Title: Gut microbiome communication with bone marrow regulates susceptibility to amebiasis.

Authors: Stacey L. Burgess*[1], Jhansi L. Leslie[1], Md. Jashim Uddin[1], Noah Oakland[1], Koji Watanabe[1, 2], Carol Gilchrist[1] , Mahmoud Saleh[1], Morgan Simpson[1], , Stephen D. Turner[3,4], Natasa Giallourou[5], Jonathan Swann[5] , Rashidul Haque[6] ,William A. Petri, Jr.*[1]

Affiliations:
1. Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia, Charlottesville, Virginia, USA

2. AIDS Clinical Center, National Center for Global Health and Medicine, Shinjuku, Tokyo, Japan

3. Department of Public Health Sciences, University of Virginia School of Medicine, Charlottesville, Virginia, USA

4. Bioinformatics Core, University of Virginia School of Medicine, Charlottesville, Virginia, USA

5. Division of Integrative Systems Medicine and Digestive Diseases, Imperial College London, United Kingdom

6. International Centre for Diarrhoeal Diseases Research, Dhaka, Bangladesh

*Correspondence to:

William A. Petri, Jr.

University of Virginia Division of Infectious Diseases and International Health

PO Box 801340

Charlottesville VA 22908-1340

434/924-5621

wap3g@virginia.edu

Abstract:

The gut microbiome may provide resistance to infection. However, the mechanisms for this are poorly understood. We found that the intestinal bacterium *Clostridium scindens* provided immunity to the parasite *Entamoeba histolytica*. Adoptive transfer of bone-marrow from *C. scindens* colonized-mice into naïve-mice protected against amebic colitis and increased intestinal neutrophils. Introduction of *C. scindens* caused expansion of marrow granulocyte-monocyte-progenitors (GMPs) and increased expression of the epigenetic mediator JMJD3 in GMPs. Deoxycholate was increased in the sera of *C. scindens* colonized mice, as well as in children protected from amebiasis. Administration of deoxycholate alone (in the absence of *C. scindens*) increased JMJD3 and GMPs and provided protection from amebiasis. This work demonstrates microbiome to bone marrow communication resulting in innate immune memory and antigen-nonspecific protection from amebiasis.

Main Text

Entamoeba histolytica is one of the leading causes of mortality due to diarrhea worldwide , with 55,000 annual deaths (*1, 2*). There is only a single class of drugs effective for invasive amebiasis, the nitroimidazoles, which are toxic and for which resistance has developed in other anaerobic protists. Symptomatic infection in children is associated with impaired growth and development as well as an increased risk of mortality (*3–6*). The clinical outcome of an *E. histolytica* exposure varies enormously and can present as diarrhea, dysentery, or amebic liver abscess (*7*). Host and parasite factors likely contribute to the outcome of infection, but do not explain the wide variation in the presentation of disease. This suggests that other environmental factors affect disease.

An emerging body of work suggests that commensal intestinal bacteria of the host may have a significant influence on the development and outcome of amebiasis and other parasitic and infectious diseases (*8, 9*). The intestinal microbiota has recently been shown to modulate bone marrow processes that give rise to immune effector cells needed for parasite clearance, such as neutrophils and inflammatory macrophages (*10, 11*). Several studies have also suggested that intestinal infection with one organism, or vaccination, may persistently alter innate immune populations to provide protection from infection with unrelated pathogens in a process coined "*trained immunity*" (*12*). However, the mechanism of how unrelated organisms might generate innate protection is not well understood.

A number of studies have suggested that microbiota-associated metabolites, such as secondary bile acids, may augment epithelial mucosal immunity to pathogens (*13*). Epigenetic changes in genes important in innate immunity have also been implicated as a mechanism for trained immunity (*14–17*). These include changes in H3K27 and H3K4 methylation of histones

associated with promotor regions of innate inflammatory genes (*16*). As such, alteration of H3K27 demethylase expression, such as JMJD3, may significantly contribute to the development of innate trained immunity (*16*, *18*, *19*). Host damage-associated molecular pattern molecules (DAMPs), that can be systemically induced by the microbiota have also been shown to be important in upregulating JMJD3 expression in both myeloid cell lines and mouse bone marrow (*20*, *21*). Collectively, this suggests that host serum soluble mediators induced by the microbiota may communicate with the bone marrow, beyond the intestinal mucosa, with the potential to influence immunity to pathogens.

We have previously shown that colonization with the *Clostridia*-related murine bacteria Segmented Filamentous Bacteria (SFB) protects from *Entamoeba histolytica* infection. Transfer of marrow derived dendritic cells from SFB-colonized mice into SFB-free recipients provided protection against *E. histolytica* (*22*). We also demonstrated that SFB persistently expanded bone marrow granulocyte monocyte progenitors (GMPs) (*21*), which produce neutrophils. This work suggests that gut colonizing *Clostridia* species might alter bone marrow hematopoiesis to confer protection against an unrelated pathogen (*12*, *23*). To explore this possibility we introduced the human commensal bacteria *Clostridium scindens* into the gut microbiome of mice and challenged them with the parasite *E. histolytica*.

Introduction of *C. scindens* to the gut microbiome provided protection from *E. histolytica* (**Figure 1 A, B, C, Figure S1)** and this protection was associated with increased intestinal neutrophil infiltration and increased bone marrow neutrophil progenitors **(Figure 1 D, I, J, K)**. Gavage with *C. scindens* slightly altered bacterial community structure **(Figure S1, A**) as measured by 16S rRNA gene V4 region sequencing. Using an algorithm for high-dimensional biomarker discovery, an OTU classified as Clostridium XIVa, to which *C. scindens* belongs, was

significantly increase in the mice administered *C. scindens* (**Figure S1, B)**. *C. scindens* was also significantly increased as measured by relative expression of baiCD oxidoreductase normalized to total bacteria (**Figure S1, C).** There was no significant difference in intestinal CD4+ and CD8+ T cells, eosinophils or inflammatory monocytes **(Figure 1 E-H)**. Previous studies have suggested that myeloid cell expansion is influenced by cytokine production by CD8+ T cells (*24*). Other studies have demonstrated that the spore-forming component of the murine indigenous intestinal microbiota, particularly *Clostridium* species, promoted intestinal T regulatory cell accumulation, as well as diminished severity of a non-infectious model of colitis (*25*). However RAG-1$^{-/-}$ mice that lack T and B cells were also protected from *E. histolytica* when colonized with *C. scindens* (**Figure 1 C).** Expansion of GMPs mediated by *C. scindens* also occurred in the absence of T cells **(Figure 1 K).** This suggested that innate immune cells primarily underlie the observed microbiota mediated changes in hematopoiesis and protection from ameba.

C. scindens is a gut bacterium capable of 7α-dehydroxylation of bile acids in the intestine (*11*, *26*). Secondary bile acids are known to increase antimicrobial peptide production from epithelium (*13*). Therefore, we explored the contribution of radio-resistant stromal and epithelial cells and radio-sensitive hematopoietic derived cells in *C. scindens* mediated protection from the gut parasite *E. histolytica*. We demonstrated that adoptive transfer of whole bone marrow from *C. scindens* colonized mice into mice not previously exposed to *C. scindens* was sufficient to provide protection from *E. histolytica* as well as recapitulate the observed increase in marrow GMPs and intestinal neutrophils **(Figure 1 L-N)**. This suggests that alterations in marrow hematopoietic cells caused by gut exposure to C. *scindens* are sufficient to confer a more robust intestinal neutrophil response to later *E. histolytica* challenge.

In exploring potential mechanisms of gut to marrow communication by *C. scindens* and protection from *E. histolytica*, we examined the serum bile acid profile of mice colonized with the bacteria. Serum bile acids are not presently known to influence bone marrow GMP expansion and have not been examined during *C. scindens* colonization. However, recent work suggests that bile acid signaling can increase differentiation of osteoblasts (*27*, *28*), myeloid derived cells that contribute to bone marrow remodeling. As we expected, colonization of mice with *C. scindens* increased serum levels of the secondary bile acid deoxycholic acid (**Figure S2**). Deoxycholate was also increased in children protected from *E. histolytica* **(Figure 2 A)**. Administration of deoxycholate prior to ameba infection increased serum levels of deoxycholate to levels comparable to *C. scindens* colonization **(Figure 2 B, C, D, Week 1)** and provided protection from infection in the animal model **(Figure 2 E, Week 3)**. This work is the first to demonstrate that deoxycholate levels can be increased systemically by introduction of *C. scindens* to the microbiome **(Figure 2 B, S2),** and to demonstrate that serum deoxycholate correlates with protection from an intestinal parasite in two independent childhood cohorts (**Figure 2 A**).

