Keestra-Gounder AM, Tsolis RM, Baumler AJ.** 2015. Now you see me, now you don't: the interaction of Salmonella with innate immune receptors. Nat Rev Microbiol **13:**206–216. http://dx.doi.org/10.1038/nrmicro3428.
7. **Fraser A, Paul M, Goldberg E, Acosta CJ, Leibovici L.** 2007. Typhoid fever vaccines: systematic review and meta-analysis of randomised controlled trials. Vaccine **25:**7848–7857. http://dx.doi.org/10.1016/j.vaccine.2007.08.027.
8. **McGregor AC, Waddington CS, Pollard AJ.** 2013. Prospects for prevention of Salmonella infection in children through vaccination. Curr Opin Infect Dis **26:**254–262. http://dx.doi.org/10.1097/QCO.0b013e32835fb829.
9. **Gordon MA, Kankwatira AM, Mwafulirwa G, Walsh AL, Hopkins MJ, Parry CM, Faragher EB, Zijlstra EE, Heyderman RS, Molyneux ME.** 2010. Invasive non-typhoid salmonellae establish systemic intracellular infection in HIV-infected adults: an emerging disease pathogenesis. Clin Infect Dis **50:**953–962. http://dx.doi.org/10.1086/651080.
10. **Morpeth SC, Ramadhani HO, Crump JA.** 2009. Invasive non-Typhi Salmonella disease in Africa. Clin Infect Dis **49:**606–611. http://dx.doi.org/10.1086/603553.
11. **McSorley SJ.** 2014. Immunity to intestinal pathogens: lessons learned from Salmonella. Immunol Rev **260:**168–182. http://dx.doi.org/10.1111/imr.12184.
12. **Santos RL, Zhang S, Tsolis RM, Kingsley RA, Adams LG, Baumler AJ.** 2001. Animal models of salmonella infections: enteritis versus typhoid fever. Microbes Infect **3:**1335–1344. http://dx.doi.org/10.1016/S1286-4579(01)01495-2.
13. **Griffin AJ, McSorley SJ.** 2011. Development of protective immunity to Salmonella, a mucosal pathogen with a systemic agenda. Mucosal Immunol **4:**371–382. http://dx.doi.org/10.1038/mi.2011.2.
14. **Tsolis RM, Xavier MN, Santos RL, Baumler AJ.** 2011. How to become a top model: impact of animal experimentation on human Salmonella disease research. Infect Immun **79:**1806–1814. http://dx.doi.org/10.1128/IAI.01369-10.
15. **Tam MA, Rydstrom A, Sundquist M, Wick MJ.** 2008. Early cellular responses to Salmonella infection: dendritic cells, monocytes, and more. Immunol Rev **225:**140–162. http://dx.doi.org/10.1111/j.1600-065X.2008.00679.x.
16. **Hess J, Ladel C, Miko D, Kaufmann SH.** 1996. Salmonella typhimurium aroA⁻ infection in gene-targeted immunodeficient mice: major role of CD4⁺ TCR-alpha beta cells and IFN-gamma in bacterial clearance independent of intracellular location. J Immunol **156:**3321–3326.
17. **Lee SJ, Dunmire S, McSorley SJ.** 2012. MHC class-I-restricted CD8 T cells play a protective role during primary Salmonella infection. Immunol Lett **148:**138–143. http://dx.doi.org/10.1016/j.imlet.2012.10.009.
18. **O'Donnell H, Pham OH, Li LX, Atif SM, Lee SJ, Ravesloot MM, Stolfi JL, Nuccio SP, Broz P, Monack DM, Baumler AJ, McSorley SJ.** 2014. Toll-like receptor and inflammasome signals converge to amplify the innate bactericidal capacity of T helper 1 cells. Immunity **40:**213–224. http://dx.doi.org/10.1016/j.immuni.2013.12.013.
