

Role of Serum Amyloid A, Granulocyte-Macrophage Colony-Stimulating Factor, and Bone Marrow Granulocyte-Monocyte Precursor Expansion in Segmented Filamentous Bacterium-Mediated Protection from *Entamoeba histolytica*

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Intestinal segmented filamentous bacteria (SFB) protect from ameba infection, and protection is transferable with bone marrow dendritic cells (BMDCs). SFB cause an increase in serum amyloid A (SAA), suggesting that SAA might mediate SFB's effects on BMDCs. Here we further explored the role of bone marrow in SFB-mediated protection. Transient gut colonization with SFB or SAA administration alone transiently increased the H3K27 histone demethylase Jmjd3, persistently increased bone marrow *Csf2ra* expression and granulocyte monocyte precursors (GMPs), and protected from ameba infection. Pharmacologic inhibition of Jmjd3 H3K27 demethylase activity during SAA treatment or blockade of granulocyte-macrophage colony-stimulating factor (GM-CSF) signaling in SFB-colonized mice prevented GMP expansion, decreased gut neutrophils, and blocked protection from ameba infection. These results indicate that alteration of the microbiota and systemic exposure to SAA can influence myelopoiesis and susceptibility to amebiasis via epigenetic mechanisms. Gut microbiota-marrow communication is a previously unrecognized mechanism of innate protection from infection.

Several studies have suggested that intestinal infection with one organism may persistently alter innate immune populations to provide protection from infection with unrelated pathogens (1–3). This idea has been referred to as innate trained immunity (4). However, the mechanism of how unrelated organisms might generate this protective yet nonspecific memory is not currently well described. Some studies have suggested that epigenetic modification of inflammatory genes in innate immune cells might underlie this effect (5, 6). Recent studies have also suggested that the microbiome might have long-term epigenetic effects on the immune system (7). Indeed, microbiota-produced serum soluble mediators and pathogen-associated molecular patterns (PAMPs) can impact myelopoiesis and hematopoiesis (8–10). We hypothesize that host-derived factors such as damage-associated molecular patterns (DAMPs) that are systemically induced by the microbiota may also have a role in altering hematopoiesis and susceptibility to infection.

We have previously demonstrated that alteration of the microbiota via introduction of segmented filamentous bacteria (SFB) (50) can alter bone marrow-derived cells and protect from infection with the protozoan parasite *Entamoeba histolytica* (11). This protection was associated with increased gut neutrophils following amebic infection. This suggested that alteration of the microbiota might have a persistent influence on the bone marrow and myelopoiesis. To address this possibility further, we explored changes in bone marrow hematopoiesis during introduction of SFB to the gut microbiota. Serum amyloid A (SAA), a DAMP, was upregulated during SFB colonization (11, 12) and is known to induce Jmjd3 (13), an epigenetic mediator and H3K27 demethylase linked to inflammation (14). SAA induced by the microbiota has also been shown to be important in neutrophil infiltration in a nonmammalian model (15). Hematopoiesis and myelopoiesis

have been shown to be influenced by granulocyte-macrophage colony-stimulating factor (GM-CSF) signaling (16). Therefore, we also assessed the ability of SAA, Jmjd3, and GM-CSF signaling to alter bone marrow hematopoiesis and susceptibility to subsequent amebic colitis.

Here we demonstrate that addition of SFB to the gut induced persistent expansion of bone marrow granulocyte monocyte precursors (GMPs) and an increase in GM-CSF alpha receptor gene (*Csf2ra*) expression, which persisted after SFB infection was cleared. Blockade of GM-CSF signaling in SFB-colonized mice prevented GMP expansion as well as gut neutrophils and mucosal protection from *E. histolytica*. Treatment of mice with SAA led to similar increases in Jmjd3 and *Csf2ra* and expansion of bone marrow GMPs, as well as protection from *E. histolytica* infection. This protection was associated with increased neutrophils. Inhibition of H3K27 demethylase (Jmjd3) activity during SAA treatment,

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both *in vivo* and *in vitro*, prevented these effects and reversed the phenotype of protection from the ameba. These results suggest that alteration of the microbiota and systemic exposure to SAA can epigenetically alter myelopoiesis and susceptibility to amebiasis via alteration of GM-CSF signaling.

MATERIALS AND METHODS

Mice. Five-week-old male CBA/J mice (Jackson Laboratories), which are susceptible to *E. histolytica* infection (17), were housed in a specific-pathogen-free facility in microisolator cages and provided autoclaved food (lab diet 5010) and water *ad libitum*. Specific-pathogen-free status was monitored quarterly. A sentinel mouse was removed from each room and was humanely euthanized for serologic evaluation, examination of pelage for fur mites, and examination of cecal contents for pinworms. The serologic assays, conducted in-house using CRL reagents, were for mouse hepatitis virus (MHV), epizootic diarrhea of infant mice (EDIM) virus, GD-7, minute virus of mice (MVM), mouse parvovirus (MPV), murine norovirus (MNV), Sendai virus, pneumonia virus of mice (PVM), RPV/Kilham's rat virus (KRV)/H-1, and *Mycoplasma pulmonis*. In the final quarter, a comprehensive serologic examination was performed, which included the above-mentioned agents plus K virus, mouse cytomegalovirus (MCMV), mouse thymic virus (MTV), lymphocytic choriomeningitis (LCM) virus, ectromelia virus, polyomavirus, reovirus 3, and mouse adenoviruses (K87 and FL). All procedures were approved by the Institutional Animal Care and Use Committee of the University of Virginia.