Deoxycholate-mediated protection from *E. histolytica* was associated with the same increased marrow GMPs as well as increased intestinal neutrophils seen with *C. scindens* **(Figure 2 F, G)**. We were interested in the mechanism by which deoxycholate or *C. scindens* increased GMPs. Due to the persistent nature of immunity to *E. histolytica* following bone marrow transplant we examined the epigenetic mediator JMJD3 (*21*, *29*) . *C. scindens* colonization or deoxycholate administration increased expression of JMJD3 specifically in sorted GMPs (**Figure 3 A, B, C**). Treatment with an inhibitor of JMJD3 during *C. scindens*

colonization abrogated bone marrow GMP expansion (**Figure 3 D**) as well as induction of intestinal neutrophils and protection from *E. histolytica* **(Figure 3 E, F)**. RNA sequencing and QPCR of sorted marrow GMPs demonstrated that genes associated with histone H3, including the demethylase JMJD3 are enriched in mice exposed to *C. scindens* **(Figure S3 A, B, C,)**. Additionally, gene centers associated with CCAAT/enhancer-binding proteins, known to be important for GMP differentiation and expansion (*30*), were enriched (**Figure S3 A, B, D**). Additionally, in vitro treatment of marrow cells with DCA increased JMJD3 expression (**Figure S3, E**).

The results presented here suggest a model whereby gut colonization with *C. scindens* increases serum deoxycholate that then acts on the marrow to increase the epigenetic mediator JMJD3. Epigenetic changes by JMJD3 increase transcription of genes that support granulocyte monocyte progenitor (GMP) expansion, such as CCAAT/enhancer-binding proteins CEBPA and CEBPB **(Figure S3, A, B, D)**. Then, when a novel challenge occurs at a mucosal site, a more robust neutrophil response results **(Figure 4).**

Future studies will examine the precise mechanisms by which *C. scindens* colonization alters bone marrow hematopoiesis, which are not fully elucidated by these studies. However his work helps yield a mechanistic understanding of how changes in the gut microbiome can result in antigen nonspecific protection from *Entamoeba histolytica* infection. The impact of the work extends beyond infectious disease to fundamental mechanisms of gut to bone marrow communication by commensal bacteria and innate trained immunity. These studies may help in development of novel treatments that modulate the severity of immune and inflammatory diseases by altering bone marrow production of inflammatory cells.

Acknowledgments

We thank Tuhinur Arju, and Mamun Kabir at icddr, b, Jeremey Gatesman at the University of Virginia Center for Comparative Medicine, AhnThu Nguyen at the Biology Department Genomics Core, and Katia Sol-Church and Alyson Prorock at the Genome Analysis and Technology Core for technical support. **Funding**. The work was supported by National Institutes of Health National Institute of Allergy and Infectious Diseases Grants R01AI-26649 (WAP) and 1R21AI130700 (SLB). **Author's contributions.** SLB, JLL, JU, NO, KW, MS, MS, and NG performed the experiments. SLB, JLL, ST, DO and NG analyzed the data. WAP, SLB, JS,RH supervised the experiments and data analysis. SLB and WAP. developed the theoretical framework. All authors discussed the results and contributed to the preparation of the manuscript. **Competing interests.** The authors declare no competing financial interests.

Data and materials availability. Data is available in the manuscript and supplemental figures S1-S3, full sequencing data is deposited to the GEO repository under the accession number GSE121503, the SRA, under PRJNA503904, all data is available upon request.

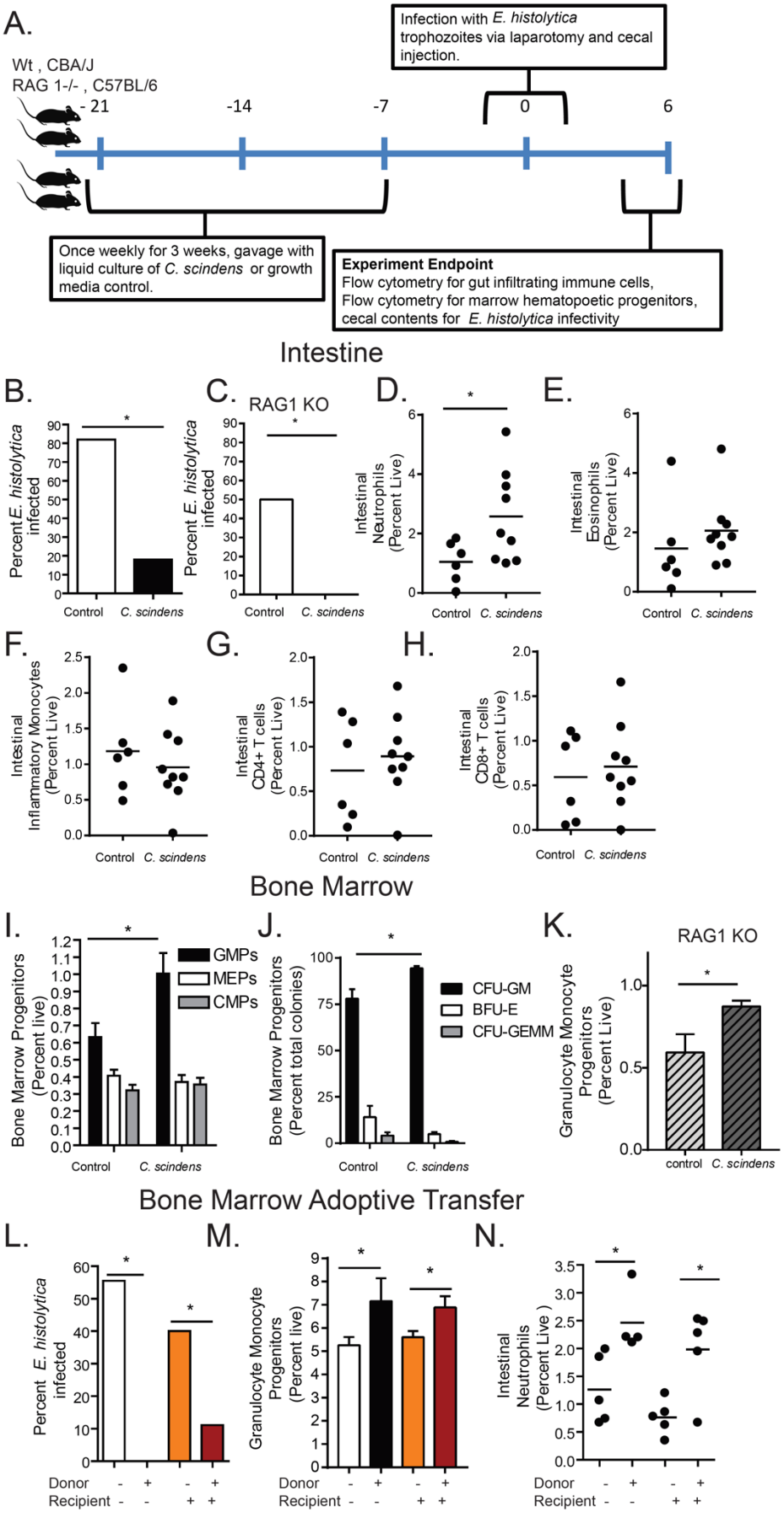

A.
Wt , CBA/J
RAG 1-/- , C57BL/6
- 21
-14
-7
0
6
Infection with E. histolytica trophozoites via laparotomy and cecal injection.
Once weekly for 3 weeks, gavage with liquid culture of C. scindens or growth media control.
Experiment Endpoint
Flow cytometry for gut infiltrating immune cells,
Flow cytometry for marrow hematopoetic progenitors,
cecal contents for E. histolytica infectivity
Intestine
B.
Percent E. histolytica infected
Control
C. scindens
C.
RAG1 KO
Percent E. histolytica infected
D.
Intestinal Neutrophils (Percent Live)
E.
Intestinal Eosinophils (Percent Live)
F.
Intestinal Inflammatory Monocytes (Percent Live)
G.
Intestinal CD4+ T cells (Percent Live)
H.
Intestinal CD8+ T cells (Percent Live)
Bone Marrow
I.
Bone Marrow Progenitors (Percent live)
GMPs
MEPs
CMPs
J.
Bone Marrow Progenitors (Percent total colonies)
CFU-GM
BFU-E
CFU-GEMM
K.
RAG1 KO
Granulocyte Monocyte Progenitors (Percent Live)
control
Bone Marrow Adoptive Transfer
L.
Percent E. histolytica infected
Donor
Recipient
M.
Granulocyte Monocyte Progenitors (Percent live)
N.
Intestinal Neutrophils (Percent Live)

