Salmonella SiiE prevents an efficient humoral immune memory by interfering with IgG$^+$ plasma cell persistence in the bone marrow

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Serum IgG, which is mainly generated from IgG-secreting plasma cells in the bone marrow (BM), protects our body against various pathogens. We show here that the protein SiiE of *Salmonella* is both required and sufficient to prevent an efficient humoral immune memory against the pathogen by selectively reducing the number of IgG-secreting plasma cells in the BM. Attenuated SiiE-deficient *Salmonella* induces high and lasting titers of specific and protective *Salmonella*-specific IgG and qualifies as an efficient vaccine against *Salmonella*. A SiiE-derived peptide with homology to laminin β1 is sufficient to ablate IgG-secreting plasma cells from the BM, identifying laminin β1 as a component of niches for IgG-secreting plasma cells in the BM, and furthermore, qualifies it as a unique therapeutic option to selectively ablate IgG-secreting plasma cells in autoimmune diseases and multiple myeloma.

immunoglobulin G | plasma cells | bone marrow | niche | *Salmonella*

The bone marrow (BM) is the central tissue for hematopoiesis as well as for immunological memory. Hematopoietic stem cells, B cell precursors, plasma cells, and memory T cells reside in distinct specialized stromal niches of the BM (1–3). These stromal niches provide cell adhesion molecules such as VCAM-1, laminin, fibronectin, and collagens, as well as cytokines and chemokines such as CXCL12, IL-7, IL-15, Kit ligand, and Flt3 ligand to support survival, expansion, and differentiation of hematopoietic cells (4, 5). In the late phase of immune responses, some antigen-experienced plasma blasts migrate into the BM in a CXCR4/CXCL12-dependent manner (6, 7) and reside there as long-lived "memory" plasma cells (8). Eosinophils and megakaryocytes play an important role as components of survival niches for plasma cells, secreting APRIL and IL-6 to promote plasma cell survival (9–11). Reynolds et al. (12) showed that IgM-secreting plasma cells do not colocalize with eosinophils as they do with IgG-secreting plasma cells, suggesting that IgM- and IgG-secreting plasma cells localize in distinct survival niches in the BM. However, it still remains unclear whether class-switched and unswitched plasma cells do indeed share the same survival niches, or have their own distinct niches.

The gram-negative bacterium *Salmonella enterica* is responsible for high mortality and morbidity in humans worldwide (13). *S. enterica* serovar Typhi causes enteric fever and kills ∼200,000 people per year. *S. enterica* serovar Typhimurium has been widely used as an experimental animal model for typhoid fever (14). Following infection via intestinal epithelia, *Salmonella* invades myeloid cells which migrate into the spleen and liver. The bacteria remains in myeloid cells for long periods of time (15–17); however, it is still unknown how *Salmonella* can survive long-term in these short-lived and mobile myeloid cells and more importantly, how it can escape from humoral immunity.

Here we show that *Salmonella* specifically reduces the number of IgG-secreting plasma cells in the BM and consequently reduces IgG titers in serum. Using chromatography and mass spectrometry, SiiE was identified as the responsible protein reducing plasma cell numbers. We determine that SiiE-deficient *Salmonella* fails to reduce plasma cell numbers and instead enhances the humoral immune response against *Salmonella* in mice, qualifying as it as an efficient vaccine against *Salmonella*. A SiiE-derived peptide with homology to murine laminin β1 also reduced the number of IgG-secreting plasma cells in the BM, suggesting that SiiE inhibits the interaction between the plasma cells and laminin β1 via the binding to integrin β1. Histological analysis revealed that laminin β1 specifically binds to IgG- but not IgM-secreting plasma cells, demonstrating that laminin β1 is a component of distinct survival niches for IgG-secreting plasma cells in the BM. We suggest that *Salmonella* secretes SiiE and inhibits the retention of IgG-secreting plasma cells in the BM as a strategy to escape from humoral immunity.

Significance

Serum IgG, which is mainly generated from IgG-secreting plasma cells in the bone marrow (BM), protects our body against various pathogens. We show here that the protein SiiE of *Salmonella* is required to prevent an efficient humoral immune response against the pathogen by selectively reducing the number of IgG-secreting plasma cells in the BM. Attenuated SiiE-deficient *Salmonella* induces high titers of specific *Salmonella*-specific IgG and qualifies as an efficient vaccine against *Salmonella*. A SiiE-derived peptide with homology to laminin β1 is sufficient to ablate IgG-secreting plasma cells from the BM, identifying laminin β1 as a component of niches for IgG-secreting plasma cells in the BM.