SFB colonization. Mice were colonized with SFB by a single oro-gastric gavage with SFB-monoassociated feces at 5 weeks of age (mono-associated feces was a gift from Yakult, Tokyo, Japan). SFB-monoassociated feces was independently confirmed to be monoassociated with SFB by quantitative PCR (qPCR), Sanger sequencing, and culture in anaerobic and aerobic media for the presence of non-SFB bacteria (11).

GM-CSF blockade. Mice were treated with 400 μ g of anti-GM-CSF antibody (Bioxcell, BE0259) or an isotype control antibody (cMAB; Bioxcell, BE0089) 1 day before and 12 days after SFB colonization and then were challenged with *E. histolytica* trophozoites on day 21.

In vitro and in vivo SAA and Jmjd3 (H3K27 demethylase) inhibitor treatment. Bone marrow cells were isolated as previously described (18). Five hundred thousand mouse bone marrow cells were treated for 16 h with 200 μ l of growth medium (RPMI 1640, 5% fetal bovine serum [FBS], 1% penicillin-streptomycin; Sigma) or medium containing ultrapure lipopolysaccharide (LPS) (10 ng/ml; InvivoGen), Pam3CSK4 (10 ng/ml; InvivoGen), SAA (10 μ g; PeptoTech, 300-13), or SAA with GSK-J5 or GSK-J4 (2.5 mg; Cayman Chemical, 12073 and 12074) and then harvested in RNeasy lysis buffer (Qiagen, Austin, TX). Mice were treated intraperitoneally on days 1, 3, and 5 with 200 μ l of PBS, SAA (15 μ g; PeptoTech, 300-13), or SAA with GSK-J5 or GSK-J4 (100 mg/kg; Cayman Chemical, 12073 and 12074) before *E. histolytica* infection on day 14.

E. histolytica culture and intracecal injection. Animal-passaged HM1:IMSS *E. histolytica* trophozoites were cultured from cecal contents of infected mice in complete trypsin-yeast-iron (TYI-33) medium supplemented with Diamond vitamin mixture (JRH Biosciences), 100 U/ml of both penicillin and streptomycin, and 5% heat-inactivated bovine serum (Sigma-Aldrich). Prior to injection, trophozoites were grown to log phase, and 2×10^6 parasites were suspended in 150 μ l culture medium and injected intracecally (11).

Quantitative real-time reverse transcription-PCR. SFB and *E. histolytica* colonization and mouse gene expression were measured by real-time PCR in stool and cecal lysate. For SFB colonization, qPCR with Sybr green was performed, and data were normalized to expression of a conserved eubacterial 16S RNA gene (EUB) (19). Primer concentrations, annealing temperatures, and cycle numbers were optimized for each primer pair. For each primer pair (Table 1), a dilution curve of a positive cDNA sample was included to enable calculation of the efficiency of the amplification. The relative message levels of each target gene were then normalized to that of the mouse housekeeping gene S14

TABLE 1 Primer sequences

Primer	Sequence
EUB forward	5'-ACTCCTACGGGAGGCAGCAGT-3'
EUB reverse	5'-ATTACCGCGGCTGCTGGC-3'
SFB forward	5'-GACGCTGAGGCATGAGAGCAT-3'
SFB reverse	5'-GACGGCACGGATTGTTATTCA-3'
<i>E. histolytica</i> probe	5'-YYTATTAGTACAAAATGGCCAATTCATTCA-Dark Quencher-3'
<i>E. histolytica</i> forward	5'-AACAGTAATAGTTTCTTTGGTTAGTAAAA-3'
<i>E. histolytica</i> reverse	5'-CTTAGAATGTCATTTCTCAATTCAT-3'
S14 forward	5'-TGGTGTCTGCCACATCTTTGCATC-3'
S14 reverse	5'-AGTCACTCGGCAGATGGTTTCCTT-3'
Jmjd3 forward	5'-CTCTGGAACCTTCATGCCGG-3'
Jmjd3 reverse	5'-CTTAGCCCCATAGTTCCTGTTT-3'
Gm-csf forward	5'-ACCACCTATGCCGATTTCAT-3'
Gm-csf reverse	5'-TCATTACGCAGGCACAAAAG-3'
Csf2ra forward	5'-GCTGGTTCAGGAGGATGATG-3'
Csf2ra reverse	5'-CTTTCGTTGACGAAGCTCAG-3'
Csf2rb forward	5'-CTGTCGCCCAAGACAGA-3'
Csf2rb reverse	5'-ATTGACCCGGGTTCTGTAT-3'

using a method described and utilized previously (11, 20, 21). Data are presented as relative expression. For *E. histolytica* quantification, qPCR with a labeled probe and a standard curve of trophozoites (22) was utilized. Data are presented as trophozoites per microliter of cecal lysate. Primers and probes were purchased from Integrated DNA Technologies, Coralville, IA, USA.

Colony formation assay for determination of bone marrow hematopoietic precursors. Bone marrow cells were isolated (18) and then cultured in methylcellulose-based medium that included 3 units/ml erythropoietin (Epo), 10 ng/ml mouse recombinant interleukin-3 (IL-3), 10 ng/ml human recombinant IL-6, and 50 ng/ml mouse recombinant stem cell factor per the manufacturer's procedures (M3434; StemCell Technologies, Vancouver, British Columbia, Canada). Colony formation of burst-forming unit erythroid cells (BFU-Es), CFU granulocytes/monocytes (CFU-GMs), and CFU granulocytes/erythrocytes/monocytes/macrophages (CFU-GEMMs) was analyzed after 7 days.

