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RESEARCH ARTICLE

Innate Immunity

Ruminococcus gnavus and *Limosilactobacillus reuteri* Regulate Reg3γ Expression through Multiple Pathways

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ABSTRACT

Epithelium-derived antimicrobial peptides represent an evolutionarily ancient defense mechanism against pathogens. Regenerating islet-derived protein 3 γ (Reg3γ), the archetypal intestinal antimicrobial peptide, is critical for maintaining host–microbe interactions. Expression of Reg3γ is known to be regulated by the microbiota through two different pathways, although it remains unknown whether specific Reg3γ-inducing bacteria act via one or both of these pathways. In recent work, we identified *Ruminococcus gnavus* and *Limosilactobacillus reuteri* as commensal bacteria able to induce *Reg3g* expression. In this study, we show these bacteria require myeloid differentiation primary response protein 88 and group 3 innate lymphoid cells for induction of Reg3γ in mice. Interestingly, we find that *R. gnavus* and *L. reuteri* suppress Reg3γ in the absence of either myeloid differentiation primary response protein 88 or group 3 innate lymphoid cells. In addition, we demonstrate that colonization by these bacteria is not required for induction of Reg3γ, which occurs several days after transient exposure to the organisms. Taken together, our findings highlight the complex mechanisms underlying microbial regulation of Reg3γ. *ImmunoHorizons*, 2023, 7: 228–234.

INTRODUCTION

Animals have evolved numerous defense mechanisms to protect themselves against infectious diseases (1), with expression of antimicrobial peptides (AMPs) representing an evolutionarily conserved immune defense mechanism present in nearly all multicellular organisms (2). These AMPs have bactericidal activity that leads to the rapid death of microbes. Most AMPs are produced by the mucosal epithelium (e.g., intestines, respiratory tract, reproductive tract) to help maintain homeostasis with the commensal microbiota and limit infection by pathogens (3). Given that the intestines represent a major route for pathogen entry and, compared with other anatomic regions, harbor the largest number of commensal microbes, it is not surprising that intestinal epithelial cells (IECs) secrete a broad range of AMPs into the lumen to maintain host–microbiota segregation and protect against enteric infection (4–6).

One of the most widely studied intestinal AMPs is regenerating islet-derived protein 3 γ (Reg3γ), which is produced by multiple intestinal epithelial lineages, including enterocytes and Paneth cells (7). Reg3γ is stored in secretory granules, has bactericidal activity against gram-positive bacteria, and is critical for maintaining spatial segregation between the intestinal epithelium and the microbiota (6, 8, 9). Regulation of Reg3γ expression is complex and involves the integration by IECs of at least two different signals. First, IEC expression of myeloid differentiation primary response protein 88 (Myd88), an adaptor protein downstream of many TLRs, is required (10–12), a finding that suggests TLR signaling is critical. Notably, expression of Myd88 specifically by Paneth cells is sufficient for Reg3γ expression (10), which demonstrates the

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Abbreviations used in this article: AMP, antimicrobial peptide; F, forward; IEC, intestinal epithelial cell; ILC3, group 3 innate lymphoid cell; KO, knockout; Myd88, myeloid differentiation primary response protein 88; qPCR, quantitative PCR; R, reverse; Reg3γ, regenerating islet-derived protein 3 γ.

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critical role for this cell type. Second, group 3 innate lymphoid cells (ILC3s) present in the lamina propria must secrete IL-22 (13, 14), which binds to IL-22Rs on the basolateral side of IECs and results in *Reg3g* expression (15). It is not clear why both Myd88 and IL-22 signaling are required or whether the same environmental signal stimulates both pathways.

Although some AMPs are constitutively expressed in the absence of microbial stimulation, others, including Reg3γ, require microbiota-derived signals for normal levels of expression (8, 10, 11, 16). However, a screen of >50 taxonomically diverse bacteria found that none was able to induce small-intestinal expression of *Reg3g* in mice (16), which demonstrates this critical function is possessed by a limited number of commensal bacteria. Using microbe–phenotype triangulation, we recently identified *Ruminococcus gnavus* and *Limosilactobacillus reuteri* [formerly *Lactobacillus reuteri* (17)] as potent inducers of *Reg3g* expression (18). We reasoned these specific Reg3γ-inducing bacteria can be exploited to gain insight into the mechanisms underlying microbiome induction of *Reg3g* expression. We find *R. gnavus* and *L. reuteri* each requires Myd88 and ILC3s for inducing expression of *Reg3g*. Moreover, these commensal bacteria induce expression of several other intestinal AMPs, with notable differences in their requirement for ILC3s. Intriguingly, *R. gnavus* and *L. reuteri* suppress AMP expression in the absence of either Myd88 or ILC3s. Taken together, our results demonstrate that microbial regulation of AMP expression is more complex than previously appreciated.