19. **McSorley SJ, Jenkins MK.** 2000. Antibody is required for protection against virulent but not attenuated Salmonella enterica serovar Typhimurium. Infect Immun **68:**3344–3348. http://dx.doi.org/10.1128/IAI.68.6.3344-3348.2000.
20. **Mastroeni P, Simmons C, Fowler R, Hormaeche CE, Dougan G.** 2000. Igh-6⁻/⁻ (B-cell-deficient) mice fail to mount solid acquired resistance to oral challenge with virulent Salmonella enterica serovar Typhimurium and show impaired Th1 T-cell responses to Salmonella antigens. Infect Immun **68:**46–53. http://dx.doi.org/10.1128/IAI.68.1.46-53.2000.
21. **Barr TA, Brown S, Mastroeni P, Gray D.** 2009. B cell intrinsic MyD88 signals drive IFN-gamma production from T cells and control switching to IgG2c. J Immunol **183:**1005–1012. http://dx.doi.org/10.4049/jimmunol.0803706.
22. **Nanton MR, Way SS, Shlomchik MJ, McSorley SJ.** 2012. Cutting edge: B cells are essential for protective immunity against Salmonella independent of antibody secretion. J Immunol **189:**5503–5507. http://dx.doi.org/10.4049/jimmunol.1201413.
23. **Hornef MW, Wick MJ, Rhen M, Normark S.** 2002. Bacterial strategies for overcoming host innate and adaptive immune responses. Nat Immunol **3:**1033–1040. http://dx.doi.org/10.1038/ni1102-1033.
24. **Bueno SM, Gonzalez PA, Schwebach JR, Kalergis AM.** 2007. T cell immunity evasion by virulent Salmonella enterica. Immunol Lett **111:**14–20. http://dx.doi.org/10.1016/j.imlet.2007.05.003.
25. **Bedoui S, Kupz A, Wijburg OL, Walduck AK, Rescigno M, Strugnell RA.** 2010. Different bacterial pathogens, different strategies, yet the aim is the same: evasion of intestinal dendritic cell recognition. J Immunol **184:**2237–2242. http://dx.doi.org/10.4049/jimmunol.0902871.
26. **Kullas AL, McClelland M, Yang HJ, Tam JW, Torres A, Porwollik S, Mena P, McPhee JB, Bogomolnaya L, Andrews-Polymenis H, van der Velden AW.** 2012. L-Asparaginase II produced by Salmonella Typhimurium inhibits T cell responses and mediates virulence. Cell Host Microbe **12:**791–798. http://dx.doi.org/10.1016/j.chom.2012.10.018.
27. **Abrahams GL, Hensel M.** 2006. Manipulating cellular transport and immune responses: dynamic interactions between intracellular Salmonella enterica and its host cells. Cell Microbiol **8:**728–737. http://dx.doi.org/10.1111/j.1462-5822.2006.00706.x.
28. **Srinivasan A, Nanton M, Griffin A, McSorley SJ.** 2009. Culling of activated CD4 T cells during typhoid is driven by Salmonella virulence genes. J Immunol **182:**7838–7845. http://dx.doi.org/10.4049/jimmunol.0900382.
29. **Nanton MR, Lee SJ, Atif SM, Nuccio SP, Taylor JJ, Baumler AJ, Way SS, McSorley SJ.** 2015. Direct visualization of endogenous Salmonella-specific B cells reveals a marked delay in clonal expansion and germinal center development. Eur J Immunol **45:**428–441. http://dx.doi.org/10.1002/eji.201444540.
30. **Cummings LA, Wilkerson WD, Bergsbaken T, Cookson BT.** 2006. In vivo, fliC expression by Salmonella enterica serovar Typhimurium is het-