Figure 1. Introduction of *Clostridium scindens* to the gut microbiota protects from infection with *E. histolytica* and adoptive transfer of bone marrow from *C. scindens* colonized donors is sufficient to provide protection to *C. scindens* naïve mice. CBA/J mice or C57BL/6 RAG1 KO mice were colonized with bile acid 7α-dehydroxylating bacteria *C. scindens* (ATCC® 35704) over three weeks prior to intracecal infection with *E. histolytica* **(A)**. Percent infectivity with ameba at day six following infection was determined via cecal culture in trophozoite supporting media **(B, C)**. Gut immune cell infiltration was determined via flow cytometry **(D-H)**, Composition of marrow hematopoietic precursors was determined via flow cytometry **(I, K)** and colony forming assays **(J)** in *C. scindens* colonized CBA/J or RAG1 KO mice. CBA/J mice colonized with *C. scindens* (+) or not (-) as above were lethally irradiated, then immediately given whole marrow from *C. scindens* (+) or *C. scindens* (-) donors then allowed to recover for 7 weeks prior to ameba challenge. Percent infectivity with ameba **(L),** change in marrow GMPs **(M)**, and gut neutrophil infiltration **(N)** was determined at 8 weeks post BMT. *= $p<0.05$, Student's t-test, Mann–Whitney U test, One Way ANOVA with Tukey posttest, bars and error bars are mean and SEM.

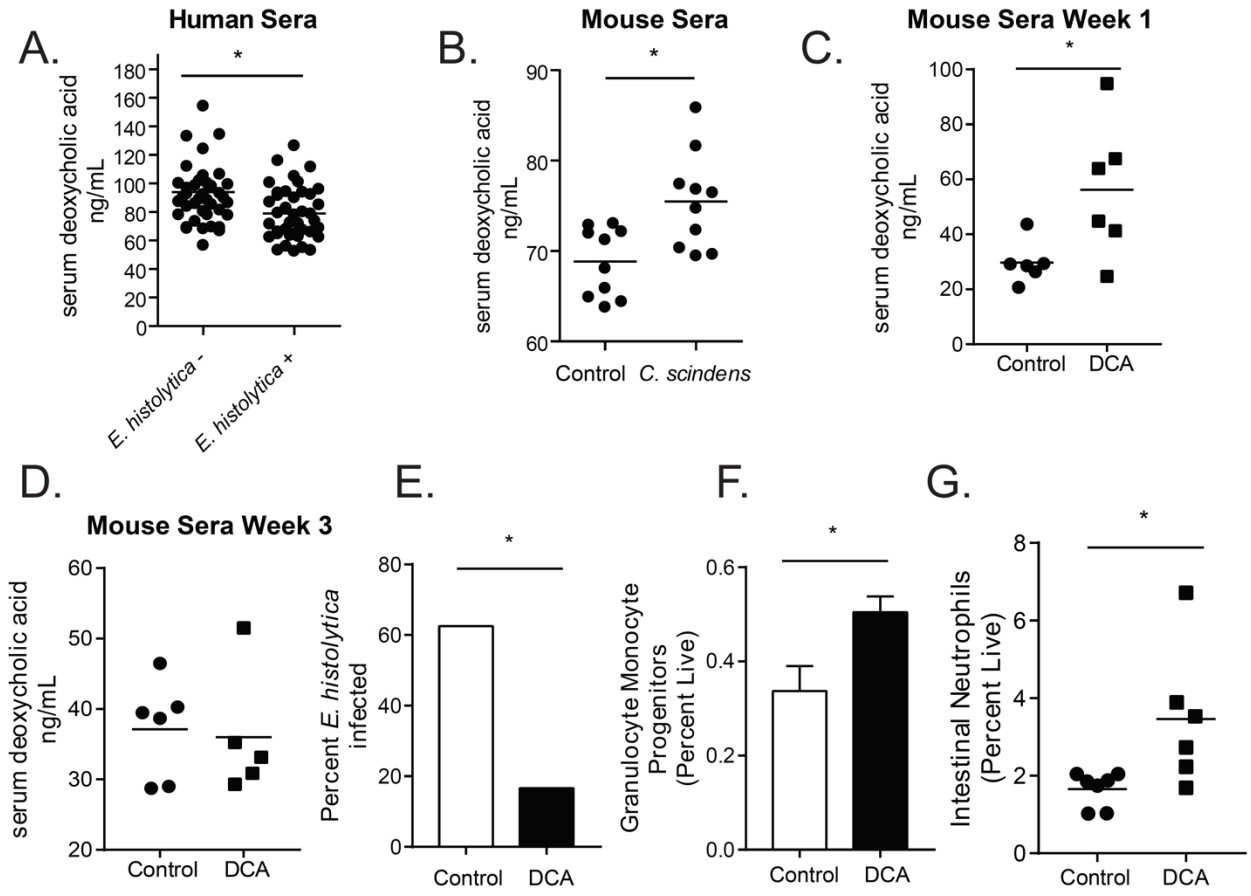


Figure 2. Increased serum deoxycholic acid (DCA) provides protection from ameba infection and increases bone marrow granulocyte monocyte progenitors. Serum DCA was measured via ELISA in 2 year old children in Bangladesh that were free of (-) or infected with (+) *E. histolytica* within 6 months of the blood draw **(A).** CBA/J mice were colonized with *C. scindens* and serum DCA was measured **(B)**. Mice were treated I.V. with DCA three times a week for two weeks and then challenged with *E. histolytica*. Serum DCA was measured during week 1 **(C)**, and at the end of the experiment **(D).** Percent infectivity **(E)**, change in marrow GMPs **(F)** and gut neutrophils **(G)** were measured at the end of the experiment**.***= $p<0.05$, Student's t-test, Mann–Whitney U test.