Determination of total H3K27me3 in bone marrow. An EpiQuik global histone H3K27 methylation assay kit (Epigentek, P-3020-48) was utilized to quantify total H3K27me3 in bone marrow from mice that had been treated with SAA and Jmjd3 inhibitors as described above.

Flow cytometry of intestinal cells. Minced intestinal tissue was digested in Liberase TL (0.17 mg/ml; Roche) and DNase (0.5 mg/ml; Sigma) for 45 min at 37°C and processed into a single-cell suspension following washing with a buffer containing EDTA. A total of 1×10^6 cells per mouse were stained with antibodies from BioLegend (CD11c-BV421, CD4-BV605, Ly6c-fluorescein isothiocyanate [FITC], CD3e-peridinin chlorophyll protein [PerCP]-Cy 5.5, Siglec F-phycoerythrin [PE], Ly6G-PE-Cy7, CD11b-allophycocyanin [APC], CD8a-AF700, and CD45-APC-Cy7). Flow cytometric analysis was performed on an LSR Fortessa (BD Biosciences) and data analyzed via FlowJo (Tree Star Inc.). All gates were set based on fluorescence-minus-one (FMO) controls.

Statistical analysis. Analysis of variance (ANOVA) followed by the Tukey-Kramer test was used for analysis of differences among multiple groups. Student's *t* test was used for comparisons between two groups. *P* values of less than 0.05 were considered significant. Statistical analysis was performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). All results are representative of at least two independent replicates.

RESULTS

Alteration of bone marrow granulocyte monocyte precursors persists after clearance of introduced commensal bacteria. Prior work has suggested that the presence of segmented filamentous