MATERIALS AND METHODS

Mice

C57BL/6J (stock number 000664), *Ahr*$^{fl/fl}$ (stock number 006203), and *Rorc-Cre* (stock number 022791) mice were purchased from Jackson laboratories, and *Myd88*$^{-/-}$ mice were obtained from A. Moseman (Duke University). Gnotobiotic HMb mice were a gift from D. Kasper (Harvard Medical School) (19). All animals were bred and maintained at Duke University, with gnotobiotic mice maintained in sterile vinyl isolators (Class Biologically Clean, Madison, WI). HMb mice were experimentally manipulated in autoclaved individually ventilated cages with autoclaved food and water. Experiments used sex- and aged-matched (ranging from 5- to 12-wk-old) mice. All procedures were approved by the Duke Institutional Animal Care and Use Committee and were conducted in accordance with National Institutes of Health guidelines.

Bacterial treatment

Mice were administered 10^7–10^8 CFUs of *Parabacteroides distasonis* (ATCC 8503), *R. gnavus* (ATCC 29149), or *L. reuteri* (BEI HM-102) by oral gavage. *R. gnavus*– and *L. reuteri*–treated mice were euthanized 3 and 5 d after administration of bacteria, respectively, unless specified otherwise.

Quantification of bacterial colonization by quantitative PCR

To determine the colonization kinetics of *R. gnavus* and *L. reuteri*, we collected fecal samples at 0, 4, 8, 24, 72, and 120 h after bacterial administration. In addition, we harvested the distal 1.5 cm of ileum from mice treated with either *R. gnavus* or *L. reuteri* at 72 or 120 h after bacterial administration, respectively. Total DNA was extracted by homogenizing samples with TRIzol (Invitrogen) and purifying with Quick-DNA MiniPrep kit (Zymo) spin columns. Using organism-specific primers for *R. gnavus* (forward [F], 5′-CCAATTACGGAAAGCTGGAT-3′; reverse [R], 5′-TCTGCTTTCCATGTATCTTCACA-3′) or *L. reuteri* (F, 5′-CAGACAATCTTTGATTGTTTAG-3′; R, 5′-GCTTGTTGGTTTGGGCTCTTC-3′), we determined copy numbers for each bacterium by quantitative PCR (qPCR; StepOnePlus; Bio-Rad) and normalized them to the entire microbial abundance using 16S rRNA universal primers 341F (5′-CCTACGGGAGGCAGCAG-3′) and 534R (5′-ATTACCGCGGCTGCTGGCA-3′).

AMP expression analysis

qPCR for AMPs was performed as previously described (18). In brief, the distal 1.5 cm of ileum was collected, frozen immediately in liquid nitrogen, and stored at −80°C until needed. Tissues were homogenized in TRIzol (Invitrogen), and RNA was purified according to the manufacturer's instructions, with a subsequent additional cleanup step (RNeasy Mini kit; Qiagen). cDNA was prepared with the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems), and qPCR was performed on a StepOnePlus (Bio-Rad) using iTaq Universal SYBR Green Supermix (Bio-Rad). The comparative cycle threshold method was used to quantify transcripts that were normalized with respect to GAPDH. Primer sequences are as follows: *GAPDH* (F, 5′-CCTCGTCCCGTAGACAAAATG-3′; R, 5′-TCTCCACTTTGCCACTGCAA-3′), *Reg3g* (F, 5′-TTCCTGTCCTCCATGATCAAAA-3′; R, 5′-CATCCACCTCTGTTGGGTTCA-3′), *Reg3b* (F, 5′-TACTGCCTTAGACCGTGCTTTCTG-3′; R, 5′-GACATAGGGCAACTTCACCTCACA-3′), *Defa5* (F, 5′-TTGGGCTCCTGCTCAACAAT-3′; R, 5′-GACACAGCCTGGTCCTCTTC-3′), and *Lyz1* (F, 5′-GAGACCGAAGCACCGACTATG-3′; R, 5′-CGGTTTTGACATTGTGTTCGC-3′).