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Results

***Salmonella* Specifically Reduces the Number of IgG-Secreting Plasma Cells in the BM.** *Salmonella* escapes from humoral immunity and can survive in the body for long periods of time, resulting in a chronic infectious disease. Long-lasting persistence requires that *Salmonella* continues to relocate between short-lived macrophages via body fluids containing antibodies. Therefore, we first examined the influence of *Salmonella* on the production of antibodies, using a chronic infection model, mimicked by intraperitoneal infection of attenuated *Salmonella* (*lon* mutant) (18, 19). C57BL/6 mice received 10^4 colony-forming units (cfu) of the attenuated *Salmonella* intraperitoneally (i.p.). Infected *Salmonella* expanded on days 4–7 in the spleen and liver and stayed there as a small population until day 20, the end of the observation period (*SI Appendix*, Fig. S1*A* and ref. 19). On day 4, at the peak of *Salmonella* expansion, polyclonal antibody-secreting cells in the spleen and BM were enumerated by ELISpot assay. Surprisingly, the number of IgG-secreting plasma cells in the BM was reduced, but the numbers of BM IgM- and IgA- and splenic IgG-secreting cells were not affected (Fig. 1*A*). The number of splenic IgM-secreting plasma cells was slightly increased, probably due to a generic bacterial stimulant, e.g., LPS. Moreover, Blimp-1^+CD138^+B220^-IgM$^-$IgA$^-$ cells, mostly consisting of IgG$^+$ plasma cells, and intracellular IgG$^+$CD138^+ B220^- cells in the BM, were also significantly reduced in number (Fig. 1 *B* and *C*). The reduction in the number of BM IgG-secreting cells was also observed after infection with wild-type *Salmonella* (Fig. 1*D*). The numerical reduction of BM IgG-secreting plasma cells, which are the main source of serum IgG, may affect the titers of IgG in serum. Indeed on day 7 after infection, polyclonal IgG, but not IgM titers in serum was significantly impaired (Fig. 1*E*). The specific numerical reduction of BM IgG-secreting plasma cells was also shown in the case of natural infection with *Salmonella*. Oral infection with attenuated *Salmonella* reduced the number of BM IgG-secreting plasma cells, but did not affect the numbers of other plasma cells in the BM, spleen and lamina propria (Fig. 1*F*).

***Salmonella* Protein SiiE Reduces the Number of IgG-Secreting Plasma Cells in the BM.** On day 4 after i.p. infection, *Salmonella* could be detected in the spleen and liver but not in the BM (Fig. 2*A*), suggesting that *Salmonella* in the spleen and liver impacts on IgG-secreting plasma cells in the BM from a distance, likely by secreted proteins. The culture supernatant of *Salmonella* includes several inducers of innate immune activation, such as LPS and flagellin. We removed LPS from the supernatant of flagellin-deficient attenuated *Salmonella* and injected it into C57BL/6 mice. Untreated and LPS-free supernatant from flagellin-deficient *Salmonella* reduced the number of BM IgG-secreting plasma cells alike (Fig. 2*B*). In contrast, untreated supernatant digested by proteinase failed to reduce them (Fig. 2*C*). To determine whether the reduction in plasma cell numbers was specific to *Salmonella*, supernatant from flagellin- and Lon-deficient *Escherichia coli* was also injected into mice. *E. coli* supernatant, however, did not affect the number of BM IgG-secreting plasma cells (Fig. 2*B*), proving that it was a *Salmonella*-specific factor altering the BM plasma cell numbers. Interestingly, the LPS/flagellin-free *Salmonella* supernatant also reduced the number of antigen-specific IgG-secreting plasma cells in the BM, which had been generated by immunization with (4-hydroxy-3-nitrophenyl) acetyl chicken gamma globulin (NP-CGG) (Fig. 2*D*). These data suggest that *Salmonella* supernatant devoid of LPS and flagellin contain a protein which impacts on BM IgG-secreting plasma cells.

To determine what this component was, we fractionated proteins in the *Salmonella* supernatant by ion-exchange chromatography and screened each fraction for its impact on BM IgG-secreting plasma cells in vivo. By SDS-polyacrylamide gel electrophoresis