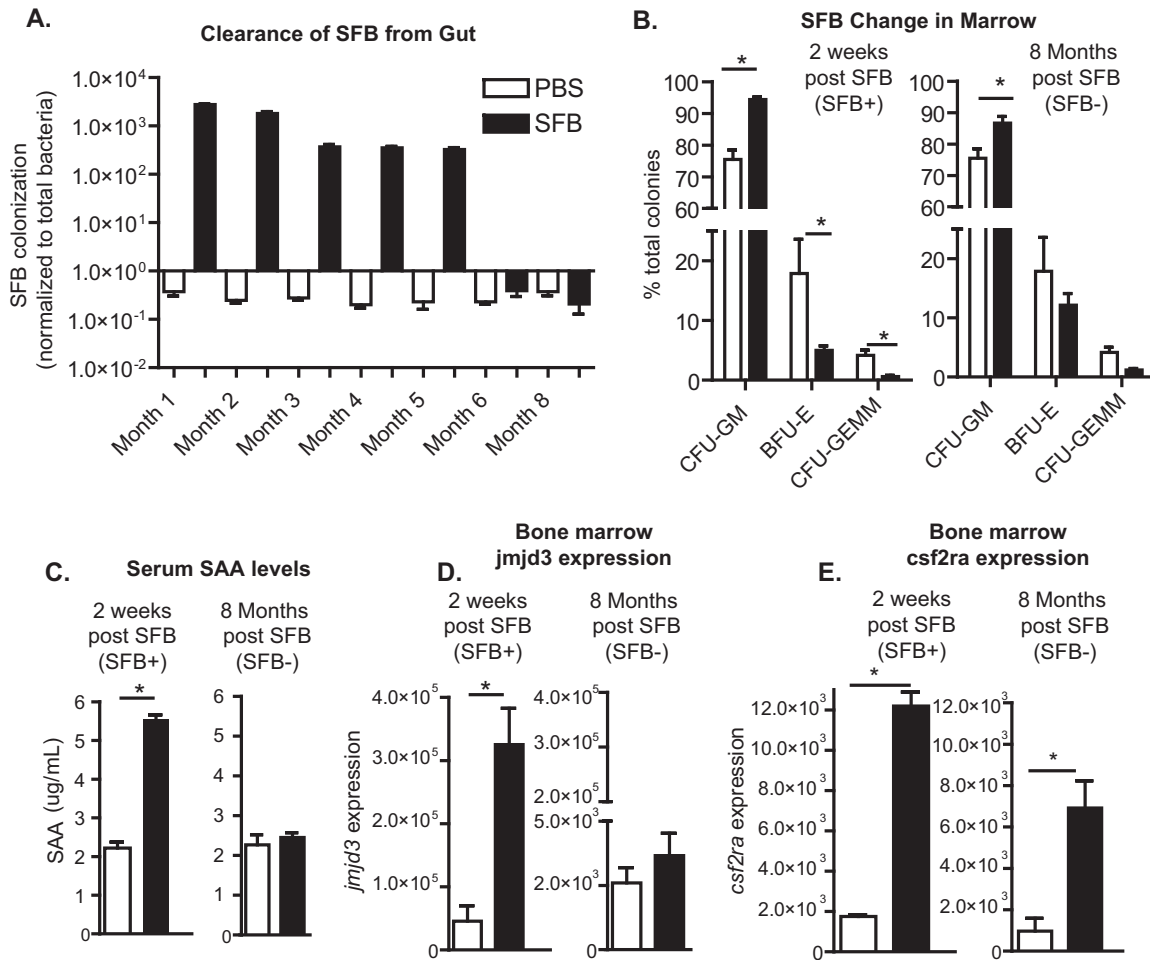


FIG 1 Expansion of bone marrow granulocyte monocyte precursors in mice with an altered microbiota persists after the introduced bacteria are cleared. (A) Male CBA/J mice were colonized with SFB at 5 weeks of age and followed until colonization was cleared. (B) Bone marrow precursors, including granulocyte-monocyte progenitors (CFU-GM), erythroid progenitors (BFU-E), and multipotential granulocyte, erythroid, macrophage, and megakaryocyte progenitors (CFU-GEMM), were measured during acute SFB colonization, 2 weeks after SFB treatment (7 weeks of age), and 2 months after SFB was cleared (8 months of age). (C) Serum levels of SAA were also measured at these time points. (D and E) Bone marrow expression of *Jmjd3* (D) and *Csf2ra* (E) was also determined at these time points. Results are means \pm standard errors of the means (SEM) from a representative experiment of two independent experiments with five mice per group. *, $P < 0.05$.

bacteria (SFB) in the gut can cause bone marrow changes that may mediate protection against subsequent infection with *E. histolytica* (11). In order to determine if alteration of the microbiota via SFB colonization would have a persistent influence on hematopoietic precursors in the bone marrow, we colonized mice with SFB at 5 weeks of age and followed colonization until SFB were no longer shed at 6 months of age (Fig. 1A). We then waited until 2 months after the last positive stool, at 8 months of age, to measure bone marrow precursor cells. Clearance of SFB in the ileum was also confirmed via Gram staining and qPCR upon euthanasia (not shown). Colonization of mice with SFB increased the CFU of GMPs in the bone marrow (Fig. 1B), and this increase in GMPs remained after the SFB were cleared (Fig. 1B). Expansion of GMPs was confirmed via flow cytometry (see Fig. S1A and B in the supplemental material). We concluded that SFB resulted in a persistent increase in GMPs.

Alteration of the gut microbiota systemically increases SAA levels as well as bone marrow *Jmjd3* and *Csf2ra* expression. Systemic DAMP signaling can alter hematopoiesis and may have

long-term effects on myeloid lineages via epigenetic mechanisms (10, 23, 24). We thus hypothesized that a serum soluble mediator that was increased during colonization with SFB might correlate with changes in the bone marrow that could support increased GMP expansion. In confirmation of our previously published data (11) we observed elevated serum amyloid A (SAA) levels in the sera of SFB-colonized mice (Fig. 1C); however, this increase did not persist after colonization was cleared (Fig. 1C). We also observed significantly, but transiently, elevated *Jmjd3* expression in the bone marrow of SFB-colonized mice (Fig. 1D). There was no difference in Toll-like receptor 2 (TLR2), TLR4, M-CSF (*Csf1*), or M-CSF receptor (M-CSFR) (*Csf1r*) expression in the bone marrow at either time point (data not shown), factors which have been shown to have a role in regulating hematopoiesis (9, 25). GM-CSF is also important in hematopoiesis and generation of GMPs (26–28). Therefore, to assess the possible contribution of GM-CSF in this model, expression of GM-CSF and the receptor subunits *Csf2ra* and *Csf2rb* was measured (29). The GM-CSF alpha receptor gene (*Csf2ra*) was upregulated in the bone marrow of SFB-

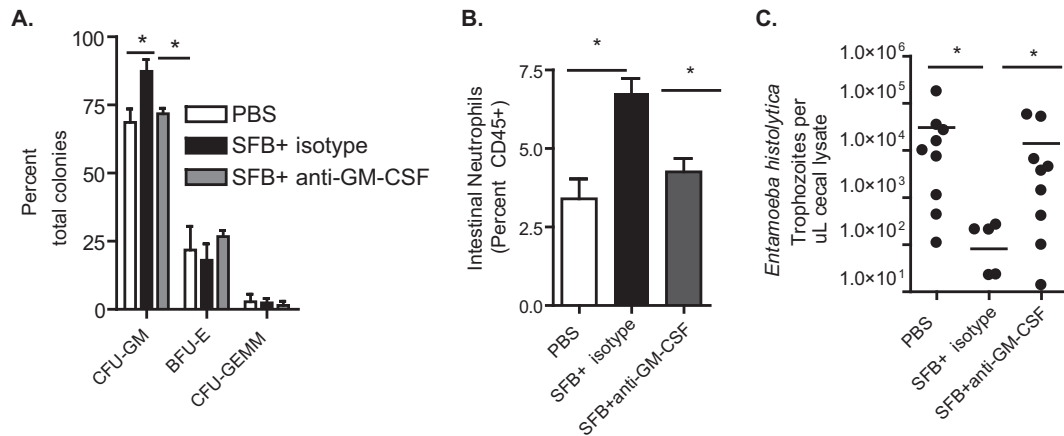


FIG 2 GM-CSF signaling is necessary for microbiota-mediated expansion of GMPs and protection from amebiasis. Mice were treated with anti-GM-CSF antibody or an isotype control antibody 1 day before and 12 days after they were colonized with SFB or treated with PBS and then challenged with *E. histolytica* trophozoites on day 21. (A) A CFU assay was utilized to determine the composition of hematopoietic precursors in the bone marrow of SFB colonized mice. (B and C) Intestinal neutrophils (B) and amebic burden (C) in the cecum were determined after 6 days via flow cytometry and qPCR. Results are Means \pm SEM from a representative experiment of two independent experiments with 12 mice per group. *, $P < 0.05$.

colonized mice, as well as after SFB were cleared (Fig. 1E). We concluded that SFB colonization resulted in transient increases in SAA and bone marrow *Jmjd3* expression and a longer-term increase in *Csf2ra* expression.