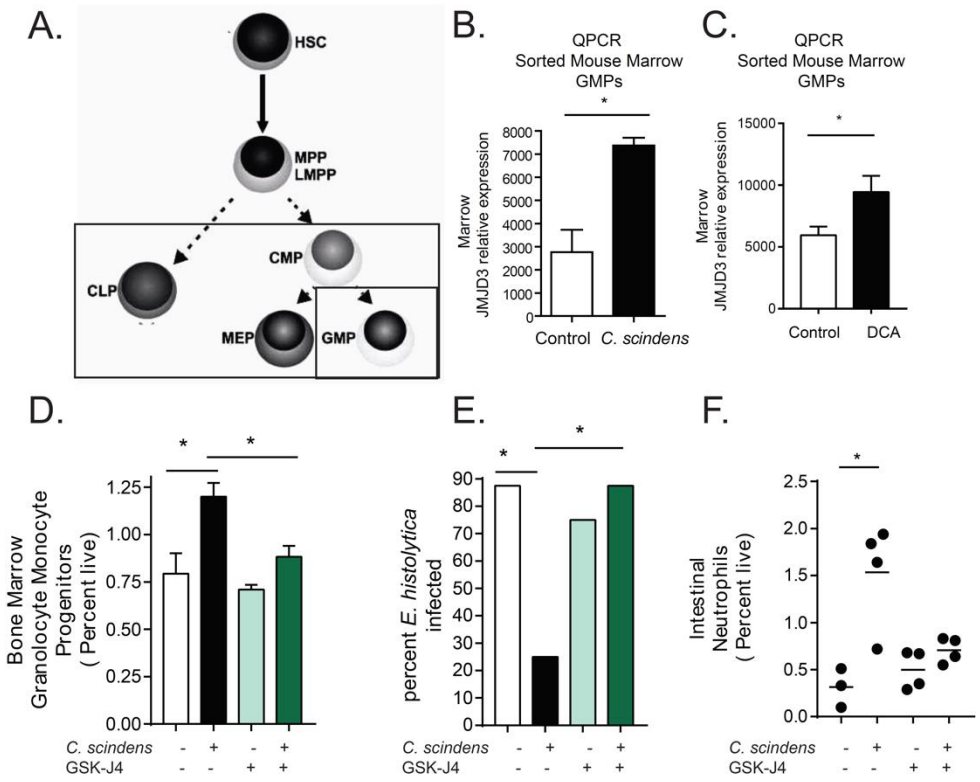


Figure 3. *C. scindens* or treatment with deoxycholate (DCA) increases expression of H3K27 demethylase JMJD3 specifically in sorted bone marrow granulocyte monocyte progenitors and blockade of JMJD3 abrogates protection from *E. histolytica.* CBA/J mice were colonized with *C. scindens* or treated with DCA and bone marrow was sorted into: Common Myeloid Progenitors (CMP); Granulocyte Macrophage Progenitors (GMPs); Megakaryocyte–erythroid Progenitors (MEP); and Common Lymphoid Progenitors (CLP). **(A)** and qPCR **(B, C)** was performed for JMJD3. CBA/J mice were treated with an inhibitor of the epigenetic mediator JMJD3 (GSK-J4) (-, +) before and during *C. scindens* colonization (-, +) but prior to infection with ameba. Bone marrow progenitor populations (**D**), percent infectivity **(E)** and intestinal neutrophils **(F)** were analyzed via flow cytometry and culture. *= $p<0.05$, Student's t-test, Mann–Whitney U test, One Way ANOVA with Tukey posttest.

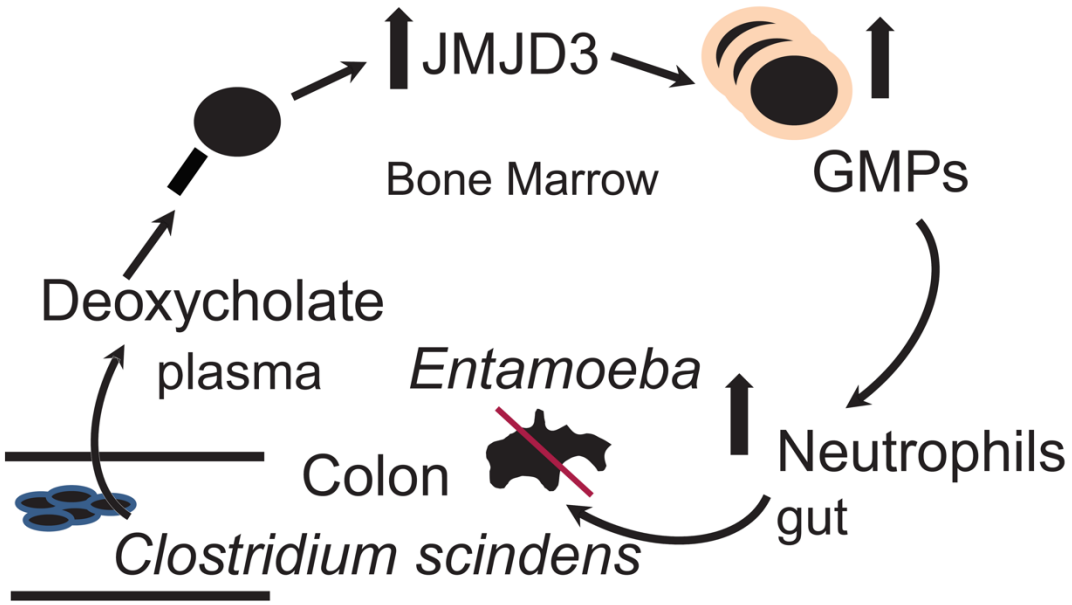


Figure 4. Potential model of *C. scindens* mediated alteration of bone marrow. Intestinal colonization with a bile acid 7α-dehydroxylating bacterium, *Clostridium scindens* increases serum deoxycholate that signals via marrow and the histone demethylase JMJD3 to increase granulocyte monocyte precursors (GMPs). GMPs in turn protect the gut from subsequent infection via more robust neutrophil granulopoiesis.

Supplementary Materials for

Title: Gut microbiome communication with bone marrow regulates susceptibility to amebiasis.

Authors: Stacey L. Burgess*[1], Jhansi L. Leslie[1], Md. Jashim Uddin[1], Noah Oakland[1], Koji Watanabe[1, 2], Carol Gilchrist[1] , Mahmoud Saleh[1], Morgan Simpson[1], , Stephen D. Turner[3,4], Natasa Giallourou[5], Jonathan Swann[5] , Rashidul Haque[6] ,William A. Petri, Jr.*[1]

Affiliations:
1. Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia, Charlottesville, Virginia, USA
2. AIDS Clinical Center, National Center for Global Health and Medicine, Shinjuku, Tokyo, Japan
3. Department of Public Health Sciences, University of Virginia School of Medicine, Charlottesville, Virginia, USA
4. Bioinformatics Core, University of Virginia School of Medicine, Charlottesville, Virginia, USA
5. Division of Integrative Systems Medicine and Digestive Diseases, Imperial College London, United Kingdom
6. International Centre for Diarrhoeal Diseases Research, Dhaka, Bangladesh

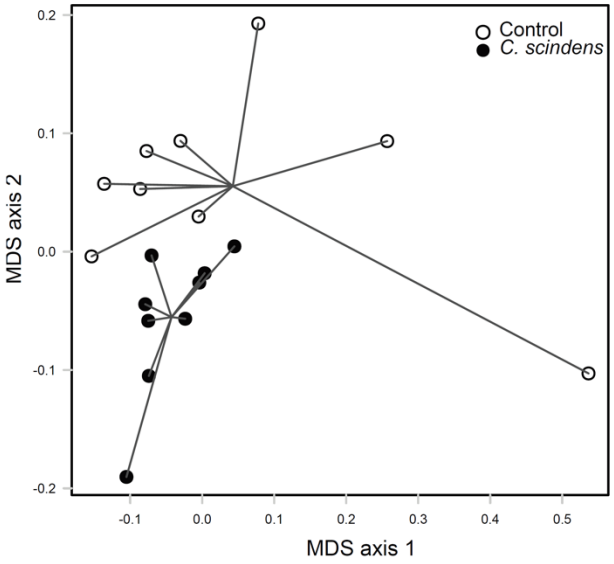
*Correspondence to: William A. Petri, wap3g@virginia.edu

This PDF file includes:

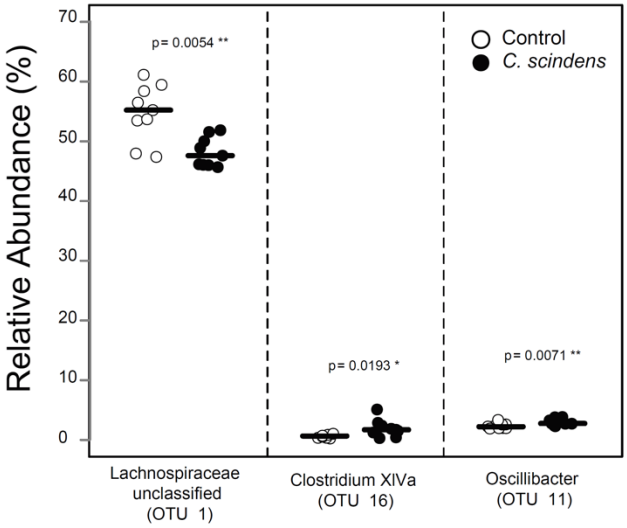
Figures S1 to S2

Materials and Methods

A.


Control
C. scindens
MDS axis 2
MDS axis 1
0.2
0.1
0.0
-0.1
-0.2
-0.1
0.0
0.1
0.2
0.3
0.4
0.5

B.


Relative Abundance (%)
70
60
50
40
30
20
10
0
p= 0.0054 **
p= 0.0193 *
p= 0.0071 **
Control
C. scindens
Lachnospiraceae unclassified (OTU 1)
Clostridium XIVa (OTU 16)
Oscillibacter (OTU 11)

C.