erogeneous, regulated by ClpX, and anatomically restricted. Mol Microbiol **61**:795–809. http://dx.doi.org/10.1111/j.1365-2958.2006.05271.x.

31. **McSorley SJ, Cookson BT, Jenkins MK.** 2000. Characterization of CD4+ T cell responses during natural infection with Salmonella typhimurium. J Immunol **164**:986–993. http://dx.doi.org/10.4049/jimmunol.164.2.986.

32. **Letran SE, Lee SJ, Atif SM, Uematsu S, Akira S, McSorley SJ.** 2011. TLR5 functions as an endocytic receptor to enhance flagellin-specific adaptive immunity. Eur J Immunol **41**:29–38. http://dx.doi.org/10.1002/eji.201040717.

33. **Jackson A, Nanton MR, O'Donnell H, Akue AD, McSorley SJ.** 2010. Innate immune activation during Salmonella infection initiates extramedullary erythropoiesis and splenomegaly. J Immunol **185**:6198–6204. http://dx.doi.org/10.4049/jimmunol.1001198.

34. **Nairz M, Schroll A, Moschen AR, Sonnweber T, Theurl M, Theurl I, Taub N, Jamnig C, Neurauter D, Huber LA, Tilg H, Moser PL, Weiss G.** 2011. Erythropoietin contrastingly affects bacterial infection and experimental colitis by inhibiting nuclear factor-kappaB-inducible immune pathways. Immunity **34**:61–74. http://dx.doi.org/10.1016/j.immuni.2011.01.002.

35. **Fruhman GJ.** 1966. Bacterial endotoxin: effects on erythropoiesis. Blood **27**:363–370.

36. **Reissmann KR, Udupa KB, Labedzki L.** 1976. Induction of erythroid colony forming cells (CFU-E) in murine spleen by endotoxin. Proc Soc Exp Biol Med **153**:98–101. http://dx.doi.org/10.3181/00379727-153-39488.

37. **Staber FG, Metcalf D.** 1980. Cellular and molecular basis of the increased splenic hemopoiesis in mice treated with bacterial cell wall components. Proc Natl Acad Sci U S A **77**:4322–4325. http://dx.doi.org/10.1073/pnas.77.7.4322.

38. **Hyland L, Villarreal-Ramos B, Clarke B, Baaten B, Hou S.** 2005. Bone marrow immunosuppression in Salmonella-infected mice is prolonged following influenza virus infection. Exp Hematol **33**:1477–1485. http://dx.doi.org/10.1016/j.exphem.2005.09.005.

39. **Koury MJ, Sawyer ST, Brandt SJ.** 2002. New insights into erythropoiesis. Curr Opin Hematol **9**:93–100. http://dx.doi.org/10.1097/00062752-200203000-00002.

40. **Elliott S, Pham E, Macdougall IC.** 2008. Erythropoietins: a common mechanism of action. Exp Hematol **36**:1573–1584. http://dx.doi.org/10.1016/j.exphem.2008.08.003.

41. **Zhang J, Socolovsky M, Gross AW, Lodish HF.** 2003. Role of Ras signaling in erythroid differentiation of mouse fetal liver cells: functional analysis by a flow cytometry-based novel culture system. Blood **102**:3938–3946. http://dx.doi.org/10.1182/blood-2003-05-1479.

42. **Gordon AR, Outram SV, Keramatipour M, Goddard CA, Colledge WH, Metcalfe JC, Hager-Theodorides AL, Crompton T, Kemp PR.** 2008. Splenomegaly and modified erythropoiesis in KLF13−/− mice. J Biol Chem **283**:11897–11904. http://dx.doi.org/10.1074/jbc.M709569200.

43. **Broxmeyer HE.** 2011. Erythropoietin surprises: an immune saga. Immunity **34**:6–7. http://dx.doi.org/10.1016/j.immuni.2011.01.004.

44. **Barclay AN.** 2009. Signal regulatory protein alpha (SIRPalpha)/CD47 interaction and function. Curr Opin Immunol **21**:47–52. http://dx.doi.org/10.1016/j.coi.2009.01.008.