Inhibition of GM-CSF signaling blocks both microbiota-mediated alteration of myelopoiesis and protection from ameba infection. Antibody neutralization of GM-CSF in SFB-colonized mice prevented GMP expansion (Fig. 2A), subsequent increases in intestinal neutrophils (Fig. 2B), and mucosal protection from amebic infection (Fig. 2C). We concluded that GM-CSF signaling mediated SFB-induced GMP expansion and downstream protection from *E. histolytica*.

SAA increases bone marrow cell *Csf2ra* expression in a *Jmjd3*-dependent manner. We postulated that exposure of bone marrow cells to increased SAA might increase *Csf2ra* expression in a *Jmjd3*-dependent manner. SAA treatment of bone marrow cells in culture increased *Jmjd3* expression and *Csf2ra* expression (Fig. 3A). Blockade of *Jmjd3* activity with an inhibitor of H3K27 demethylase activity, GSK-J4, but not the inactive control, GSK-J5, (30, 31) reversed the increase in *Csf2ra* expression (Fig. 3B). Treatment of bone marrow cells with TLR2 and TLR4 agonists (pam3csk4 and LPS) did not have a significant effect on *Csf2ra* or *Jmjd3* expression at this time point, suggesting that the effect was not due to PAMPs potentially contaminating the SAA preparation.

Treatment of mice with SAA increases bone marrow *Csf2ra* expression and alters hematopoiesis in an H3K27 demethylase-dependent manner. Because SAA increased *Jmjd3* and *Csf2ra* expression in bone marrow cells, we hypothesized that treatment of mice with SAA might alter hematopoiesis in an H3K27 demethylase-dependent manner. Treatment of mice with recombinant SAA transiently and significantly increased serum levels of SAA and bone marrow expression of the H3K27 demethylase *Jmjd3* (Fig. 4A and B). SAA treatment decreased total bone marrow H3K27me3 and increased *Csf2ra* expression in a *Jmjd3*-dependent manner (Fig. 4C and D, day 14). Treatment of mice with SAA also caused expansion of GMPs, which was reversed with the *Jmjd3* inhibitor (Fig. 4E, day 14; see Fig. S1C in the supplemental

material). We concluded that SAA treatment *in vivo* increased bone marrow *Csf2ra* expression and GMP expansion in an H3K27 demethylase-dependent manner.

SAA provides protection from *E. histolytica* infection in an H3K27 demethylase-dependent manner. We hypothesized that SAA would protect from *E. histolytica* infection as it increased GMP expansion in an H3K27 demethylase-dependent manner. Bone marrow GMPs remained elevated in SAA-treated mice that were not administered a *Jmjd3* inhibitor after ameba infection (Fig. 5A). There was no significant change in intestinal expression of IL-17A (Fig. 5B), IL-23 (Fig. 5C), or IL-22 (Fig. 5D) or a significant difference in CD4⁺ T cells in SAA-treated mice (Fig. 5E). As neutrophils arise from bone marrow GMPs (32), we postulated that they would be increased in the intestine following SAA administration and *E. histolytica* infection. An increase in neutrophil

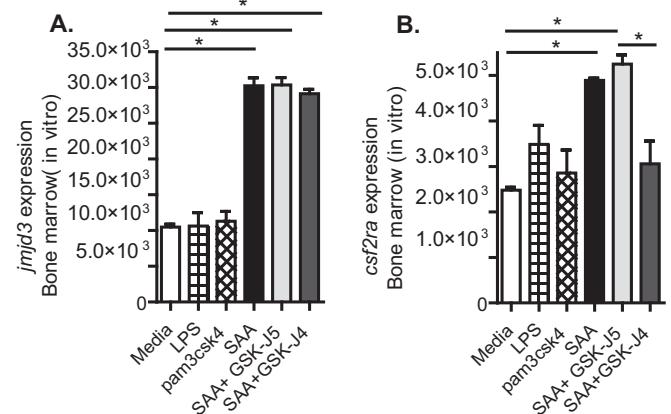


FIG 3 Serum amyloid A (SAA) increases isolated bone marrow cell *Csf2ra* expression in a *Jmjd3*-dependent manner. Isolated total bone marrow cells in growth medium were treated with recombinant SAA with or without an inhibitor of *Jmjd3*, GSK-J4, or its inactive control GSK-J5. Cells were collected at 16 h posttreatment and *Jmjd3* (A) and *Csf2ra* (B) expression determined. Results are means \pm SEM from a representative experiment of three independent experiments with 3 mice per group. *, $P < 0.05$.