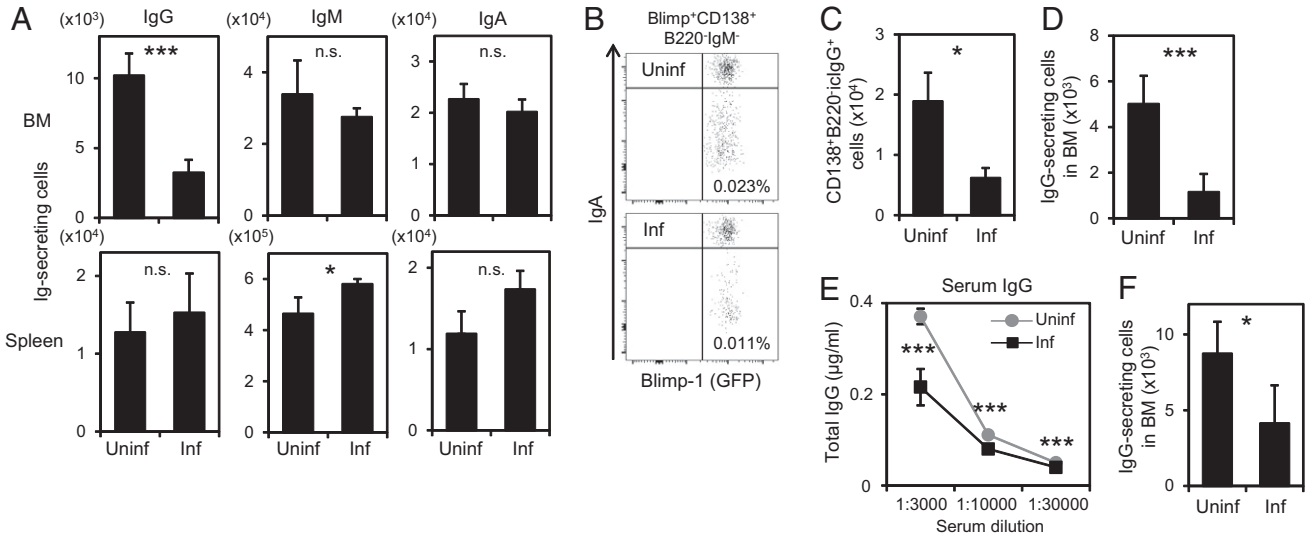

Fig. 1. Numerical reduction of BM IgG-secreting plasma cells by infection with *Salmonella*. (*A*) *Salmonella* reduces the number of IgG-secreting cells in the BM. C57BL/6 mice were infected intraperitoneally (i.p.) with 10^4 cfu of attenuated *Salmonella* and were killed on day 4 after infection. Cells in the spleen and femurs of *Salmonella*-infected (Inf) or uninfected (Uninf) mice were analyzed for IgG-, IgM-, or IgA-secreting cells by ELISpot assay ($n = 10$). (*B*) *Salmonella* reduces the number of Blimp-1^+IgM$^-$IgA$^-$ cells in the BM. Blimp^{gfp} mice were infected as described above. Flow cytometry plots show the expression of Blimp-1 and IgA in Blimp-1^+CD138^+B220^-IgM$^-$ live cells of the BM ($n = 4$). (*C*) *Salmonella* numerically reduces intracellular (ic) IgG$^+$ plasma cells in the BM. BM cells from mice infected as described above were analyzed by flow cytometry for the expression of intracellular IgG in B220^-CD138^+ cells ($n = 6$). (*D*) Wild-type *Salmonella* reduces the number of BM IgG-secreting plasma cells. C57BL/6 mice were infected with 10^2 cfu of *Salmonella* wild-type strain and on day 5 were analyzed for IgG-secreting plasma cells by ELISpot assay ($n = 6$–7). (*E*) *Salmonella* reduces IgG titers in serum. Sera from C57BL/6 mice on day 7 postinfection with 10^4 cfu of attenuated *Salmonella* were analyzed for titers of total IgG by ELISA ($n = 4$). (*F*) Numerical reduction of BM IgG-secreting plasma cells by oral infection with *Salmonella*. C57BL/6 mice were infected orally with 0.5–2 × 10^9 cfu of attenuated *Salmonella* and were analyzed on day 7 for IgG-secreting cells by ELISpot assay ($n = 7$). These data are representative of at least two independent experiments. $^*P < 0.05$, $^{***}P < 0.001$, n.s., not significant.

