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Increased Susceptibility to Salmonella Infection in Signal Regulatory Protein \(\alpha\)-Deficient Mice

Lin-Xi Li,* Shaikh M. Atif,* Shirdi E. Schmiel,† Seung-Joo Lee,* and Stephen J. McSorley*

Recent studies have shed light on the connection between elevated erythropoietin production/spleen erythropoiesis and increased susceptibility to Salmonella infection. In this article, we provide another mouse model, the Sirp\(\alpha\)-deficient (Sirp\(\alpha\)/−/−) mouse, that manifests increased erythropoiesis as well as heightened susceptibility to Salmonella infection. Sirp\(\alpha\)/−/− mice succumbed to systemic infection with attenuated Salmonella, possessing significantly higher bacterial loads in both the spleen and the liver. Moreover, Salmonella-specific Ab production and Ag-specific CD4 T cells were reduced in Sirp\(\alpha\)/−/− mice compared with wild-type controls. To further characterize the potential mechanism underlying SIRP\(\alpha\)-dependent Ag-specific CD4 T cell priming, we demonstrate that lack of SIRP\(\alpha\) expression on dendritic cells results in less efficient Ag processing and presentation in vitro. Collectively, these findings demonstrate an indispensable role of SIRP\(\alpha\) for protective immunity to Salmonella infection. The Journal of Immunology, 2012, 189: 2537–2544.

Chronic bacterial infections initiate both innate and adaptive immune responses in the host, primarily within the secondary lymphoid organs, including spleen and lymph nodes (1, 2). Systemic infection of Salmonella enterica, a gram-negative intracellular bacterium, leads to a characteristic pathological condition called splenomegaly in both mice and humans (3, 4). At the peak of infection, the spleen of infected C57BL/6 mice expands to >10-fold its size in the naive state (5). It was commonly thought that the enlarged spleen accompanying Salmonella infection is caused by local leukocyte expansion or recruitment. Indeed, splenic lymphocytes, especially CD4 and CD8 T cells, expand after Salmonella infection, and a large increase in phagocyte populations is also observed (5–7). Although wild-type (WT) C57BL/6 mice typically survive and exhibit a self-limiting infection with an attenuated S. typhimurium strain, immunodeficient mice lacking CD4, MHC class II, IFN-\(\gamma\), or T-bet fail to clear a primary Salmonella infection, demonstrating an indispensable role for CD4 T cells in Salmonella protective immunity (8–10). In addition, Ab responses are also known to contribute to resistance to Salmonella infection (11–13), although the mechanism of protection is unclear.

More recently, our laboratory reported that RBCs (defined as CD71−Ter119+) and reticulocyte precursors (CD71+Ter119+) expand greatly during Salmonella infection, increasing from ~20% of naive splenocytes to >80% of the infected spleen (5). Given this erythroid expansion, these observations provide an alternative mechanism to explain Salmonella-induced splenomegaly. Importantly, blocking erythropoiesis induced by elevated levels of the hormone erythropoietin (EPO) significantly reduced host susceptibility to Salmonella infection (5, 14). These data suggest that increased erythropoiesis is an evasive mechanism that Salmonella adopts to facilitate persistent infection of the host.

In the steady state, erythropoiesis in the bone marrow and RBC turnover in the periphery are tightly regulated (15, 16). An important signaling module that regulates RBC turnover is the SIRP\(\alpha\) transmembrane glycoprotein also known as SHPS-1 (Src homology 2 domain-containing protein tyrosine phosphatase substrate-1), CD172a, BIT (brain Ig-like molecule with a tyrosine-based activation motif), MFR (macrophage fusion receptor), or p84 (17–21). It is expressed primarily on myeloid cells, such as macrophages, granulocytes, and myeloid dendritic cells (DCs), but is barely detectable on B or T lymphocytes (22–26). The extracellular domain of SIRP\(\alpha\) comprises three Ig-like domains, which interact with its ligand CD47, another member of the Ig superfamily known as a marker of self on RBCs (24, 27, 28). The binding of CD47 on RBCs with SIRP\(\alpha\) on macrophages delivers an inhibitory “do not eat me” signal that prevents unwanted phagocytosis and maintains the peripheral RBC pool (27–31).