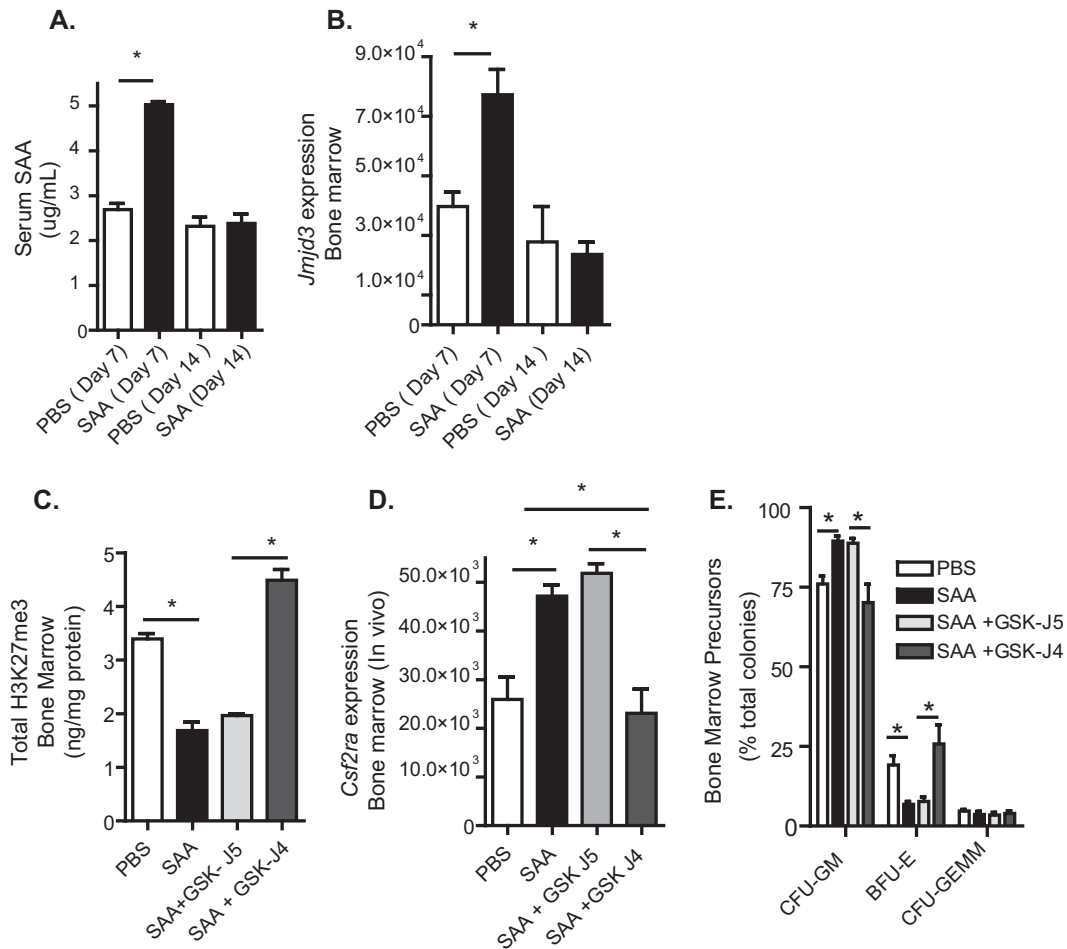


FIG 4 Treatment of mice with serum amyloid A (SAA) increases bone marrow *Csf2ra* expression and granulocyte-monocyte precursors in an H3K27 demethylase-dependent manner. Mice were injected intraperitoneally with recombinant SAA (days 1, 3, and 5) with or without an inhibitor of *Jmjd3*, GSK-J4, or its inactive control GSK-J5. (A and B) Serum and bone marrow were collected at days 7 and 14, and SAA and *Jmjd3* expression were measured. (C to E) Bone marrow was collected at day 14, and total H3K27me3 (C), *Csf2ra* (D), and bone marrow precursors (E) were determined. Results are means \pm SEM from a representative experiment of three independent experiments with 3 (A and B) or 5 (C to E) mice per group. *, $P < 0.05$.

frequency (Fig. 5F) and number (data not shown) in the cecal lamina propria in SAA-treated mice was observed during, but not prior to, challenge with *E. histolytica*. There was no increase in eosinophils or inflammatory monocytes, which also arise from GMPs (32), at either time point (not shown). SAA administration prior to infection provided protection from *E. histolytica* infection, and this protection, along with increased gut neutrophils and bone marrow GMPs, was dependent on H3K27 demethylase activity (Fig. 5A, F, and G, day 21). We concluded that SAA provided protection from *E. histolytica* in an H3K27 demethylase-dependent manner.

DISCUSSION

The key finding of this study is that addition of SFB to the gut microbiota epigenetically induced an increase in GM-CSF alpha receptor expression and expansion of bone marrow GMPs, which persisted after SFB infection was cleared. Blockade of GM-CSF signaling in SFB-colonized mice prevented GMP expansion as well as gut neutrophils and mucosal protection from *E. histolytica*. Therefore, microbiota-marrow signaling via GM-CSF resulted in

GMP expansion, gut neutrophil infiltration, and protection from enteric infection.