Tissue preparation and histological analysis

Ileal tissue was fixed in 10% formalin, embedded in paraffin, and 4-μm-thick sections were stained with H&E. Paneth cells, identified as crypt cells containing eosinophilic granules, were enumerated in 10 crypts per sample. Immunohistochemistry was performed as previously described (20). In brief, 4-μm-thick tissue sections were deparaffinized in xylene followed by alcohol rehydration. Ag retrieval was performed in 1× Borg Decloaker solution (Biocare Medical) at 95°C for 3 min in a pressure cooker (Instant Pot). Ab staining was performed with anti-mouse Reg3γ (1:100 dilution; Invitrogen) followed by a biotin-conjugated secondary (1:500 dilution; Jackson Immunoresearch), and the protein was visualized with Vectastain Elite ABC peroxidase detection kit (Vector Laboratories) followed by incubation with liquid DAB+Substrate (Dako). Image acquisition was performed using a Zeiss Axio Imager microscope, and images were processed using Fiji (ImageJ) software.

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Statistical analysis

Data are represented as mean ± SE (SEM) throughout the figures. Prism 9 (GraphPad Software) was used for all statistical analyses. qPCR analyses were compared using a Mann–Whitney *U* test, one-way ANOVA, or Kruskal–Wallis test, as appropriate. Bacterial 16S rRNA gene copy numbers were compared using a Wilcoxon test. All data were considered statistically significant for $p < 0.05$.

RESULTS

Reg3γ induction does not require probiotic colonization

We previously demonstrated that a single oral dose of *R. gnavus* or *L. reuteri* induces ileal *Reg3g* expression in gnotobiotic Swiss Webster mice colonized with a human microbiota (18). However, it is not clear how quickly, or for how long, *Reg3g* induction occurs after treatment with these Reg3γ-inducing organisms. Therefore, we treated SPF C57BL/6 mice with a single oral dose of *R. gnavus* or *L. reuteri* and harvested ileal tissue at different time points after treatment. Although *R. gnavus* induced *Reg3g* expression 3 and 5 d after treatment, this increased expression was no longer present at 7 d (Fig. 1A). In contrast, induction of *Reg3g* expression by *L. reuteri* displayed slower kinetics, with an increase present only on day 5 (Fig. 1B). Given that exogenously administered bacteria often do not persist in the intestinal tract (21–23), we determined the colonization kinetics of *R. gnavus* and *L. reuteri* by analyzing the fecal abundance of these bacteria at various time points. Surprisingly, even though these organisms induce *Reg3g* expression at 3–5 d after treatment, we found both organisms were absent from the fecal microbiota by 24 h after treatment (Fig. 1C, 1D). Moreover, neither *R. gnavus* nor *L. reuteri* was detected in stool or the distal ileum at time points when *Reg3g* expression was first noted to be increased (i.e., day 3 for *R. gnavus*, day 5 for *L. reuteri*). Taken together, these results indicate that persistent intestinal colonization of *R. gnavus* or *L. reuteri* is not required for *Reg3g* induction.