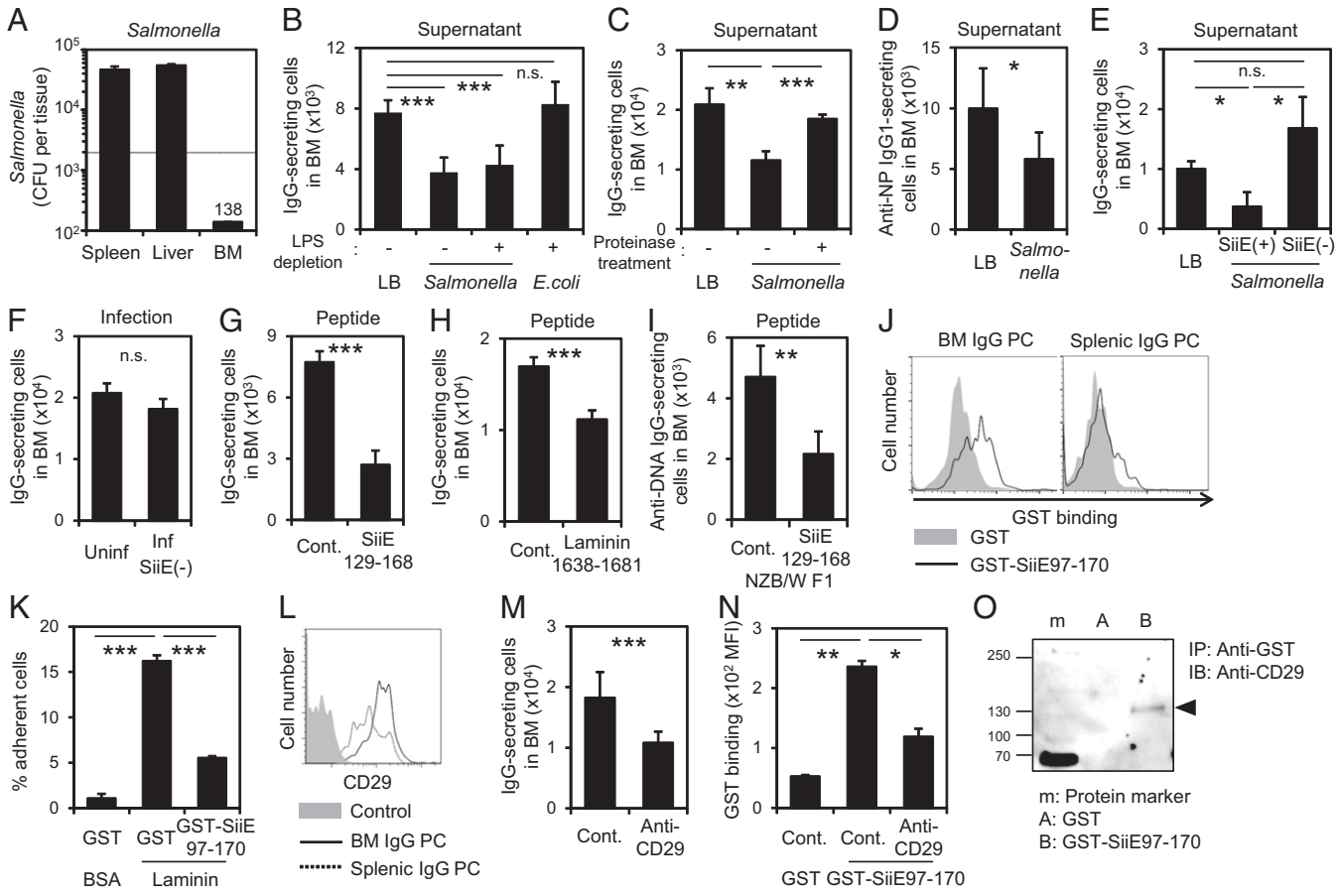

Fig. 2. Identification of the microbial component reducing the number of BM IgG-secreting plasma cells. (*A*) *Salmonella* colonizes in the spleen and liver but not in the BM. The cfu of *Salmonella* in the spleen, liver, and BM of mice on day 4 after i.p. infection with 10^4 cfu of attenuated *Salmonella* was counted. The dotted line represents the limit of quantification ($n = 3$–6). (*B*) A *Salmonella*-specific microbial component reduces the number of BM IgG-secreting plasma cells. C57BL/6 mice received 200 μL of LB medium, untreated or LPS-depleted culture supernatants from flagellin-deficient attenuated *Salmonella* or *E. coli* and on the next day (24 h later) were analyzed for IgG-secreting cell numbers in the BM by ELISpot assay ($n = 8$). (*C*) A *Salmonella*-derived protein reduces the number of BM IgG-secreting plasma cells. C57BL/6 mice received 200 μL of LB medium, untreated or proteinase K treated (0.1 mg/mL, 1 h at 37 °C) culture supernatants from attenuated *Salmonella* and analyzed as described above ($n = 4$). (*D*) Supernatant from attenuated *Salmonella* reduces the number of antigen-specific IgG-secreting plasma cells in the BM. C57BL/6 mice were primed i.p. with 100 μg of (4-hydroxy-3-nitrophenyl) acetyl chicken gamma globulin (NP-CGG) in incomplete Freund's adjuvant (day 0) and were boosted i.v. with 50 μg of NP-CGG in PBS 4 wk after priming (day 28). On day 41 after priming, mice were injected i.p. with 200 μL of LB or LPS/flagellin-free supernatant and analyzed for anti-NP IgG1-secreting cells in the BM on the next day ($n = 6$). (*E*) Supernatant from SiiE-deficient attenuated *Salmonella* fails to reduce BM IgG-secreting plasma cell numbers. C57BL/6 mice received i.p. 200 μL of LB medium or LPS-depleted supernatants from SiiE-deficient [SiiE(–)] or SiiE-producing [SiiE(+)] attenuated *Salmonella* and on the next day were analyzed for IgG-secreting cells in the BM by ELISpot assay ($n = 5$). (*F*) SiiE-deficient attenuated *Salmonella* fails to reduce BM IgG-secreting plasma cell numbers. C57BL/6 mice were infected i.p. with 10^4 cfu of SiiE(–) or SiiE(+) attenuated *Salmonella* and analyzed as described above ($n = 5$). (*G*) A 40 amino acid-long peptide from the N terminus of SiiE protein reduces the number of BM IgG-secreting plasma cells. C57BL/6 mice received i.p. 100 μg peptide coding SiiE amino acid 129–168 and were analyzed as described above ($n = 5$). (*H*) Laminin β1-derived peptide with homology to SiiE reduces the number of BM IgG-secreting plasma cells. C57BL/6 mice received i.p. 100 μg peptide coding mouse laminin β1 amino acid 1638–1681 and were analyzed as described above ($n = 4$). (*I*) SiiE peptide reduces the number of DNA-specific IgG-secreting plasma cells in the BM of NZB/W F1 mice. NZB/W F1 5-mo-old female mice received i.p. 100 μg peptide coding SiiE amino acid 129–168 on days 0, 3, 7, and 10 and were analyzed for anti-DNA IgG-secreting cells in the BM by ELISpot assay on day 11 ($n = 5$–6). (*J*) SiiE fragment binds to IgG-secreting plasma cells in the BM but not spleen. BM or splenic cells from C57BL/6 mice were incubated with 30 μg/mL of GST-SiiE 97–170 or GST, stained with phycoerythrin (PE)-labeled anti-GST antibodies, and analyzed by flow cytometry ($n = 3$). (*K*) SiiE fragment inhibits the adhesion of BM IgG-secreting plasma cells to laminin in vitro. Sorted $CD138^+B220^-IgM^-IgA^-$ cells from the BM of C57BL/6 mice were treated with GST-SiiE 97–170 or GST and then incubated with laminin-coated plates. Adherent cells were counted after washing. (*L*) More IgG-secreting plasma cells in the BM express high levels of integrin β1 compared with those in the spleen. Live $Blimp\text{-}1^+CD138^+B220^-IgM^-IgA^-$cells of the BM and spleen are shown ($n = 2$). (*M*) Injection of anti-integrin β1 (CD29) antibody reduces the number of BM IgG-secreting plasma cells. C57BL/6 mice received 200 μg of anti-integrin β1 (HMβ1–1) or isotype control and analyzed as described above ($n = 6$). (*N*) Antibodies against integrin β1 interfere the binding of SiiE to BM IgG-secreting plasma cells. As described above (*J*), IgG-secreting plasma cells were stained with GST-SiiE 97–170 or GST following treatment with anti-integrin β1 antibody (HMβ1–1) or isotype control ($n = 3$). (*O*) SiiE interacts with integrin β1. BM cell lysates were immunoprecipitated with GST-SiiE 97–170 or GST and anti-GST antibodies followed by immunoblotting analysis with anti-integrin β1 antibodies. These data are representative of at least two independent experiments. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. n.s., not significant.