45. **Ishikawa-Sekigami T, Kaneko Y, Okazawa H, Tomizawa T, Okajo J, Saito Y, Okuzawa C, Sugawara-Yokoo M, Nishiyama U, Ohnishi H, Matozaki T, Nojima Y.** 2006. SHPS-1 promotes the survival of circulating erythrocytes through inhibition of phagocytosis by splenic macrophages. Blood **107**:341–348. http://dx.doi.org/10.1182/blood-2005-05-1896.

46. **Li LX, Atif SM, Schmiel SE, Lee SJ, McSorley SJ.** 2012. Increased susceptibility to Salmonella infection in signal regulatory protein alpha-deficient mice. J Immunol **189**:2537–2544. http://dx.doi.org/10.4049/jimmunol.1200429.

47. **Strugnell R, Dougan G, Chatfield S, Charles I, Fairweather N, Tite J, Li JL, Beesley J, Roberts M.** 1992. Characterization of a Salmonella typhimurium aro vaccine strain expressing the P.69 antigen of Bordetella pertussis. Infect Immun **60**:3994–4002.

48. **Weiskopf K, Ring AM, Ho CC, Volkmer JP, Levin AM, Volkmer AK, Ozkan E, Fernhoff NB, van de Rijn M, Weissman IL, Garcia KC.** 2013. Engineered SIRPalpha variants as immunotherapeutic adjuvants to anticancer antibodies. Science **341**:88–91. http://dx.doi.org/10.1126/science.1238856.

49. **Brown DE, McCoy MW, Pilonieta MC, Nix RN, Detweiler CS.** 2010. Chronic murine typhoid fever is a natural model of secondary hemophagocytic lymphohistiocytosis. PLoS One **5**:e9441. http://dx.doi.org/10.1371/journal.pone.0009441.

50. **Salazar-Gonzalez RM, Srinivasan A, Griffin A, Muralimohan G, Ertelt JM, Ravindran R, Vella AT, McSorley SJ.** 2007. Salmonella flagellin induces bystander activation of splenic dendritic cells and hinders bacterial replication in vivo. J Immunol **179**:6169–6175. http://dx.doi.org/10.4049/jimmunol.179.9.6169.

51. **Hattangadi SM, Wong P, Zhang L, Flygare J, Lodish HF.** 2011. From stem cell to red cell: regulation of erythropoiesis at multiple levels by multiple proteins, RNAs, and chromatin modifications. Blood **118**:6258–6268. http://dx.doi.org/10.1182/blood-2011-07-356006.

52. **Nairz M, Sonnweber T, Schroll A, Theurl I, Weiss G.** 2012. The pleiotropic effects of erythropoietin in infection and inflammation. Microbes Infect **14**:238–246. http://dx.doi.org/10.1016/j.micinf.2011.10.005.

53. **McSorley SJ, Asch S, Costalonga M, Rieinhardt RL, Jenkins MK.** 2002. Tracking Salmonella-specific CD4 T cells in vivo reveals a local mucosal response to a disseminated infection. Immunity **16**:365–377. http://dx.doi.org/10.1016/S1074-7613(02)00289-3.

54. **Jelkmann W.** 1998. Proinflammatory cytokines lowering erythropoietin production. J Interferon Cytokine Res **18**:555–559. http://dx.doi.org/10.1089/jir.1998.18.555.

55. **Vannucchi AM, Grossi A, Rafanelli D, Statello M, Cinotti S, Rossi-Ferrini P.** 1994. Inhibition of erythropoietin production in vitro by human interferon gamma. Br J Haematol **87**:18–23. http://dx.doi.org/10.1111/j.1365-2141.1994.tb04864.x.

56. **Frede S, Fandrey J, Pagel H, Hellwig T, Jelkmann W.** 1997. Erythropoietin gene expression is suppressed after lipopolysaccharide or interleukin-1 beta injections in rats. Am J Physiol **273**:R1067–R1071.