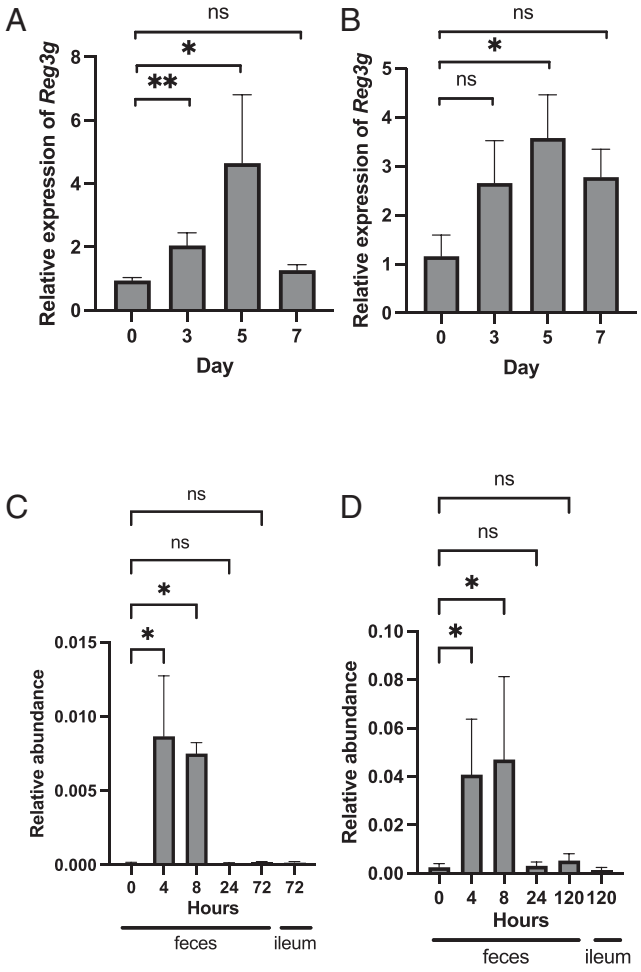

FIGURE 1. Induction of Reg3γ expression does not require colonization by *R. gnavus* or *L. reuteri*.

(**A** and **B**) qPCR analysis of small-intestinal *Reg3g* expression in mice treated with *R. gnavus* (A) or *L. reuteri* (B) at the indicated time points. (**C** and **D**) Mice were treated with *R. gnavus* (C) or *L. reuteri* (D), and fecal burden of these organisms was measured by qPCR at the indicated time points. *$p < 0.05$, **$p < 0.01$.

R. gnavus *and* L. reuteri *increase ileal Reg3γ protein levels without influencing Paneth cell numbers*

To ensure this change in *Reg3g* expression results in altered protein levels, we performed immunohistochemistry for Reg3γ in ileal samples obtained from mice treated with *R. gnavus* or *L. reuteri*. Compared with control animals, treatment with either *R. gnavus* or *L. reuteri* resulted in greater Reg3γ protein levels at the crypt base and along the villi (Fig. 2), which reflects Reg3γ in both Paneth cells and enterocytes. These results demonstrate that induction of *Reg3g* expression by *R. gnavus* and *L. reuteri* results in increased levels of protein.

This increase in Reg3γ protein in the crypt base could reflect either an increase in the number of Paneth cells or increased Reg3γ production within each Paneth cell. To discriminate between these possibilities, we enumerated Paneth cells in H&E-stained ileal sections and found no change in Paneth cell numbers in mice treated with either *R. gnavus* or *L. reuteri* (Fig. 3). These results demonstrate that the increase in Reg3γ is not a consequence of increased Paneth cell numbers but is more likely caused by increased Reg3γ production within each Paneth cell.

R. gnavus *and* L. reuteri *regulate Reg3γ expression via multiple mechanisms*

Previous studies have revealed that microbiota-induced expression of Reg3γ requires Myd88 and secretion of IL-22 by ILC3s (6, 13). These prior studies largely focused on the microbiota as a whole; however, given the pleiotropy of the microbiota, it is possible that specific microbes differ in their mechanism of Reg3γ induction. As such, we investigated whether Myd88 and/or ILC3s are required for induction of *Reg3g* by *R. gnavus* and *L. reuteri*. Neither *R. gnavus* nor *L. reuteri* was able to induce *Reg3g* expression in *Myd88*$^{-/-}$ mice (Fig. 4A), a finding that indicates Myd88 is required for induction of *Reg3g* by these

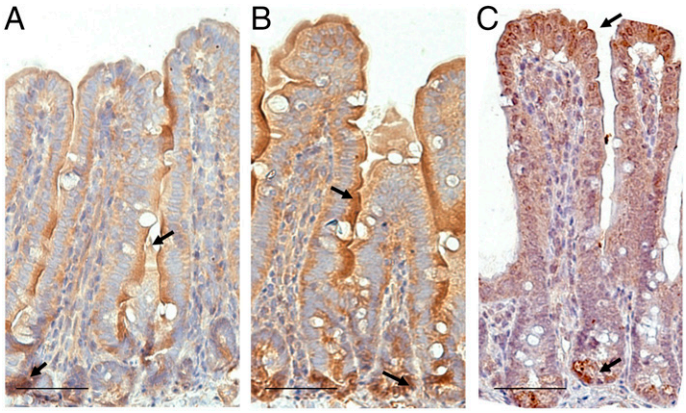

FIGURE 2. Ileal Reg3γ protein level is increased in *R. gnavus*– and *L. reuteri*–treated mice.