Although more recent studies have implied that SIRP\(\alpha\) plays an important role in regulating the homeostasis of T cells, NK cells, and DCs (32–34), little is known regarding the function of SIRP\(\alpha\) in bacterial infection and the development of adaptive immunity. In this current study, we have examined the immune response to Salmonella infection in Sirp\(\alpha\)-deficient mice, and report an indispensable role for SIRP\(\alpha\) in resolving Salmonella primary infection and establishing an effective Salmonella-specific adaptive immune response.

Materials and Methods

Mice

C57BL/6 (B6) mice were purchased from the National Cancer Institute (Frederick, MD) and The Jackson Laboratory (Bar Harbor, ME). Congenic Sirp\(\alpha\)/−/− mice (B6.129P2-Sirpa\(-/-\)tm1Nog \(\times\) Rbrc) were provided by the RIKEN BioResource Center through the National BioResource Project of the Ministry of Education, Culture, Sports, Science, and Technology, Japan. These mice had been backcrossed to C57BL/6 for >10 generations. Rag\(\gamma\)-/- mice were kindly provided by Dr. D. Masopust (University of Minnesota, Minneapolis, MN). CD90.1 or CD45.1 congenic, Rag-deficient SM1 TCR transgenic mice have been previously described (35, 36). Briefly, the SM1
TCR transgenic mice express a monocolonal TCR specific for Salmonella flagellin peptide (427–441)-1-α. These mice were backcrossed to RAG-2-deficient C57BL/6 mice to obtain RAG-deficient SM1 offspring that contained SM1 CD4 T cells but no other lymphocytes. Mice used for experiments were 6–12 wk old, unless otherwise noted. All animal experiments were approved by the University of California, Davis, Institutional Animal Care and Use Committee.

Salmonella and Chlamydia infection

S. typhimurium strain BRD509 (aroAaroD) was grown overnight in Luria-Bertani broth without shaking, and bacterial concentration was estimated using a spectrophotometer (OD, 600 nm). Chlamydia muridarum strain Nigg (American Type Culture Collection, Manassas, VA) was cultivated, purified, aliquoted, titrated, and stored at −80°C, as previously described (37). Mice were infected i.v. in the lateral tail vein with 5 × 10^5 CFU S. typhimurium or 1 × 10^6 inclusion forming units of C. muridarum diluted in 200 μl PBS. The actual Salmonella bacterial dose administered was always confirmed by plating serial dilutions of the bacterial culture onto MacConkey agar plates. A fresh-thawed suspension was always confirmed by plating serial dilutions of the bacterial culture described (37). Mice were infected i.v. in the lateral tail vein with 5 × 10^3 CFU S. typhimurium or 1 × 10^6 inclusion forming units of C. muridarum diluted in 200 μl PBS. The actual Salmonella bacterial dose administered was always confirmed by plating serial dilutions of the bacterial culture onto MacConkey agar plates. A fresh-thawed suspension was always used for each experiment. For survival studies, mice were monitored daily for signs of illness and sacrificed at the moribund stage. 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 on MacConkey agar plates, and bacterial counts were calculated.

Flow cytometry

Spleens were harvested from naive and infected mice. Single-cell preparations were made using PBS with 2% FCS. Aliquots of single-cell suspension were stained with a panel of Abs (listed below) and analyzed on a FACSCanto or an LSRFortessa flow cytometer (BD Biosciences, San Jose, CA). Abs used included FITC-CD71, APC-TER119, eFlour450-CD3, FITC-CD11a, APC-IFN-γ, PE-CD45.1, APC-eFlour565, APC-CD19, FITC-CD69, PE-CD62L, PE-CY7-CD44, APC-CY7-CD25 (eBioscience, San Diego, CA), and PerCP-CD4 and APC-CY7-CD8 (BD Biosciences, San Diego, CA). Data were analyzed using FlowJo software (Tree Star, Ashland, OR).