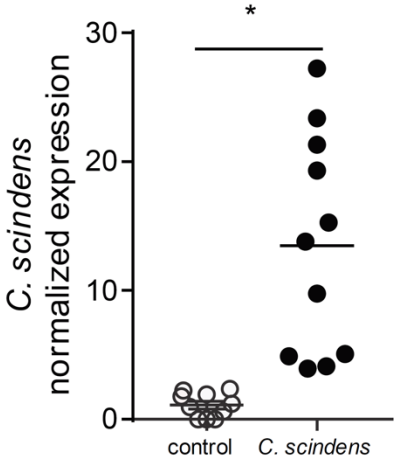

*
C. scindens normalized expression
30
20
10
0
control
C. scindens

Figure S1. Introduction of *Clostridium scindens* to the gut microbiota alters community structure. CBA/J mice were colonized with bile acid 7α-dehydroxylating bacteria *C. scindens* (ATCC® 35704) over three weeks prior to intracecal infection with *E. histolytica.* Composition of the cecal microbiota community structure after *C. scindens* colonization was determined by analysis of 16S rRNA gene V4 region sequences. Non metric multidimensional scaling (NMDS) plot of Bray-Curtis dissimilarity. The groups are significantly different with a perMANOVA p value of 0.002. **(A)** and linear discriminant analysis effect size (LEfSe) on phylotyped OTUs **(B)**. Relative expression of baiCD stereo-specific 7alpha/7beta-hydroxy-3-oxo-delta4-cholenoic acid oxidoreductase normalized to total eubacterial 16S rRNA was determined via qPCR **(C).** *= $p<0.05$.

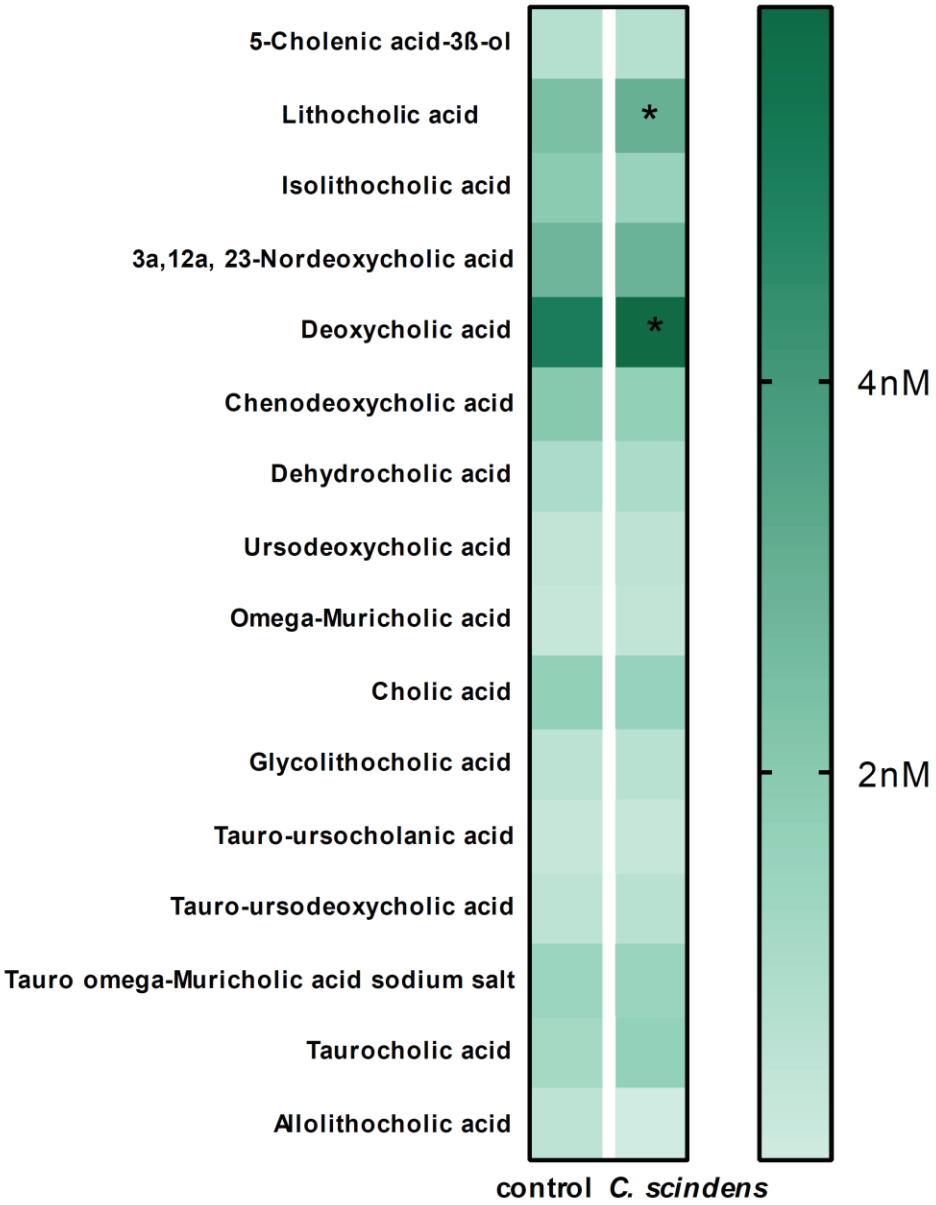

5-Cholenic acid-3ß-ol
Lithocholic acid
Isolithocholic acid
3a,12a, 23-Nordeoxycholic acid
Deoxycholic acid
Chenodeoxycholic acid
Dehydrocholic acid
Ursodeoxycholic acid
Omega-Muricholic acid
Cholic acid
Glycolithocholic acid
Tauro-ursocholanic acid
Tauro-ursodeoxycholic acid
Tauro omega-Muricholic acid sodium salt
Taurocholic acid
Allolithocholic acid
*
*
4nM
2nM
control
C. scindens

Figure S2. *Clostridium scindens* increases serum secondary bile acids.

CBA/J mice were colonized with *C. scindens* (ATCC® 35704), a bacterium capable of the 7α-dehydroxylation of bile acids, over three weeks prior to intracecal infection with *E. histolytica.* Serum bile acids were measured using ultra-performance liquid chromatography-mass spectrometry in *C. scindens* gavaged and control animals**.** *= $p<0.05$, Fishers LSD.