Immunohistochemistry of Reg3γ (visualized in brown) in ileal tissue obtained from mice treated with sterile bacterial media (control) (**A**), *R. gnavus* (**B**), or *L. reuteri* (**C**). Arrows highlight Reg3γ in crypts and along the villi. Each image is representative of four mice. Samples for (A) and (B) were obtained 3 d after treatment, whereas samples for (C) were obtained after 5 d. Original magnification ×40. Scale bar, 50 μm.

organisms. Surprisingly, *Myd88*$^{-/-}$ mice treated with either bacterium had suppressed levels of *Reg3g* expression, which indicates that, in the absence of Myd88 signaling, these bacteria inhibit *Reg3g* expression.

To determine the role of ILC3s in *R. gnavus* and *L. reuteri* stimulation of *Reg3g* expression, we generated ILC3-deficient mice (ILC3 knockout [KO]) by crossing *Ahr*$^{fl/fl}$ mice to *Rorc-cre* mice as previously described (24). Consistent with previous work demonstrating the role of ILC3s in *Reg3g* expression (13), ILC3 KO mice have reduced *Reg3g* expression compared with *Ahr*$^{fl/fl}$ littermate controls (Fig. 4B). Neither *R. gnavus* nor *L. reuteri* induced *Reg3g* expression levels in ILC3 KO mice (Fig. 4B), which demonstrates that ILC3s are critical for induction of *Reg3g* by these organisms. Similar to our results with *Myd88*$^{-/-}$ mice, we found *R. gnavus* and *L. reuteri* suppressed *Reg3g* expression in ILC3 KO animals (Fig. 4B). Taken together, our results establish that Myd88 and ILC3s are both required for *Reg3g* induction by *R. gnavus* and *L. reuteri*. In addition, these bacteria suppress *Reg3g* expression in an Myd88- and ILC3-independent manner.

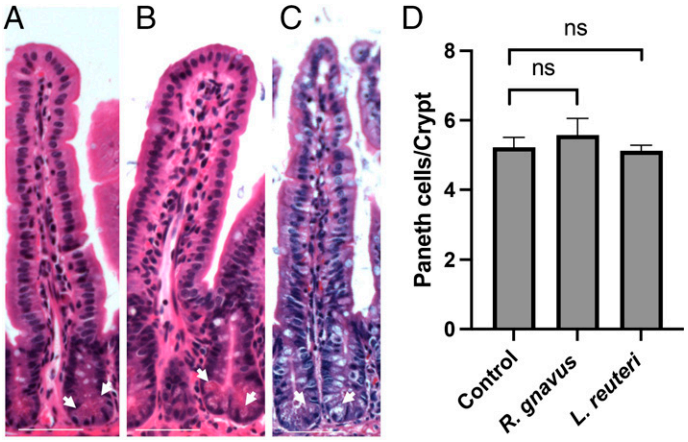

FIGURE 3. Paneth cell numbers are not affected by *R. gnavus* or *L. reuteri* treatment.

(**A**–**C**) H&E-stained ileal tissue from mice treated with sterile bacterial media (control; A), *R. gnavus* (B), or *L. reuteri* (C). Arrows highlight Paneth cells at the crypt base. Samples for (A) and (B) were obtained 3 d after treatment, whereas samples for (C) were obtained after 5 d. Original magnification ×40. Scale bar, 50 μm. (**D**) Average number of Paneth cells per crypt in control, *R. gnavus*–treated, and *L. reuteri*–treated mice. A minimum of 10 crypts/mouse were assessed (n = 4 mice/group).

R. gnavus *and* L. reuteri *differ in their requirement for Myd88 and ILC3s to induce expression of multiple AMPs*

The intestinal epithelium secretes several AMPs in addition to Reg3γ (7, 25). Because *R. gnavus* and *L. reuteri* induce ileal expression of Reg3γ, we investigated whether these organisms also induce expression of other AMPs. We treated mice with either *Parabacteroides distasonis* (an irrelevant bacterial control), *R. gnavus*, or *L. reuteri* and examined ileal expression of *Reg3b*, *Defa5* (α-defensin 5), and *Lyz1* (lysozyme). Although both *R. gnavus* and *L. reuteri* significantly induce *Reg3b* expression, only *R. gnavus* increased expression of *Defa5* and *Lyz1* (Fig. 5). These results demonstrate that *R. gnavus* and *L. reuteri* differ in their ability to induce diverse AMPs.