Salmonella-specific antibody ELISA

Mice were bled retro-orbitally at various time points postinfection. Serum was collected and analyzed by ELISA for Salmonella-specific Ab, as previously described (13). Briefly, serial dilutions of serum samples were added to heat-killed (HK) S. typhimurium-coated microtitre plates (Costar, Corning, NY). Salmonella-specific Abs were detected using biotinylated isotype-specific Abs (eBioscience) and ExtrAvidin-Peroxidase substrate (Sigma-Aldrich, St. Louis, MO).

Salmonella-specific ELISPOT assay

At 2 wk after Salmonella infection, mice were treated with enrofloxacin (Baytril) at 2 mg/ml in drinking water to eradicate bacteria. After removal of antibiotic water, mice were rested for an additional week before spleens were harvested and single-cell suspensions prepared. After RBC lysis, CD4+ T cells were enriched via magnetic selecting LS MACS column and anti-PE magnetic beads (Miltenyi Biotec) (41). The enriched cells were stained with surface markers and Tetramer-specific cells were enriched via magnetic selecting LS MACS column and anti-PE magnetic beads (Miltenyi Biotec). Enriched DCs were seeded at 1 × 10^3 cells per well in a 96-well plate and cocultured with 1 × 10^5 purified SM1 T cells plus Ag. At 24 h, T cells were recovered 16 h later, stained with surface markers, and analyzed by flow cytometry.

Detection of C. muridarum-specific CD4 T cells

The C. muridarum-specific epitope PmpG-1 has been previously described (40). PE-labeled MHC class II tetramers (I-Ab) containing C. muridarum polymorphic membrane protein G-1 (PmpG-1) residue 303–311 were made in our laboratory (to be published elsewhere). Single-cell preparations from spleens were incubated for 1 h at room temperature with a 1:100 dilution of PmpG-1 tetramer in Fc block. Tetramer-specific cells were enriched via magnetic selecting LS MACS column and anti-PE magnetic beads (Miltenyi Biotec) (41). The enriched cells were stained with surface markers and analyzed by flow cytometry.

Results

Sirpa^-/- mice exhibit a marked increase in splenic erythroid cells

We first examined the splenic cellular profile of naive Sirpa^-/- mice. Consistent with previous reports, Sirpa^-/- mice exhibited mild splenomegaly, with a total cell number that is normal (Fig. 1B). The marked increase in RBCs in Sirpa^-/- mice is normal (Fig. 1B). The marked increase in RBCs in Sirpa^-/- mice also correlated with decreased lymphocyte cell numbers. CD4 T cell, CD8 T cell, and B cell numbers were ~2-fold reduced in Sirpa^-/- naive mice compared with WT controls (Fig. 1D, 1E). These results explain the previous finding that splenic white pulmonary was reduced in Sirpa^-/- mice (30, 33).

Table I. Salmonella-specific MHC class II epitopes

<table>
<thead>
<tr>
<th>Salmonella Peptide</th>
<th>Salmonella Protein</th>
<th>Epitope Sequence</th>
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<tbody>
<tr>
<td>FliC 427-441</td>
<td>Bacterial flagellum</td>
<td>VQRNFNSATLNGLNT</td>
</tr>
<tr>
<td>SseJ 268-280</td>
<td>SPI2-secreted effector protein</td>
<td>LIYYTDFSNSSIA</td>
</tr>
<tr>
<td>SseJ 329-341</td>
<td>SPI2-secreted effector protein</td>
<td>CYYETADAFKVIM</td>
</tr>
<tr>
<td>PagC 163-174</td>
<td>phoP-activated gene</td>
<td>GYGEGSNISTK</td>
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Salmonella flagellin was purified as described previously (39). Flagellin peptide (flagellin 427-441) was purchased from Invitrogen (Carlsbad, CA). Spleens were harvested and digested with collagenase D (Roche Diagnostics, Indianapolis, IN) (39), and DCs were enriched to >85–95% purity via magnetic selecting LS MACS column and CD11c magnetic beads (Miltenyi Biotec). Enriched DCs were seeded at 1 × 10^3 cells per well in a 96-well plate and cocultured with 1 × 10^5 purified SM1 T cells plus Ag. At 24 h, T cells were recovered 16 h later, stained with surface markers, and analyzed by flow cytometry.
Salmonella infection was mapped to a deficiency in hematopoietic Sirpα the bone marrow and spleen of WT and Sirpα−/− mice. This small, but statistically significant, difference indicates that innate immune response in WT and Sirpα−/− mice is impaired in cleaning bacterial infection. A much greater difference in bacterial counts between WT and Sirpα−/− mice became obvious as infection progressed to week 2 (Fig. 2C, 2D). Although many Sirpα−/− mice died between 2 and 3 wk, surviving Sirpα−/− mice exhibited a dramatic increase in Salmonella colonization in both spleen and liver at 3 wk postinfection (Fig. 2C, 2D). In conclusion, Sirpα−/− mice fail to control Salmonella replication in vivo and ultimately succumb to an infection that is self-limiting in WT controls.