(SDS/PAGE) and mass spectrometry, we then identified a protein, SiiE, as the most likely active component. SiiE is a large protein of 5,559 amino acids, with two distinct regions in the N and C terminus and 53 repeated bacterial Ig domains in between (*SI Appendix*, Fig. S1*B*) (20). SiiE is secreted and is involved in the adhesion to gut intestinal epithelial cells (21). Here we demonstrate the presence of free SiiE protein of *Salmonella* in the spleen, as detectable by immunofluorescence (*SI Appendix*, Fig. S1*C*). Supernatant from

attenuated SiiE-deficient *Salmonella* and the SiiE-deficient bacteria themselves failed to reduce the number of IgG-secreting plasma cells in the BM (Fig. 2 *E* and *F*). The SiiE-deficient strain carrying pGST-miniSiiE, which encodes a hybrid protein of the N-terminal and the C-terminal domain of SiiE and can functionally adhere to an epithelial cell line (22), significantly reduced plasma cell numbers (*SI Appendix*, Fig. S1*D*). From a search with the Basic Local Alignment Search Tool (BLAST, the National Library of Medicine), two sequences of the N-terminal region had high homologies (score < 0.01) to murine laminin β1 and myosin 7A. Since myosin is an intracellular protein, we focused on laminin β1, assuming that secreted SiiE competes with laminin β1 for interaction with IgG-secreting plasma cells. Injection of SiiE 129–168, a synthetic 40-amino acid-long peptide with high homology to a conserved sequence of laminin β1 in many species (*SI Appendix*, Fig. S1*B*), markedly reduced the number of BM IgG-secreting plasma cells, as did injection of laminin β1 1638–1681 peptide (Fig. 2 *G* and *H*). In the NZB/W murine model of lupus nephritis, autoantibody-secreting plasma cells of the BM contribute to the development of nephritis in F1 female mice (23). In this model, injection of SiiE 129–168 also reduced the number of anti-DNA IgG-secreting plasma cells in the BM (Fig. 2*I*). Furthermore, a SiiE 97–170 fragment stained IgG-secreting plasma cells of the BM, but not those of the spleen (Fig. 2*J*). This fragment also inhibited the adhesion of BM IgG-secreting plasma cells to laminin in vitro (Fig. 2*K*). Laminin receptors are categorized as integrins and nonintegrin molecules, e.g., the 67-kD laminin receptor (RPSA, ref. 24). Integrins as receptors for laminins comprise six members of the β1 subfamily (α1β1, α2β1, α3β1, α6β1, α7β1, and α9β1), three αV subfamily members (αVβ3, αVβ5, and αVβ8), and additionally the α6β4 integrin (24). To identify the responsible receptor for laminin β1 and SiiE, we first analyzed the expression and function of integrin β1 (CD29), one of the major laminin receptors. More IgG-secreting plasma cells in the BM expressed integrin β1 at a high level than those in the spleen (Fig. 2*L*). Injection of an antibody against integrin β1 significantly reduced the number of IgG-secreting plasma cells in the BM, but not the spleen (Fig. 2*M*). To test whether SiiE interacts with integrin β1, flow cytometry and immunoprecipitation analyses were performed. These showed that the binding of GST-SiiE 97–170 on BM IgG-secreting plasma cells could be inhibited by preincubation of an anti-integrin β1 antibody (Fig. 2*N*), and also that GST-SiiE 97–170 interacts with integrin β1 in BM lysates (Fig. 2*O*). These data suggest that SiiE interacts with integrin β1, competing with laminin β1. As integrin β1 is a heterodimer, we also wanted to examine its heterodimer partner. Therefore, we compared the expression of integrin α1, α2, α3, α6, α7, and α9 on IgG^+, IgM^+, and IgA^+ plasma cells in the BM and spleen (*SI Appendix*, Fig. S3*A*). None of the receptors were exclusively expressed on BM IgG+ plasma cells, except for a subpopulation of BM IgG-secreting plasma cells expressing integrin α2. Some BM IgM^- IgA^-Blimp-1^+ plasma cells also expressed integrin α2, although the loss or blockage of integrin α2 failed to affect the number of IgG-secreting plasma cells in the BM (*SI Appendix*, Fig. S3 *B* and *C*). We further examined another subfamily of αV integrin and nonintegrin 67-kD laminin receptor. The expression and function of integrin αV and the expression of 67-kD laminin receptor did not impact BM IgG-secreting plasma cells (*SI Appendix*, Fig. S3 *A* and *D*). These data strongly suggest that SiiE of *Salmonella* interacts with integrin β1 on BM IgG-secreting plasma cells by competing with their binding to laminin β1, although the heterodimer partner of integrin β1 still remains unknown.