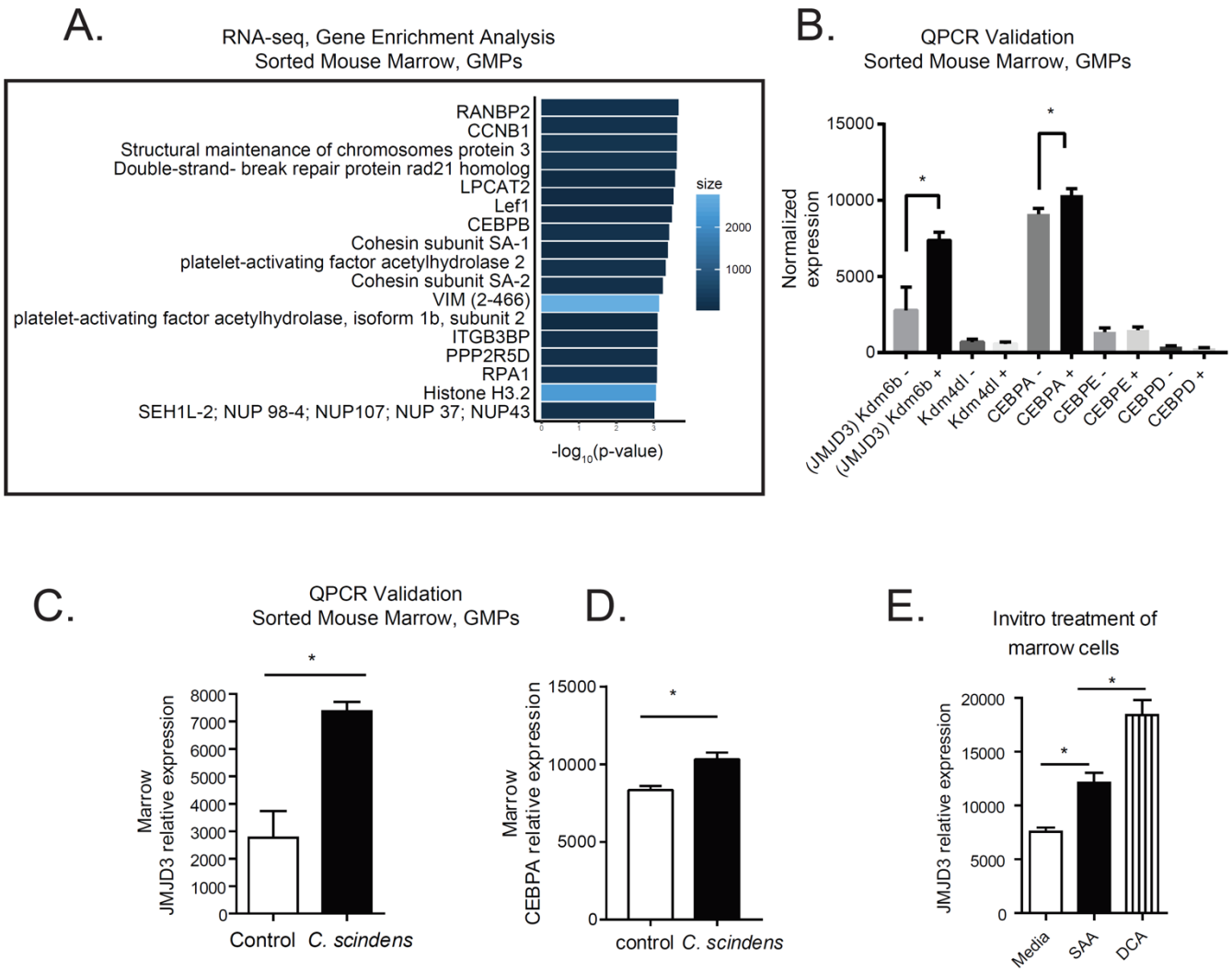

A.
RNA-seq, Gene Enrichment Analysis
Sorted Mouse Marrow, GMPs
RANBP2
CCNB1
Structural maintenance of chromosomes protein 3
Double-strand- break repair protein rad21 homolog
LPCAT2
Lef1
CEBPB
Cohesin subunit SA-1
platelet-activating factor acetylhydrolase 2
Cohesin subunit SA-2
VIM (2-466)
platelet-activating factor acetylhydrolase, isoform 1b, subunit 2
ITGB3BP
PPP2R5D
RPA1
Histone H3.2
SEH1L-2; NUP 98-4; NUP107; NUP 37; NUP43
size
2000
1000
-log10(p-value)
B.
QPCR Validation
Sorted Mouse Marrow, GMPs
Normalized expression
15000
10000
5000
0
*
*
(JMJD3) Kdm6b -
(JMJD3) Kdm6b +
Kdm4dl -
Kdm4dl +
CEBPA -
CEBPA +
CEBPE -
CEBPE +
CEBPD -
CEBPD +
C.
QPCR Validation
Sorted Mouse Marrow, GMPs
Marrow JMJD3 relative expression
8000
7000
6000
5000
4000
3000
2000
1000
0
*
Control
C. scindens
D.
Marrow CEBPA relative expression
15000
10000
5000
0
*
control
C. scindens
E.
Invitro treatment of marrow cells
JMJD3 relative expression
20000
15000
10000
5000
0
*
*
Media
SAA
DCA

Figure S3. Intestinal colonization with *Clostridium scindens* increases expression of H3K27 demethylase JMJD3 and granulopoiesis promoting transcription factor CEBPA specifically in bone marrow granulocyte monocyte progenitors and secondary bile acid treatment (DCA) increases JMJD3 in marrow cells in vitro. CBA/J mice were colonized with *Clostridium* scindens and bone marrow was sorted into CMP, GMP, MEP and CLP. RNA-seq analysis **(A)** and qPCR **(B-D)** was performed to validate enriched genes from RNA-seq on cDNA prepared from RNA isolated from sorted GMPs. For RNAseq a bias-corrected method was used to map reads directly to the transcriptome and a gene list was generated for up or down regulated genes compared to *C. scindens* negative mice. An enrichment analysis utilizing ConsensusPathDB for most over-represented gene centered on the top 50% of genes by unadjusted P value was performed **(A) .** Key genes located in or with protein products acting in select gene centers in **(A)** were validated via qPCR **(B)**. Expression of noted genes was normalized to a housekeeping gene (S14) **(B, C, D)**. An additional qPCR run was performed on independent samples **(C, D)** for significant genes in **(B).** Whole marrow cells were treated in vitro with DCA and positive control SAA and JMJD3 expression measured via qPCR at 4 hours **(E).** *= $p<0.05$, Student's t-test, One Way ANOVA with Tukey posttest.

Methods

Mice

Five week old male CBA/J mice (Jackson Laboratories or RAG 1 KO mice (Jackson Laboratories) were housed in a specific pathogen–free facility in micro isolator cages and provided autoclaved food (Lab diet 5010) and water ad libitum. Specific pathogen free status was monitored quarterly. Quarterly, a sentinel mouse (or rat) was removed from each room and humanely euthanized for serologic evaluation, examination of pelage for furmites, and examination of cecal contents for pinworms. The serologic assays, conducted in-house using CRL reagents, were MHV, EDIM, GD-7, MVM, MPV, and MNV, (Sendai, PVM, RPV/ KRV/H-1, M. pulmonis, and SDAV for rats). In the final quarter, a comprehensive serology was run which included the above agents plus K-virus, MCMV, MTV, LCM, Ectromelia, 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. All experiments were performed according to provisions of the USA Animal Welfare Act of 1996 (Public Law 89.544).

Clostridium scindens colonization

CBA/J mice were colonized with bile acid 7α-dehydroxylating bacteria *C. scindens* (ATCC® 35704) over three weeks prior to intracecal infection with *E. histolytica.* Mice were gavaged with 100ul of overnight culture at an optical density of 1.4 at 600nm or media control (BHI, Anerobe Systems, AS-872) once per week.

Intravenous deoxycholate treatment

CBA/J mice were treated intravenously via tail vein injection by a trained veterinary technician 3 times a week, over two weeks with 400uL of PBS or 400uL of 0.20mg/mL DCA (Sigma) in PBS per treatment.

Adoptive Marrow Transplant

Donor and recipient CBA/J mice were colonized with bile acid 7α-dehydroxylating bacteria *C. scindens* (ATCC® 35704) or treated with media controls as above. CBA/J mice colonized with *C. scindens* (+) or not were lethally irradiated, then immediately given whole marrow from *C. scindens* (+) or *C. scindens* (-) donors then allowed to recover for 7 weeks prior to ameba challenge. Irradiation was performed with 900 Rad in a single dose from a Shepard irradiator, Mark 1 Model 68A Dual, Serial Number 1163 with a Cs-137 source. All irradiation experiments were supervised by a member of the University of Virginia Environmental Health and Safety team. Mice were place on placed on sulfa water 3 days prior and 21 days post bone marrow transplant.

***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 3 x10^6 parasites were suspended in 100 µL culture media and injected intracecally

(6). Data analysis and graphing was performed with Graphpad Prism 7.0. Final figures were modified and arranged in Adobe Illustrator.

JMJD3 Blockade

Mice were treated intraperitoneally with hybridoma grade DMSO in PBS (Sigma) or GSK-J4 in DMSO/PBS (100uL, DMSO/PBS, 25 mg/kg, Cayman Chemical,12074) one day before and during *C. scindens* colonization (once per week) but not during *E. histolytica* infection.

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. $1x10^6$ cells per mouse were stained with antibodies from Bio Legend, CD11c-BV421, CD4-BV605, Ly6c-FitC, CD3e-PerCp CY 5.5, SIGLEC F-PE, Ly6G PE Cy7, CD11b-APC, CD8a AF700, 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. Further data analysis and graphing was performed with Graphpad Prism 7.0. Final figures were modified and arranged in Adobe Illustrator.

Bone marrow flow cytometry and cell sorting

Bone marrow cells were isolated from dissected and cleaned and opened on one end femur, fibia and tibia of mice by centrifugation in custom made microcentrifuge tubes composed of a 0.5ml microcentrifuge tube with a hole punched in the bottom nested inside a 1.5mL microscentrifuge

tube (VWR). Bone marrow cells were stained with PerCP-Cy5.5-labeled lineage (Lin) markers (TCRb, CD3e,CD49b, B220Gr1, CD11c, CD11b), anti-CD34-Brilliant Violet 421, c-Kit-Brilliant Violet 605, CD127-PE-Cy7, FcgRII-III (CD16/CD42)-APC-Cy7, and Sca-1-APC, CD150 PE, Live dead staining with Zombie Aqua (Bioledgend). Flow cytometric analysis was performed on an LSR Fortessa (BD Biosciences) or cells sorted on a Becton Dickinson Influx Cell Sorter into RNA later (Qiagen) or Cryostore CS10 (Stemcell) with 7AAD as a live dead stain . Data analyzed via FlowJo (Tree Star Inc.). All gates were set based on fluorescence minus one (FMO) controls. CLP are Lin-IL-7R+c-Kit^{int}Sca-1^{int}, CMP are Lin-c-Kit+Sca-1-CD34+FcgRII-III^{int}; GMP are Lin-c-Kit+Sca-1-CD34+FcgRII-III^{hi}; MEP are Lin-c-Kit+Sca-1-CD34-FcgRII-III-.