**Sirpα is indispensable for protective immunity to S. typhimurium infection**

To examine the role that Sirpα plays in Salmonella resistance, we infected WT, Sirpα−/−, and Sirpα−/− mice (littermates) i.v. with 5 × 10⁷ attenuated S. typhimurium (BRD509) and monitored infected mice for signs of disease. Given the previous association between EPO and susceptibility to Salmonella infection, we hypothesized that increased erythropoiesis in Sirpα−/− mice would heighten susceptibility to Salmonella infection. Indeed, although WT and Sirpα−/− mice survived for months after Salmonella infection, all Sirpα−/− mice died between 2 and 4 wk postinfection (Fig. 2A). The inability of Sirpα−/− mice to resolve Salmonella infection was mapped to a deficiency in hematopoietic cell lineages because Sirpα−/− bone marrow chimeras exhibit a similar survival pattern (Fig. 2B).

We next accessed the bacterial burden in WT and Sirpα−/− mice at various time points after Salmonella infection. WT C57BL/6 mice typically resolve attenuated Salmonella infection ~5 wk postinfection. At 1 wk postinfection, Sirpα−/− mice have a slightly higher bacterial burden than do WT mice in both spleen and liver (Fig. 2C, 2D). This small, but statistically significant, difference indicates that innate immune response in Sirpα−/− mice is impaired in cleaning bacterial infection. A much greater difference in bacterial counts between WT and Sirpα−/− mice became obvious as infection progressed to week 2 (Fig. 2C, 2D). Although many Sirpα−/− mice died between 2 and 3 wk, surviving Sirpα−/− mice exhibited a dramatic increase in Salmonella colonization in both spleen and liver at 3 wk postinfection (Fig. 2C, 2D). In conclusion, Sirpα−/− mice fail to control Salmonella replication in vivo and ultimately succumb to an infection that is self-limiting in WT controls.

**Defects in Salmonella-specific CD4 T cell response in Sirpα−/− mice**

Our laboratory and others have previously documented a similar inability to resolve Salmonella infection in MHC class II-deficient, CD28−/−, and T-bet−/− mice (8, 10, 13), suggesting that Sirpα deficiency may affect the CD4 T cell response to Salmonella infection. To test this hypothesis, we examined Salmonella epitope-specific Th1 responses in Sirpα−/− mice, using IFN-γ ELISPOTs and four different natural Salmonella epitopes (Table I). To allow time for Salmonella-specific CD4 T cell responses to develop in vivo while keeping Sirpα−/− mice alive, mice were treated with antibiotics during weeks 3 and 4 postinfection. At 5 wk after initial infection, we observed that SseI-, SseJ-, FliC-, and PagC-specific CD4 T cell responses were diminished in Sirpα−/− mice (Fig. 3A). In contrast, WT mice maintained an elevated frequency of CD4 T cells specific for each of these Salmonella epitopes (Fig. 3A).