Loss of SiiE Enhances the Production of Anti-*Salmonella* IgG. As shown above, SiiE inhibits the residency of BM IgG-secreting plasma cells. We thus expected that SiiE prevents an efficient humoral immune response against *Salmonella*. C57BL/6 mice infected with the *siiE* mutant bacteria were analyzed for their titers of anti-*Salmonella* IgG. SiiE-producing and *siiE* mutant strains of *Salmonella* expanded normally in vitro and in vivo (Fig. 3*A*) and both secreted proteins and LPS into their supernatants. The SiiE-deficient strain, however, induced markedly more anti-*Salmonella* IgG and more efficient removal of the bacteria compared with the SiiE-producing strain (Fig. 3 *B* and *C*). To evaluate the potential of the mutant strain as a protective vaccine, mice primed by SiiE-producing or SiiE-deficient *Salmonella* were challenged with a lethal dose of wild-type *Salmonella*. On day 7 after challenge, all naive mice died, while both groups of the vaccinated mice survived. Mice vaccinated with SiiE-deficient *Salmonella* strongly suppressed the expansion of virulent *Salmonella* in the spleen, compared with mice vaccinated with SiiE-producing *Salmonella* (Fig. 3*D*). These data characterize SiiE-deficient *Salmonella* as an efficient vaccine.

Laminin $\beta1^+CXCL12^+$ Stromal Cells Organize the Survival Niche for BM IgG-Secreting Plasma Cells. Is laminin β1 an essential component of the BM survival niche for IgG-secreting plasma cells? Histological analysis showed that laminin β1 was ubiquitously distributed in the marrow (Fig. 4*A*, *Left*). We have shown that BM IgG^+ plasma cells reside in $CXCL12^+$ stromal cells (7). Approximately 34.2% (±12.7%, $n = 3$) of $CXCL12^+$ stromal cells also costained for laminin β1 (Fig. 4*A*). Laminin β1 expression was distributed on the cell surface and cellular processes of $CXCL12^+$ stromal cells (Fig. 4*A*, *Middle* and *Right*). To determine whether laminin β1 interacts with IgG^+ or IgM^+ Blimp-1^+ plasma cells in the BM and spleen, we stained frozen sections of femurs and spleen of $Blimp^{gfp}$ mice for laminin β1 and IgG or IgM. Approximately 90% of BM IgG^+Blimp-1^+ plasma cells bound to laminin β1, while less than 10% of BM IgM^+Blimp-1^+, and less than 30% of splenic IgG^+Blimp-1^+ plasma cells, attached to laminin β1 (Fig.

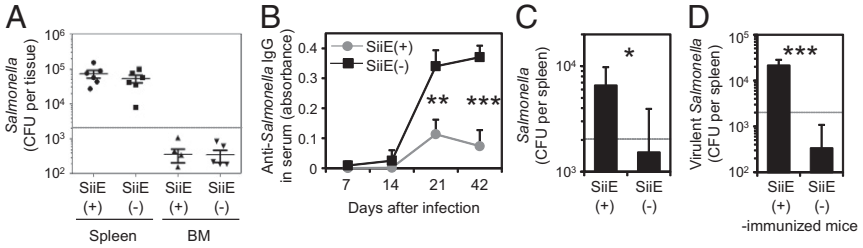

Fig. 3. Loss of SiiE enhances the humoral immune response against *Salmonella*. (*A*) SiiE-deficient attenuated *Salmonella* normally expands in the spleen. C57BL/6 mice were infected i.p. with 10^4 cfu of SiiE-deficient [SiiE(–)] or SiiE-producing [SiiE(+)] attenuated *Salmonella* and 4 d later were analyzed for cfu of *Salmonella* in the spleen and BM. The dotted line represents the limit of quantification ($n = 6$). (*B* and *C*) SiiE-deficient *Salmonella* enhances the provision of anti-*Salmonella* antibodies resulting in more efficient removal of the bacteria. C57BL/6 mice infected i.p. with 10^4 cfu of SiiE-deficient or SiiE-producing attenuated *Salmonella*, were bled on days 7, 14, 21, and 42 after infection for measurement of anti-*Salmonella* IgG by ELISA (*B*) and were analyzed on day 21 for cfu of *Salmonella* in the spleen (*C*). ($n = 5$–6). (*D*) Vaccination of SiiE-deficient attenuated *Salmonella* efficiently protects against a lethal dose of *Salmonella*. C57BL/6 mice which were vaccinated i.p. with 10^4 cfu of SiiE-deficient or SiiE-producing attenuated *Salmonella*, were challenged i.p. with 10^3 cfu of wild-type *Salmonella* on day 21 after vaccination. On day 28, the number of virulent *Salmonella* in the spleen of the challenged mice was enumerated using antibiotic selection ($n = 5$). These data are representative of at least two independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