In young mice after weaning, SFB colonization is known to follow distinct kinetics of colonization, with intestinal levels of the bacteria increasing dramatically shortly after birth and then decreasing significantly as the mice age (33). In adult mice, we also observed a short-term period of high levels of SFB colonization, followed by a longer period of lower-level colonization, followed by a cessation of shedding. As GMPs remained elevated even after clearance of SFB, we hypothesized that transient intestinal SFB colonization may have induced epigenetic changes that influenced bone marrow hematopoiesis. Consistent with this hypothesis, we observed increased expression of *Jmjd3*, encoding an H3K27 demethylase, in the bone marrow of SFB-colonized mice during acute colonization. Our studies have not yet identified the precise mechanism by which SFB colonization increases bone marrow GMPs, which may be multifactorial in nature. Previous studies have linked changes in M-CSF to alteration of hematopoiesis and protection from infection (34). We did not observe changes in M-CSF cytokine or receptor (*Csf1r*) expression in the bone mar-

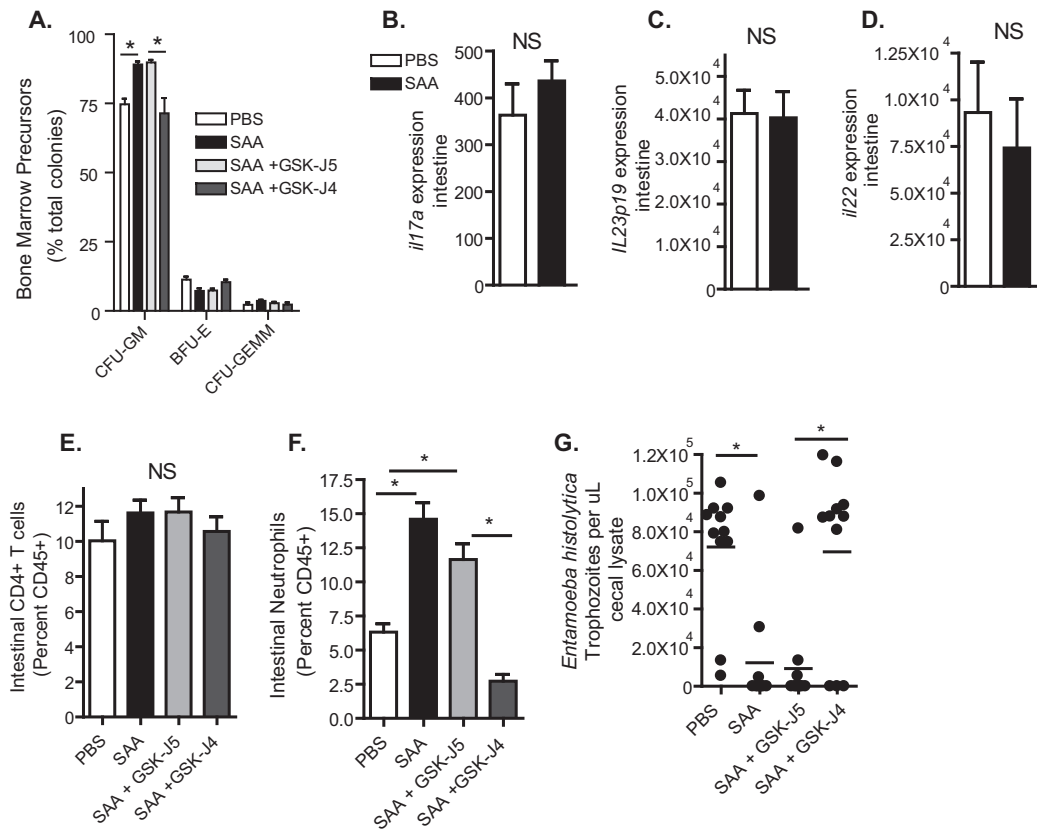


FIG 5 Serum amyloid A (SAA) provides protection from *E. histolytica* infection in an H3K27 demethylase-dependent manner. Mice were injected intraperitoneally with recombinant SAA (days 1, 3, 5) with or without an inhibitor of Jmjd3, GSK-J4, or its inactive control GSK-J5. Mice were then infected with *E. histolytica* 9 days after the final injection of SAA (day 14) and sacrificed 7 days later (day 21). (A) Bone marrow was collected at day 21, and bone marrow precursors were determined. (B to G) Intestinal expression of *il17a* (B) *IL23p19* (C), and *IL22* (D), CD4⁺ T cells (E), neutrophils (F), and infection burden (G) were measured (day 21). Results are means \pm SEM from a representative experiment of three independent experiments with 5 (A) or 12 (B to G) mice per group. *, $P < 0.05$.

row with SFB treatment. This was not entirely unexpected, however, as it has been shown that Jmjd3 has little effect on M-CSF-mediated inflammatory monocyte generation (35). However, GM-CSF signaling is also known to be important in hematopoiesis and generation of GMPs, and the magnitude and duration of signaling influence outcomes, with weak signaling favoring cell survival and stronger signaling promoting both survival and proliferation (26–28). The GM-CSF alpha receptor gene (*Csf2ra*) was upregulated in the bone marrow of acutely SFB colonized mice, as well as after SFB was cleared. Blockade of GM-CSF during SFB colonization prevented GMP expansion, increased intestinal neutrophils, and protection from ameba infection. These data demonstrated that GM-CSF signaling during SFB colonization is important in protection from amebiasis.