It was possible that CD4 T cells in Sirpα−/− mice produced other Th1 cytokines such as IL-2 instead of IFN-γ, or that they polarized down the Th2 pathway by producing IL-4. To test these possibilities, we used cytokine ELISA to measure IFN-γ, IL-2, and IL-4 production in response to Salmonella Ags. Similar to what we discovered in IFN-γ ELISPOT assay, Sirpα−/− mice

**FIGURE 1.** Increased splenic erythropoiesis in Sirpα−/− naive mice. (A) Total splenocytes recovered from the spleens of WT and Sirpα−/− mice. (B) The percentages of RBCs (Ter119−CD71+) and RBC precursors (Ter119−CD71+) in the bone marrow and spleen of WT and Sirpα−/− mice, as measured by flow cytometry. (C) Total RBCs and RBC precursors recovered from the spleens of WT and Sirpα−/− mice. (D) Total CD4 and CD8 T lymphocytes recovered from the spleen of WT and Sirpα−/− mice. Data shown are representative of three similar experiments. Error bars represent the mean ± SEM. **p < 0.01.
produced less IFN-γ and IL-2 overall (Fig. 3B, 3C). In contrast, none of the mice produced IL-4, indicating that CD4 T cells do not skew toward the Th2 lineage in either WT or Sirpα−/− mice (data not shown).

To assess the total polyclonal Salmonella-specific CD4 T cell response to Salmonella Ags, we next measured cytokine production in response to HK S. typhimurium. Similar to single-Ag stimulations, HK S. typhimurium induced high levels of IFN-γ and IL-2 production by WT mice, whereas these cytokines were only marginally produced by Sirpα−/− mice (Fig. 3D, 3E). Again, no IL-4 production was detected, regardless of the dose of HK S. typhimurium used (data not shown).

Taken together, we conclude that Sirpα−/− mice develop an Ag-specific deficiency in CD4 T cell responses to single Salmonella Ags (SseL, SseJ, flagellin, and PagC), and the overall polyclonal Salmonella-specific Th1 response is also reduced.

SIRPα deficiency on DCs leads to a defect in Ag presentation

SIRPα is expressed by a subpopulation of APCs in mice (22). It seemed possible that the defect we detected in peptide-specific CD4 T cell responses in Sirpα−/− mice was due to the lack of SIRPα expression by DCs. To test this hypothesis, we examined the ability of SIRPα-deficient DCs to activate Ag-specific CD4 T cells in vitro. We cocultured Salmonella flagellin-specific SM1 T cells (35) with WT or SIRPα-deficient DCs in the presence or absence of crude (flagellin) or processed (flagellin peptide) Ag. At 16 h after cocultivation, SM1 T cells upregulated the activation markers CD69, CD44, and CD25 and downregulated CD62L in a dose-dependent manner when flagellin was processed and presented by WT DCs (Fig. 4A–D). However, the activation level of SM1 T cells was considerably lower in the presence of SIRPα-deficient DCs when compared with WT DCs (Fig. 4A–D), indicating that SIRPα-deficient DCs failed to process and present flagellin to the same level as WT DCs. In contrast, when SM1 T cells were activated by flagellin peptide, expression of SIRPα on DCs was not essential (Fig. 4A–D). With all these findings taken together, we conclude that SIRPα expression on DCs plays a crucial role in Ag processing and presentation to CD4 T cells.

**Defects in Ab response in Sirpα−/− mice**

Although Salmonella is an intracellular bacterium, it is known that Ab responses play an important role in protective immunity (11–13). We therefore examined Salmonella-specific serum Ab postinfection responses of WT and Sirpα−/− mice. Both WT and Sirpα−/− mice developed elevated titers of Salmonella-specific IgM and IgG2c at 2 and 3 wk postinfection; however, the overall Ab titers were lower in Sirpα−/− mice than in WT controls (Fig. 5A–D). No Salmonella-specific IgG1 response was detected in either WT or Sirpα−/− mice (data not shown). These data show that mice deficient in SIRPα generate Salmonella-specific Ab responses after Salmonella infection but that these responses are lower than those in WT mice.