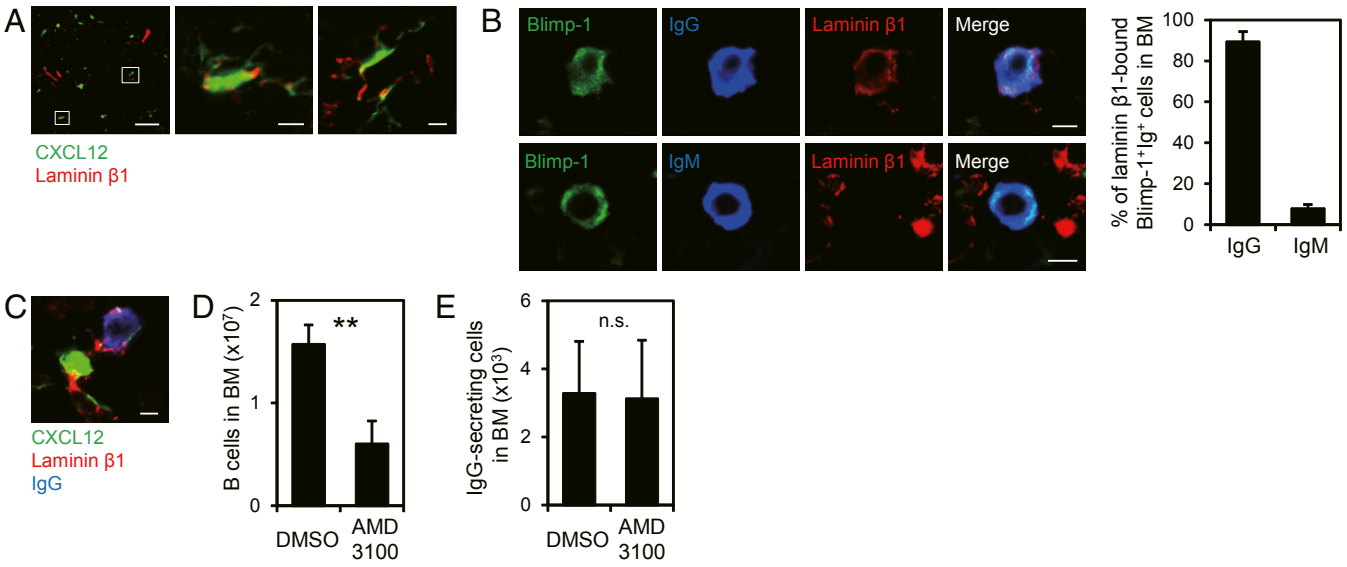

Fig. 4. BM IgG-secreting plasma cells persist in laminin β1$^+$ CXCL12-expressing stromal cells. (*A*) The distribution of laminin β1 on CXCL12$^+$ stromal cells in the BM. BM frozen sections from CXCL12/GFP knock-in mice were stained for laminin β1 (scale bars, 50 μm) (*Left*) and 5 μm (*Middle* and *Right*). (*B*) IgG- but not IgM-secreting plasma cells in the BM contact laminin β1. BM frozen sections from Blimp^{gfp} mice were stained for laminin β1 and IgG or IgM. The bar graph shows the percentages of laminin β1-bound Ig$^+$ plasma cells in total Ig$^+$ plasma cells (n = 80–100; three mice) (scale bars, 5 μm). (*C*) IgG$^+$ cells contact laminin β1$^+$CXCL12$^+$ stromal cells in the BM. BM frozen sections from CXCL12/GFP knock-in mice were stained for laminin β1 and IgG (scale bars, 5 μm). (*D* and *E*) AMD3100 numerically reduces B cells but not IgG-secreting plasma cells in the BM. C57BL/6 mice were injected i.p. twice a day with 5 μg of AMD3100 for 4 d. B220$^+$ cells and IgG-secreting plasma cells were analyzed on day 4 by flow cytometry (*D*) and by ELISpot assay (*E*), respectively (n = 5–6). These data are representative of at least two independent experiments. $^{**}P < 0.01$, n.s., not significant.

4*B* and *SI Appendix*, Fig. S2). We have previously shown that ∼90% of IgG$^+$ plasma cells are in contact with CXCL12-expressing cells (7, 25). In fact, most IgG$^+$ cells contacted laminin β1-coated CXCL12$^+$ cells (Fig. 4*C*). These histological data suggest that BM IgG-secreting plasma cells preferentially reside in laminin β1$^+$ CXCL12$^+$ stromal niches. *Salmonella* is also known to reduce the expression of CXCL12 in the BM (26). Therefore, to determine whether loss of CXCL12 by *Salmonella* affects the reduction of IgG-secreting plasma cell numbers in the BM, we examined the effect of CXCR4 antagonists on the persistence of BM IgG-secreting plasma cells. On day 4 after the first injection of the CXCR4 antagonist AMD3100, the number of IgG-secreting plasma cells in the BM was not affected, while B cell numbers were reduced (Fig. 4 *D* and *E*). We conclude that the reduced number of IgG-secreting plasma cells in the BM was caused by SiiE from *Salmonella*, but not by the down-regulation of CXCL12.

Discussion

We show here that *Salmonella* specifically reduces the number of IgG-secreting plasma cells in the BM, which are the main source of serum IgG, in a SiiE-dependent and CXCL12-independent manner. Since no *Salmonella* could be detected in the BM and the reduction was also induced by culture supernatant of *Salmonella*, we assumed that a secreted component of *Salmonella* was responsible. We have identified the *Salmonella* protein SiiE as the responsible component. A SiiE-derived peptide, which has high homology to murine laminin β1, was able to reduce the number of BM IgG-secreting plasma cells. Data from cytometric and molecular analyses suggest that SiiE interacts with integrin β1 and competes with laminin β1 for binding. Moreover, attenuated SiiE-deficient *Salmonella* induced high titers of protective IgG against *Salmonella*, identifying it as an efficient vaccine for *Salmonella*. Histological analyses of the BM revealed that IgG- but not IgM-secreting plasma cells bind to laminin β1. Thus, laminin β1$^+$CXCL12$^+$ stromal cells are an integral part of the survival niche for IgG-secreting plasma cells in the BM, a lesson learned from *Salmonella*.