Colony forming assay for determination of bone marrow hematopoietic precursors

Bone marrow cells were isolated (*32*) and then cultured in methylcellulose-based medium that included, 3 units/mL Epo, 10 ng/mL mouse recombinant IL-3, 10 ng/mL human recombinant IL-6, and 50 ng/mL mouse recombinant stem-cell factor per manufacturer procedures (M3434; StemCell Technologies, Vancouver, BC). Colony formation of burst-forming unit–erythroid (BFU-Es), colony-forming unit–granulocyte/monocyte (CFU-GMs), and CFU granulocyte/erythrocyte/monocyte/macrophage (CFU-GEMMs) were analyzed after 7 days.

RNA sequencing and data analysis

RNA was isolated from approximately 17,000 sorted GMPs utilizing the Qiagen RNeasy Micro Kit. Ribosomal reduction was performed to concentrate for mRNA. Directional cDNA libraries were generated and samples sequenced in multiplex (50PE reads) using the Illumina HiSeq v4

platform. For library prep, the NEBNext® Ultra™ Directional RNA Library Prep Kit for Illumina® and NEBNext® rRNA Depletion Kit (Human/Mouse/Rat) and alternate protocol for low yield RNA and 15 PCR cycles of amplification was utilized. For sequencing, libraries were sequenced with the NGS NextSeq kit - 150 cycle High Output Kit, paired end 75x75 bp read. Data analysis was performed by the UVA Bioinformatics Core. Gene reads were aligned to the mouse transcriptome and read counts quantified using EDGE-pro software. The Deseq2 Bioconductor package was then used to normalize read counts between libraries and estimate dispersion for each gene prior to differential gene analysis. These data were then fit to a negative binomial model, which was used for differential expression analysis. A bias-corrected method was used to map reads directly to the transcriptome and a gene list was generated for up or down regulated genes compared to *C. scindens* negative mice. An enrichment analysis utilizing ConsensusPathDB for most over-represented gene centers in the top 50% of genes by unadjusted P value was performed. The sequences associated with analysis are deposited to the GEO repository under the GEO accession number GSE121503.

16S rRNA Gene V4 region sequencing

DNA was isolated from cecal lysate (QIAamp DNA Stool Mini Kit). The V4 region of the 16S rRNA gene was amplified from each sample using the dual indexing sequencing strategy as described previously (Kozich et al., 2013). Sequencing performed on the Illumina MiSeq platform, using a MiSeq Reagent Kit V2 500 cycles (Illumina cat# MS102-2003), according to the manufacturer's instructions with modifications found in the Schloss SOP: https://github.com/SchlossLab/MiSeq_WetLab_SOP. The mock community produced

ZymoBIOMICS Microbial Community DNA Standard (Zymo Research cat# D6306) was sequenced to monitor sequencing error. The overall error rate was 0.02%.

Sequence Curation and Analysis

Raw sequences were curated using the software package mothur version 1.39.5 (Schloss et al., 2009) following the Illumina MiSeq standard operating procedure. Briefly, paired end reads were assembled into contigs and aligned to the V4 region using the SLIVA 16S rRNA sequence database (Quast et al., 2013), sequences that failed to align or were flagged as possible chimeras were removed. A naïve Bayesian classifier using the Ribosomal Database Project (Wang et al., 2007) classified sequences. Operational Taxonomic Units (OTUs) using a 97% similarity cutoff were generated using the Opticlust clustering algorithm (Westcott et al., 2017).

The number of sequences in each sample was then rarefied to 35,000 sequences to minimize bias due to uneven sampling. Following curation in mothur, further data analysis and figure generation was carried out in R (v 3.4.1) using the package vegan (Oksanen et al, 2018). This includes determining the axes for the multidimensional scaling (MDS) plots using Bray-Curtis dissimilarity calculated from sequence abundance. Additionally, vegan was used to determine significance between groups using PERMANOVA. The sequences associated with analysis will be deposited to the SRA under the PRJNA503904. Final figures were modified and arranged in Adobe Illustrator CC.

***C. scindens* culture, marrow and cecal lysate qPCR**

Purity of *C. scindens* culture was confirmed via qPCR and sanger sequencing with broad specificity eubacteria primers, EUB forward 5'-ACTCCTACGGGAGGCAGCAGT-3'EUB

reverse 5'-ATTACCGCGGCTGCTGGC-3', and *C. scindens* baiCD stereo-specific 7alpha/7beta-hydroxy-3-oxo-delta4-cholenoic acid oxidoreductase primers, BaiCD F - 5′-CAGCCCRCAGATGTTCTTTG-3′ BaiCD R - 5′-GCATGGAATTCHACTGCRTC-3′ *C. scindens* colonization was measured via qPCR from cecal lysate (QIAamp DNA Stool Mini Kit). qPCR for baiCD with Sybr green was performed and data were normalized to expression of a conserved *Eubacteria* 16s RNA gene (EUB) (*33*). Primer concentrations, annealing temperatures, and cycle number were optimized for each primer pair. For each primer pair, 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 EUB or the mouse housekeeping gene S14 using a method described and utilized previously (*21, 22, 34*). Data is presented as relative expression. For sorted marrow qPCR, RNA was isolated from approximately 17,000 sorted GMPs utilizing the Qiagen RNeasy Micro Kit. S14 forward 5'-TGGTGTCTGCCACATCTTTGCATC-3', S14 reverse 5' AGTCACTCGGCAGATGGTTTCCTT-3',Jmjd3 forward 5'-CTCTGGAACTTTCATGCCGG-3' Jmjd3 reverse, 5'-CTTAGCCCCATAGTTCCGTTTG-3,' CebpA forward 5'-CAAAGCCAAGAAGTCGGTGGACAA, CebpA reverse 5' -TCATTGTGACTGGTCAACTCCAGC

CebpE forward 5'-TGTGGGCACCAGACCCTAAG, CebpE reverse 5'-GCTGCCATTGTCCACGATCT, CebpD forward 5'- CTTTTAGGTGGTTGCCGAAG, CebpD reverse 5' GCAACGAGGAATCAAGTTTCA, Kdm4dl forward 5'-CATGGTCACCTTTCCCTATGG, Kdm4dl reverse 5'-AAAATTGATGGCCTCTGCG. Primers were purchased from Integrated DNA Technologies Coralville, Iowa, USA.

Serum deoxycholate ELISA

Serum Deoxycholate was measured via ELISA (Cloud-Clone Corp. CES089Ge) in 80 children each from two birth cohorts in Mirpur Dhaka, Bangladesh. Full details of the design of these birth cohort studies have been described (*35*, *36*) and all studies were approved by the Ethical Review Committee of the ICDDR, B and the Institutional Review Boards of the Universities of Virginia and informed consent was obtained after the nature and possible consequences of the studies were explained in all cohort studies. Children were between one and half and two years of age and serum was selected by identifying diarrheal stools within 6 months of the blood draw that were *E. histolytica* positive (n=40) and negative (n=40) as identified via qPCR (*37*). Serum was measured in 6 mice from each of two experiments that were gavaged with *C. scindens* or media control as described utilizing the same kit or in DCA treated mice. For both children and mice 25uL of serum was utilized at a 1:2 (humans) or 1:4 (mice) dilution following kit protocol with a 10 minute development step following administration of substrate solution.