**SIRPα deficiency does not affect the development of Chlamydia-specific CD4 T cells**

We next asked whether the failure to develop pathogen-specific CD4 T cell responses in Sirpα−/− mice was a peculiar feature of Salmonella infection. We infected WT and Sirpα−/− mice i.v. with 1 × 10⁷ C. muridarum, another intracellular bacterium that requires a Th1 response for resolution (42, 43). Of interest, when comparing Chlamydia epitope-specific CD4 T cell responses in WT and Sirpα−/− mice, using MHC class II tetramers, we did not observe any defect in Sirpα−/− mice (Fig. 6A, 6B). Indeed, Sirpα−/− mice developed similar clonal expansion of Chlamydia (PnpG1)-specific CD4 T cells following infection. Therefore, the defects in CD4 T cell responses we detected in Salmonella infection were not universal but appear restricted to the CD4 T cell response to Salmonella. Together, these data point to a role for SIRPα in the generation of Salmonella-specific Th1 responses and the regulation of erythropoiesis (Fig. 7).

**Discussion**

In an earlier study, we reported a marked increase in splenic erythropoiesis after systemic Salmonella infection of mice (5). We concluded that massive expansion of erythrocytes accounts for...
Salmonella-induced splenomegaly and also increased susceptibility to infection (5). As a key regulatory molecule for peripheral RBC turnover, SIRPα is widely expressed on myeloid lineage cells such as macrophages (27). Consistent with previous observations (30), we found that Sirpα−/− mice exhibit an increase in the number of erythroid cells in the spleen. Surprisingly, we found that Sirpα−/− mice are considerably more susceptible to Salmonella infection, as demonstrated by uncontrolled bacterial replication and increased mortality after challenge with attenuated bacteria. Thus, our current study provides another example of

**FIGURE 3.** Impaired Ag-specific CD4+ T cell response in Sirpα−/− mice after Salmonella infection. WT and Sirpα−/− mice were infected i.v. with 5 × 10⁶ attenuated Salmonella (BRD509) for a total of 5 wk. All mice were treated with antibiotics during weeks 3 and 4. (A) Purified splenic CD4+ T cells from infected mice were cocultured with Salmonella-specific CD4 T cell Ag (SseJ, SseI, FliC, or PagC) in the presence of irradiated APCs for 20 h in an ELISPOT plate precoated with anti-IFN-γ. Ag-specific CD4 T cell numbers were determined. Data represent three similar experiments with at least three mice per group. (B and C) A total of 1 million splenocytes from infected WT and Sirpα−/− mice were cocultured with Salmonella-specific CD4 T cell Ags (SseJ, SseI, FliC or PagC) for 24–48 h. Cytokine productions in culture supernatants were determined by cytokine ELISA. Data represent three similar experiments with at least three mice per group. (D and E) A total of 1 million total splenocytes from infected WT and Sirpα−/− mice were cocultured with serial diluted HK S. typhimurium for 24–48 h. Cytokine productions in culture supernatants were determined by cytokine ELISA. Data represent three similar experiments with at least three mice per group.

**FIGURE 4.** Reduced activation of Salmonella-specific CD4 T cells by SIRPα-deficient DCs in vitro. CD11c+ DCs (1 × 10⁵) purified from WT or SIRPα-deficient mice were cocultured with 1 × 10⁵ flagellin-specific SM1 T cells in the presence or absence of various doses of Ag flagellin or 10 µg/ml flagellin peptide. Graphs depict the percent expression of CD69 (A), CD44 (B), CD25 (C), or loss of CD62L (D) on gated SM1 T cells 16 h poststimulation. Error bars represent mean ± SEM.
a correlation between increased erythropoiesis and increased susceptibility to *Salmonella* infection.

There are likely to be multiple mechanisms by which erythropoiesis and SIRPα affect susceptibility to *Salmonella* (Fig. 7). We previously demonstrated that increased EPO production encourages bacterial growth and speculated that this resulted from an alteration in the architecture of the spleen that inhibited the adaptive immune response (5). An alternative possibility is that EPO can directly inhibit macrophage bactericidal activity, thus increasing susceptibility to *Salmonella* (14). Our new data suggest that SIRPα deficiency also induces erythropoiesis, impairs host immunity, and encourages *Salmonella* growth. Together these processes suggest a growing link between erythroid development and susceptibility to bacterial infection.