In addition to GM-CSF signaling, we explored the potential role of SAA in SFB-induced GMP expansion. Our laboratory has previously observed that SFB colonization can increase serum levels of SAA (11), but this had not been explored beyond acute colonization with the bacteria. During acute colonization with SFB, serum levels of the DAMP SAA were significantly increased, as was bone marrow expression of *Jmjd3*, encoding an H3K27 demethylase (36). However, SAA and Jmjd3 did not remain elevated after SFB was cleared. From these data, we surmised that transiently increased serum SAA levels might have influenced

bone marrow GMP expansion in an epigenetic manner. Treatment of mice with SAA was able to momentarily increase serum levels of SAA to a magnitude comparable to that observed during SFB colonization. SAA administration, both *in vitro* and *in vivo*, increased expression of the GM-CSF alpha receptor gene (*Csf2ra*) in bone marrow in a manner similar to that observed during SFB colonization. SAA treatment led to a significant decrease in bone marrow H3K27me3 levels and expansion of GMPs. Blockade of Jmjd3 activity in SAA-treated mice prevented changes in H3K27me3 levels, *Csf2ra* expression, and GMP expansion. Hematopoiesis, including expansion of GMPs and their differentiation into myeloid subsets, is known to be associated with global changes in histone methylation (24). Our results demonstrated that SAA treatment specifically could increase bone marrow GMPs in an H3K27 demethylase-dependent manner. SAA treatment therefore skewed bone marrow development toward the generation of myeloid-derived cells, which might at least partially explain how SFB in the gut resulted in expansion of GMPs. Inflammatory stimuli induced by *E. histolytica* infection alone may also induce epigenetic changes in the bone marrow, and this possibility will be examined in future studies.

SAA administration prior to ameba infection provided protection from amebiasis. Protection was associated with increased intestinal neutrophils following ameba infection. There was no in-

crease in eosinophils or inflammatory monocytes, which can also arise from GMPs (32), either before or after ameba infection. This protection, along with intestinal neutrophil infiltration during infection, was dependent on H3K27 demethylase activity. The lack of change in inflammatory monocytes was somewhat expected, as *Jmjd3* has been shown to be dispensable for inflammatory monocyte generation (35). It is not yet clear why eosinophils were unaffected. However, as they have a unique precursor population downstream of GMPs, they might behave differently than neutrophils (37). We previously have demonstrated that neutrophil infiltration is protective during *E. histolytica* infection in murine models (38) and that increased neutrophil infiltration correlated with protection during SFB colonization (11). The observed increase in intestinal neutrophils after SAA treatment and ameba infection is consistent with these studies. This increase in neutrophils during ameba infection and following SAA treatment is likely a consequence of the expanded bone marrow GMPs. Blockade of H3K27 demethylase activity prevented both SAA-mediated GMP expansion and increases in intestinal neutrophils. This suggests that systemic exposure to SAA can influence myelopoiesis and susceptibility to amebiasis, potentially via epigenetic mechanisms. However, the specific cause of increased intestinal neutrophils during SAA treatment is not yet known.

Our previous work suggested that IL-17A induction after adoptive transfer of bone marrow dendritic cells (BMDCs) from SFB-colonized mice may be important in induction of neutrophils and protection from amebiasis (11). It has also been demonstrated that SAA administration can induce a Th17 response and increased neutrophilia in the lung (39). It has further been recognized that SAA-mediated Th17 induction during SFB colonization is most robust in the small intestine and that Th17 responses are SFB specific (40–43). IL-22, associated with Th17 induction, has also recently been shown to provide colonization resistance against intra-abdominal bacterial infection and to induce SAA (44). In this study, we did not observe a significant increase in IL-17A, IL-23, IL-22, or CD4⁺ T cells with SAA treatment. This suggests that SAA administration does not completely recapitulate the effect on the intestinal mucosal that was observed during SFB administration. Alteration of the microbiota during SFB administration may also contribute to protection, and this possibility will be examined in future studies. Recent studies have also shown that neutrophilia can occur independently of IL-17A induction in a fungal infection model (45). Therefore, while IL-17A may be important in neutrophil infiltration to mucosal sites during infection with *E. histolytica* (46), it is not necessarily the sole factor governing it.

In conclusion, these data reveal key details of host-pathogen interaction. Alteration of the microbiota via introduction of SFB can have a persistent effect on hematopoiesis and GM-CSF signaling. GM-CSF signaling is also needed for microbiota-mediated expansion of myeloid precursor cells and protection from ameba infection. These data further demonstrate that a host DAMP (SAA) can, independently of SFB colonization, have epigenetic mediator (*Jmjd3*)-dependent and GM-CSF-associated effects on myelopoiesis and thereby provide protection from amebiasis. To our knowledge, this is the first demonstration that a systemic, yet transient, increase in a host-derived DAMP can alter bone marrow hematopoiesis, GM-CSF signaling, and susceptibility to parasitic infection. SAA and GM-CSF may therefore provide useful targets to explore in the treatment of intestinal parasitic infection. Future

studies will determine if commensal clostridia found in populations exposed to *E. histolytica* can alter the progression of amebiasis and will explore which time points are key for protection. The ameba burden may significantly influence the long-term sequelae of amebic colitis and the progression from asymptomatic (47) to fulminant colitis in patients. Increased *E. histolytica* burden has also been shown to be associated with diarrhea in children (48). Therefore, a decreased ameba burden caused by a component of the microbiota may have a significant influence on amebic colitis in patients.

These observations may have broader implications for the field of immunology and infectious disease. SAA is upregulated in the serum during many infectious and inflammatory diseases (49). We suspect that this effect is generalizable and that increased serum SAA via many mechanisms might lead to more robust mucosal immune responses to subsequent infectious or inflammatory challenges. Therefore, mechanisms observed in the amebiasis model might help to better explain the newly emerging field of innate trained immunity (4), as well as the poorly understood, but widespread, observation of postinfectious chronic gut dysfunction (50).

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