Given that AMPs differ in their regulation (7, 11), we investigated whether Myd88 and ILC3s are required for *R. gnavus*– or *L. reuteri*–mediated induction of these additional AMPs. We quantified ileal expression of *Reg3b*, *Defa5*, and *Lyz1* in *Myd88*$^{-/-}$ and ILC3 KO mice treated with either *R. gnavus* or *L. reuteri* (Fig. 5B). *R. gnavus* and *L. reuteri* are unable to induce expression of these additional AMPs in *Myd88*$^{-/-}$ mice (Fig. 5), a finding that establishes Myd88 is required in this process. ILC3s are required for normal expression of *Reg3b* and *Lyz1* as evidenced by lower expression in ILC3 KO mice compared with *Ahr*$^{fl/fl}$ littermate controls; neither *R. gnavus* nor *L. reuteri* induces these AMPs in ILC3 KO mice, which establishes that these commensal bacteria induce *Reg3b* and *Lyz1* expression in an ILC3-dependent manner. Interestingly, we observed no difference in *Defa5* expression between *Ahr*$^{fl/fl}$ and ILC3 KO mice, a finding that indicates ILC3s are not required for *Defa5* expression (Fig. 5C); however, *R. gnavus* treatment no longer induces *Defa5* expression in the ILC3 KO mice, establishing that this bacterium that normally induces expression of *Defa5* requires ILC3s to do so. Similar to our findings with Reg3γ, *R. gnavus* and *L. reuteri* suppressed expression of *Reg3b* and *Lyz1* in a Myd88- and ILC3-independent manner, whereas suppression of *Defa5* was observed only in Myd88$^{-/-}$ mice and not ILC3 KO mice. Interestingly, even though *L. reuteri* was unable to induce *Defa5* or *Lyz1* expression in wild-type mice, it can suppress expression of these genes in some genetic backgrounds. Taken together, our findings highlight differences between the AMP-inducing activities of *R. gnavus* and *L. reuteri* and demonstrate the complexity

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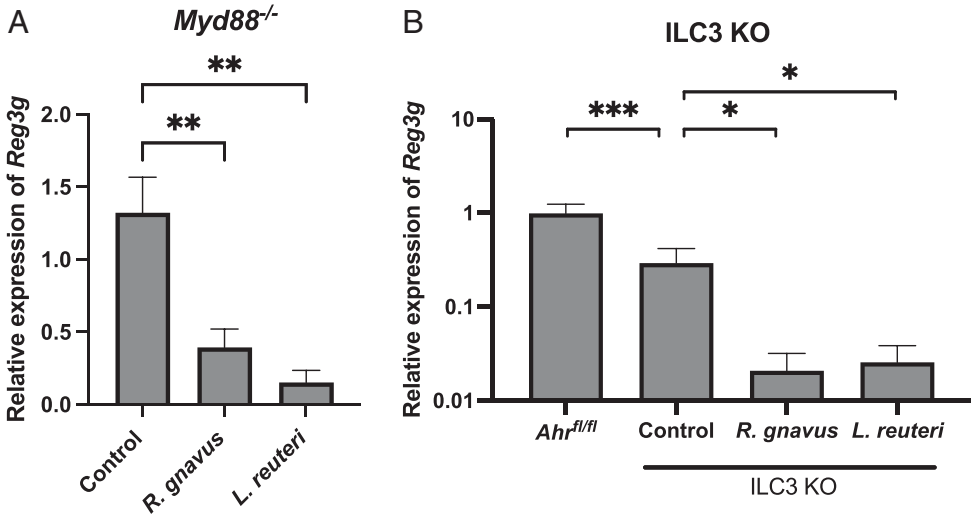

FIGURE 4. *R. gnavus* and *L. reuteri* require Myd88 and ILC3s for Reg3γ induction.
(**A** and **B**) qPCR analysis of small-intestinal *Reg3g* in mice treated with sterile bacterial media (control), *R. gnavus*, or *L. reuteri* in Myd88$^{-/-}$ mice (A) or *Ahr*$^{fl/fl}$ *Rorc-Cre* mice (ILC3 KO; B). Small-intestinal samples were obtained from the control and *R. gnavus*–treated animals 3 d after treatment; samples were obtained from *L. reuteri*–treated mice 5 d after treatment. In (B), *Ahr*$^{fl/fl}$ littermates were used as an ILC3-sufficient control, and the *y*-axis is shown as a log scale. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

underlying microbial regulation of these genes, the specifics of which differ between AMPs.