The increased susceptibility of SIRPα-deficient mice to bacterial infection may also be independent of increased erythropoiesis and directly caused by defects in Ag-specific CD4 T cell responses. Our ELISPOT results show that 5 wk post- *Salmonella* infection, *Sirpα<sup>−/−</sup>* mice lack Ag-specific CD4 T cells of all known *Salmonella* epitopes: SseI, SseJ, FliC, and PagC. These epitopes represent the only well-defined epitopes in the *Salmonella* mouse model and therefore suggested a broad reduction in the Th1 response to *Salmonella* (38, 44). The overall Th1 cytokine production by *Sirpα<sup>−/−</sup>* mice after *Salmonella* infection parallels that observed in mice lacking the Th1-specific transcription factor T-bet, suggesting a similar deficiency in these mouse models (10). Similar requirement for SIRPα in induction of Th1 responses has also been reported in a recent study using the parasite *Leishmania major* infection model (45).

In addition to macrophages, SIRPα is also abundantly expressed on DCs (22). CD8α<sup>+</sup>CD11c+ DCs in the spleen express a much higher level of SIRPα than do CD8α<sup>−/−</sup>CD11c+ DCs (26, 46, 47). Lack of expression of SIRPα or its ligand, CD47, results in reduced numbers of CD8α<sup>−/−</sup>CD11c+ DCs (26). Thus, it is possible that the diminished Ag-specific CD4 T cell responses in *Sirpα<sup>−/−</sup>* mice are due to the reduced number of this CD8α<sup>−/−</sup>CD11c+ DC population and/or less efficient Ag presentation by SIRPα-deficient DCs. Indeed, our data clearly show that the ability of Ag-specific CD4 T cell priming by SIRPα-deficient DCs in vitro was reduced when compared with that of WT DCs. These results provide a mechanistic explanation for the reduced *Salmonella* Ag-specific CD4 T cell response in *Sirpα<sup>−/−</sup>* mice in vivo. Similar

**FIGURE 5.** *Salmonella*-specific Ab responses in WT and *Sirpα<sup>−/−</sup>* mice. WT and *Sirpα<sup>−/−</sup>* mice were infected i.v. with 5 × 10<sup>5</sup> attenuated *Salmonella* (BRD509). Serum was collected 2 (A, B) and 3 (C, D) wk postinfection (p.i.). *Salmonella*-specific IgM (A, C) and IgG2c (B, D) titers were determined by ELISA. Graphs show OD readings (mean ± SEM) of IgM and IgG2c responses. Data represent three independent experiments with at least four mice per group.

**FIGURE 6.** SIRPα expression is not required for Ag-specific CD4 T cell response to *C. muridarum* infection. (A) FACS plots of PmpG-1 MHC class II tetramer-specific CD4 T cell staining in naive, WT, and *Sirpα<sup>−/−</sup>* mice 5 wk after *C. muridarum* i.v. infection. (B) Total PmpG-1–specific CD4 T cell numbers in WT and *Sirpα<sup>−/−</sup>* mice.

**FIGURE 7.** Predicted model of how SIRPα deficiency leads to increased susceptibility to *Salmonella* infection.
in vitro studies have also reported that interaction of SIRPα on DCs with CD47 on T cells contributes to the activation of Ag-specific cytotoxic T cells (25).

In addition to reduced cell-mediated immunity, the development of Salmonella-specific Ab responses was also reduced in Sirpe−/− mice. As described earlier, increased erythropoiesis in Sirpe−/− mice disrupts the splenic architecture after Salmonella infection. The enlarged spleen could lead to disruption of efficient B cell and T helper cell interactions, thus making Ab production less efficient.

Overall, our present study demonstrates an indispensable role for SIRPα in protective immunity against Salmonella infection (Fig. 7). Deficiency of SIRPα correlated with a reduction of CD4 Th1 responses to Salmonella-specific Ags, inefficient Ag presentation, and reduced Salmonella-specific Ab. Our data also strengthen the association between susceptibility to Salmonella and erythroid development and suggest that focusing on this interaction may assist the development of vaccines or therapeutics for bacterial infection.

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Disclosures

The authors have no financial conflicts of interest.

References