57. **Boutin AT, Weidemann A, Fu Z, Mesropian L, Gradin K, Jamora C, Wiesener M, Eckardt KU, Koch CJ, Ellies LG, Haddad G, Haase VH, Simon MC, Poellinger L, Powell FL, Johnson RS.** 2008. Epidermal sensing of oxygen is essential for systemic hypoxic response. Cell **133**:223–234. http://dx.doi.org/10.1016/j.cell.2008.02.038.

58. **Lifshitz L, Prutchi-Sagiv S, Avneon M, Gassmann M, Mittelman M, Neumann D.** 2009. Non-erythroid activities of erythropoietin: functional effects on murine dendritic cells. Mol Immunol **46**:713–721. http://dx.doi.org/10.1016/j.molimm.2008.10.004.

59. **Nix RN, Altschuler SE, Henson PM, Detweiler CS.** 2007. Hemophagocytic macrophages harbor Salmonella enterica during persistent infection. PLoS Pathog **3**:e193. http://dx.doi.org/10.1371/journal.ppat.0030193.

60. **Silva-Herzog E, Detweiler CS.** 2008. Intracellular microbes and haemophagocytosis. Cell Microbiol **10**:2151–2158. http://dx.doi.org/10.1111/j.1462-5822.2008.01192.x.

61. **Gardenghi S, Renaud TM, Meloni A, Casu C, Crielaard BJ, Bystrom LM, Greenberg-Kushnir N, Sasu BJ, Cooke KS, Rivella S.** 2014. Distinct roles for hepcidin and interleukin-6 in the recovery from anemia in mice injected with heat-killed Brucella abortus. Blood **123**:1137–1145. http://dx.doi.org/10.1182/blood-2013-08-521625.

62. **Behrens EM, Canna SW, Slade K, Rao S, Kreiger PA, Paessler M, Kambayashi T, Koretzky GA.** 2011. Repeated TLR9 stimulation results in macrophage activation syndrome-like disease in mice. J Clin Invest **121**:2264–2277. http://dx.doi.org/10.1172/JCI43157.

63. **Ohyagi H, Onai N, Sato T, Yotsumoto S, Liu J, Akiba H, Yagita H, Atarashi K, Honda K, Roers A, Muller W, Kurabayashi K, Hosoi-Amaike M, Takahashi N, Hirokawa M, Matsushima K, Sawada K, Ohteki T.** 2013. Monocyte-derived dendritic cells perform hemophagocytosis to fine-tune excessive immune responses. Immunity **39**:584–598. http://dx.doi.org/10.1016/j.immuni.2013.06.019.

64. **McDonald EM, Pilonieta MC, Nick HJ, Detweiler CS.** 2016. Bacterial stimulation of Toll-like receptor 4 drives macrophages to hemophagocytose. Infect Immun **84**:47–55. http://dx.doi.org/10.1128/IAI.01149-15.

65. **McCoy MW, Moreland SM, Detweiler CS.** 2012. Hemophagocytic macrophages in murine typhoid fever have an anti-inflammatory phenotype. Infect Immun **80**:3642–3649. http://dx.doi.org/10.1128/IAI.00656-12.

66. **Brown DE, Nick HJ, McCoy MW, Moreland SM, Stepanek AM, Benik R, O'Connell KE, Pilonieta MC, Nagy TA, Detweiler CS.** 2015. Increased ferroportin-1 expression and rapid splenic iron loss occur with anemia caused by Salmonella enterica serovar Typhimurium infection in mice. Infect Immun **83**:2290–2299. http://dx.doi.org/10.1128/IAI.02863-14.

67. **Nagy TA, Moreland SM, Detweiler CS.** 2014. Salmonella acquires ferrous iron from haemophagocytic macrophages. Mol Microbiol **93**:1314–1326. http://dx.doi.org/10.1111/mmi.12739.

68. **Weiss G, Houston T, Kastner S, Johrer K, Grunewald K, Brock JH.** 1997. Regulation of cellular iron metabolism by erythropoietin: activation of iron-regulatory protein and upregulation of transferrin receptor expression in erythroid cells. Blood **89**:680–687.