CXCL12 is known to be required for the formation of long-lived memory plasma cells in the BM, since CXCR4-deficient plasma cells do not develop into long-lived BM plasma cells (6, 7). It has been controversial whether CXCL12 is required for the immigration and/or the maintenance of plasma cells in (to) the BM. Slocombe et al. (26) reported that chronic inflammation including infection causes mobilization of plasma cells from the BM in a CXCL12- and TNFα-dependent manner. However, Hauser et al. (27) showed that the migratory capacity of newly generated plasma cells is lost between day 8 and 12 after boost, suggesting that CXCL12 is required for the initial migration of plasma cells into their survival niches. As *Salmonella* is known to affect the expression of CXCL12, we speculated that this alteration in expression could result in the reduction in IgG plasma cell numbers seen in the BM. However, while we found that the CXCR4 antagonist AMD3100 inhibited the retention of B cells in the BM, it did not affect established IgG-secreting plasma cells. Our data directly support the hypothesis that CXCL12 is not required for the retention and maintenance of IgG-secreting plasma cells in the BM.

Infection with *S. typhi*, which is restricted to humans and causes severe and often fatal typhoid fever, can be prevented by vaccination with attenuated strains, e.g., Ty21a (28). The *siiE* gene in *S. typhi* has been reported as two distinct ORFs (9,852 bp and 6,771 bp, Typhimurium has 16,680 bp), suggesting that it is a pseudogene (29). Furthermore, the *siiE* gene in *S. typhi* has a mutation (148 A > T) within the sequence with close homology to laminin β1. A lack of SiiE, or nonfunctional SiiE in *S. typhi* may be a reason why vaccination against *S. typhi* is effective. In contrast, a vaccine against *S. typhimurium*, which causes severe food poisoning in humans, cattle, swine, sheep, horses, rodents, and galliformes is not yet available. Diseases caused by these invasive nontyphoidal *Salmonella* (iNTS), including *S. typhimurium*, have been largely neglected, despite causing an estimated 155,000 deaths per year, akin to infection with *S. typhi*, and having a fatality

rate of 20–25%, which is higher than that by infection with *S. typhi* (30, 31). We show here that the SiiE-deficient mutant of *S. typhimurium* can induce efficient immune responses, compared with SiiE-producing *Salmonella*. To establish vaccines against iNTS, including *S. typhimurium*, we propose using SiiE-deficient attenuated *Salmonella* as a vaccine. It has been reported that *Salmonella* inhibits acquired immunity, e.g., B cell activation, germinal center formation, and the expression of MHC class II (32–34); we here further add the inhibition of humoral memory by SiiE to this list, although *siiE* was previously listed among 118 gene candidates potentially related to long-term persistence of *Salmonella* in a previous genome-wide screening (35). We know that *Salmonella* secretes SiiE (21); however, it remains unclear how invading *Salmonella* secretes SiiE extracellularly from inside the infected myeloid cells, although presumably this must be the case as we could not detect large quantities of free *Salmonella* in the spleen. The lifestyle of long-term persisting *Salmonella* in short-lived myeloid cells in vivo should be further investigated to better understand the SiiE secretion mechanism.

The SiiE peptide homologous to laminin β1 significantly reduced the number of anti-DNA IgG-secreting plasma cells in the BM in the NZB/W murine model of lupus nephritis. This property could therefore be further exploited for the treatment of autoimmune diseases. Autoimmune diseases with a substantial contribution of pathogenic IgG autoantibodies, like systemic lupus erythematosus, can be refractory to conventional treatment, because BM plasma cells secreting these autoantibodies are protected in their BM niches (23, 36, 37). Multiple myeloma is caused by redundant titers of antibodies generated from plasma cell myeloma in the BM. It has already been reported that myeloma cell lines preferentially contact laminin in vitro (38, 39), suggesting that targeting of adhesion molecules including laminin should be considered as therapy (40). The depletion of BM plasma cell myeloma by SiiE may directly ameliorate disease.

Method Summary

CXCL12/GFP knock-in mice (41) and Blimp^{gfp} mice (42) were kindly provided by Dr. Takashi Nagasawa and Dr. Stephen L. Nutt, respectively. All experiments were approved by the federal state institution "Landesamt für Gesundheit und Soziales" in Berlin, Germany. All *Salmonella* strains used in this study were derivatives of *S. typhimurium* χ3306 and are detailed in *SI Appendix*, Table S1. To count Ig-secreting cells, MultiScreen filter plates were coated with goat anti-mouse Ig, F(ab')$_2$ fragment and cells were incubated for 5 h at 37 °C. Alkaline phosphatase-conjugated anti-IgG, IgM, or IgA was added and spots were counted by ELISpot reader. For cell staining, cells were stained with antibodies and measured by BD FACS Fortessa flow cytometer.

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