Targeted profiling of serum bile acids using UPLC-MS

Plasma bile acids were quantified as previously described (*38*) by ACQUITY ultraperformance liquid chromatography (UPLC) (Waters, Ltd., Elstree, UK). Briefly, 100 µl of plasma were spiked with isotopically labeled bile acid standards followed by the addition of 300 µl of ice-cold methanol to facilitate protein precipitation. Bile acids were separated over a 15-minute gradient on an ACQUITY BEH C8 column (1.7 µm, 100 mm x 2.1 mm), detected by a Xevo TQ-S mass spectrometer (Waters, Manchester, UK) operating in the negative ionization mode (ESI-) and assayed using multiple reaction monitoring (MRM).

References

1. C. Troeger *et al.*, Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015, *Lancet Infect. Dis.* **17**, 909–948 (2017).
2. R. Lozano *et al.*, Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010., *Lancet (London, England)* **380**, 2095–128 (2012).
3. D. Mondal, W. A. Petri, R. B. Sack, B. D. Kirkpatrick, R. Haque, Entamoeba histolytica-associated diarrheal illness is negatively associated with the growth of preschool children: evidence from a prospective study., *Trans. R. Soc. Trop. Med. Hyg.* **100**, 1032–8 (2006).
4. W. A. Petri *et al.*, Enteric infections, diarrhea, and their impact on function and development., *J. Clin. Invest.* **118**, 1277–90 (2008).
5. H. P. Verkerke, W. A. Petri, C. S. Marie, The dynamic interdependence of amebiasis, innate immunity, and undernutrition., *Semin. Immunopathol.* **34**, 771–85 (2012).
6. K. L. Kotloff *et al.*, Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study., *Lancet* **382**, 209–22 (2013).
7. R. Haque, C. D. Huston, M. Hughes, E. Houpt, W. A. J. Petri, Amebiasis., *N. Engl. J. Med.* **348**, 1565–73 (2003).
8. C. A. Gilchrist *et al.*, Role of the Gut Microbiota of Children in Diarrhea Due to the Protozoan Parasite Entamoeba histolytica., *J. Infect. Dis.* **213**, jiv772- (2015).
9. S. L. Burgess, C. A. Gilchrist, T. C. Lynn, W. A. Petri, Parasitic protozoa and interactions with the host intestinal microbiota., *Infect. Immun.* , IAI.00101-17 (2017).
10. A. Khosravi *et al.*, *Gut Microbiota Promote Hematopoiesis to Control Bacterial Infection* (Elsevier, 2014; http://www.pubmedcentral.nih.gov/articleren der.fcgi?artid=4144825&tool=pmcentrez&rendertype=abstract).
11. C. G. Buffie, E. G. Pamer, Microbiota-mediated colonization resistance against intestinal pathogens., *Nat. Rev. Immunol.* **13**, 790–801 (2013).
12. M. G. Netea *et al.*, Trained immunity: A program of innate immune memory in health and disease, *Science (80-.).* **352**, aaf1098-aaf1098 (2016).
13. E. Blacher, M. Levy, E. Tatirovsky, E. Elinav, Microbiome-Modulated Metabolites at the Interface of Host Immunity., *J. Immunol.* **198**, 572–580 (2017).
14. S. Saeed *et al.*, Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity, *Science (80-.).* **345**, 1251086–1251086 (2014).
15. J. W. M. van der Meer, L. A. B. Joosten, N. Riksen, M. G. Netea, Trained immunity: A smart way to enhance innate immune defence, *Mol. Immunol.* **68**, 40–44 (2015).
16. Q. Yan *et al.*, Jmjd3-mediated epigenetic regulation of inflammatory cytokine gene expression in serum amyloid A-stimulated macrophages., *Cell. Signal.* **26**, 1783–1791 (2014).
17. A. Christ *et al.*, Western Diet Triggers NLRP3-Dependent Innate Immune Reprogramming, *Cell* **172**, 162–175.e14 (2018).
18. E. Niemitz, Jmjd3, PHF20 and reprogramming, *Nat. Genet.* **45**, 477–477 (2013).
19. A. Salminen, K. Kaarniranta, M. Hiltunen, A. Kauppinen, Histone demethylase Jumonji D3 (JMJD3/KDM6B) at the nexus of epigenetic regulation of inflammation and the aging process., *J. Mol. Med. (Berl).* **92**, 1035–43 (2014).
20. F. De Santa *et al.*, Jmjd3 contributes to the control of gene expression in LPS-activated

macrophages., *EMBO J.* **28**, 3341–52 (2009).
21. S. L. Burgess *et al.*, Role of Serum Amyloid A, GM-CSF and Bone Marrow Granulocyte-Monocyte Precursor Expansion in Segmented Filamentous Bacteria-mediated Protection from *Entamoeba histolytica* ., *Infect. Immun.* , IAI.00316-16 (2016).
22. S. L. Burgess *et al.*, Bone Marrow Dendritic Cells from Mice with an Altered Microbiota Provide Interleukin 17A-Dependent Protection against Entamoeba histolytica Colitis., *MBio* **5**, e01817-14- (2014).
23. S. L. Burgess, W. A. Petri, Jr, The Intestinal Bacterial Microbiome and E. histolytica Infection., *Curr. Trop. Med. reports* **3**, 71–74 (2016).
24. C. M. Schürch, C. Riether, A. F. Ochsenbein, Cytotoxic CD8+ T Cells Stimulate Hematopoietic Progenitors by Promoting Cytokine Release from Bone Marrow Mesenchymal Stromal Cells, *Cell Stem Cell* **14**, 460–472 (2014).
25. K. Atarashi *et al.*, Induction of colonic regulatory T cells by indigenous Clostridium species, *Science (80-.).* **331**, 337–341 (2011).
26. G. N. MORRIS, J. WINTER, E. P. CATO, A. E. RITCHIE, V. D. BOKKENHEUSER, Clostridium scindens sp. nov., a Human Intestinal Bacterium with Desmolytic Activity on Corticoids, *Int. J. Syst. Bacteriol.* **35**, 478–481 (1985).
27. S. W. Cho *et al.*, Positive regulation of osteogenesis by bile acid through FXR., *J. Bone Miner. Res.* **28**, 2109–21 (2013).
28. H. Id Boufker *et al.*, Role of farnesoid X receptor (FXR) in the process of differentiation of bone marrow stromal cells into osteoblasts, *Bone* **49**, 1219–1231 (2011).
29. R. Bosselut, Pleiotropic Functions of H3K27Me3 Demethylases in Immune Cell Differentiation, *Trends Immunol.* **37**, 102–113 (2016).
30. H. S. Radomska *et al.*, CCAAT/enhancer binding protein alpha is a regulatory switch sufficient for induction of granulocytic development from bipotential myeloid progenitors., *Mol. Cell. Biol.* **18**, 4301–14 (1998).
31. E. R. Houpt *et al.*, The mouse model of amebic colitis reveals mouse strain susceptibility to infection and exacerbation of disease by CD4+ T cells., *J. Immunol.* **169**, 4496–503 (2002).
32. M. Swamydas, M. S. Lionakis, Isolation, purification and labeling of mouse bone marrow neutrophils for functional studies and adoptive transfer experiments., *J. Vis. Exp.* , e50586 (2013).
33. P. S. Hoffman *et al.*, Preclinical Studies of Amixicile: A Systemic Therapeutic Developed for Treatment of Clostridium difficile Infections also shows Efficacy against Helicobacter pylori., *Antimicrob. Agents Chemother.* (2014), doi:10.1128/AAC.03112-14.
34. P. Y. Muller, H. Janovjak, A. R. Miserez, Z. Dobbie, Processing of gene expression data generated by quantitative real-time RT-PCR., *Biotechniques* **32**, 1372–4, 1376, 1378–9 (2002).
35. D. Mondal *et al.*, Contribution of enteric infection, altered intestinal barrier function, and maternal malnutrition to infant malnutrition in Bangladesh., *Clin. Infect. Dis.* **54**, 185–92 (2012).
36. B. D. Kirkpatrick *et al.*, The “Performance of Rotavirus and Oral Polio Vaccines in Developing Countries” (PROVIDE) Study: Description of Methods of an Interventional Study Designed to Explore Complex Biologic Problems, *Am. J. Trop. Med. Hyg.* **92**, 744–51 (2015).
37. S. Roy *et al.*, Real-time-PCR assay for diagnosis of Entamoeba histolytica infection., *J.*

Clin. Microbiol. **43**, 2168–72 (2005).
38. M. H. Sarafian *et al.*, Bile Acid Profiling and Quantification in Biofluids Using Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry, *Anal. Chem.* **87**, 9662–9670 (2015).