DISCUSSION

AMPs play a critical role in protecting the host against infection, and their proper regulation is paramount. Earlier studies demonstrated that induction of Reg3γ, a canonical AMP, requires microbial signals to activate Myd88 and ILC3s (8, 10, 13); however, these studies compared conventional mice with a complex microbiota with germ-free mice. As such, it is possible that some bacteria use a Myd88 pathway, whereas others exploit an ILC3-dependent pathway to induce Reg3γ. In this study, we used *R. gnavus* and *L. reuteri* as archetypal Reg3γ-inducing commensal

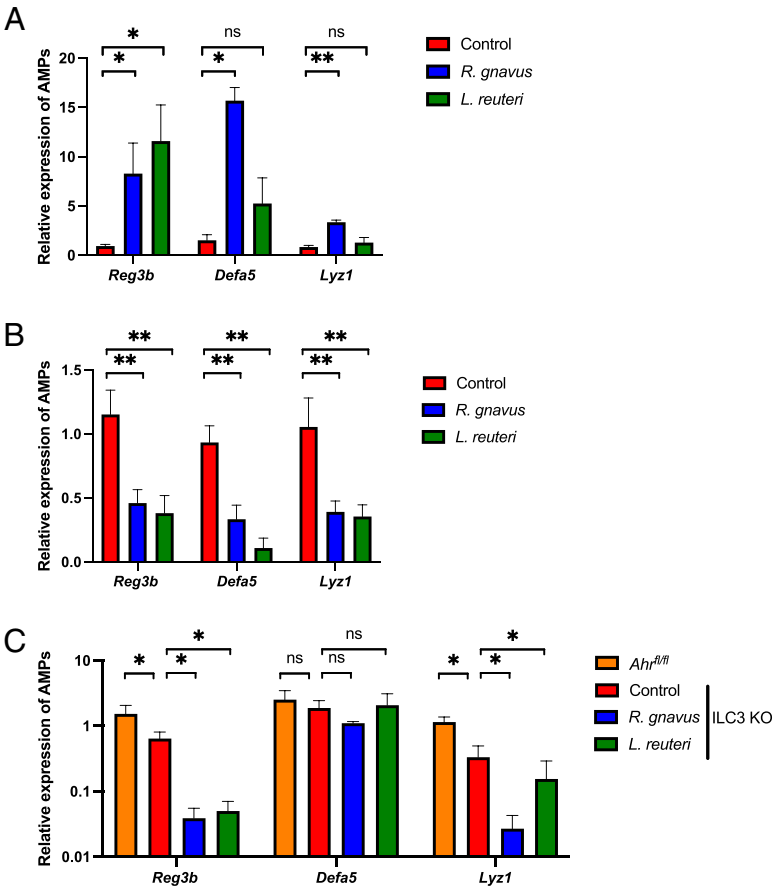

FIGURE 5. *R. gnavus* and *L. reuteri* differ in their requirement for Myd88 and ILC3s to induce expression of multiple AMPs.
(**A**) qPCR analysis of small-intestinal *Reg3b*, *Defa5* (α-defensin 5), and *Lyz1* (lysozyme) in mice treated with *P. distasonis* (control bacterium), *R. gnavus*, or *L. reuteri*. (**B** and **C**) qPCR analysis of small-intestinal *Reg3b*, *Defa5*, and *Lyz1* in mice treated with a media control, *R. gnavus*, or *L. reuteri* in *Myd88*$^{-/-}$ mice (B) or *Ahr*$^{fl/fl}$ littermates and ILC3-deficient mice (C). Small-intestinal samples were obtained from the control and *R. gnavus*–treated animals 3 d after treatment; samples were obtained from *L. reuteri*–treated mice 5 d after treatment. In (C), the *y*-axis is shown as a log scale. *$p < 0.05$, **$p < 0.01$.

bacteria to clarify the mechanism(s) of Reg3γ induction by specific bacteria. We find neither bacterium can induce *Reg3g* expression in the absence of either Myd88 or ILC3s, findings that demonstrate individual bacteria require both pathways for AMP expression.

For these studies, we used mice that lacked Myd88 in all cells. Although Myd88 expression by Paneth cells is sufficient for Reg3γ expression (10), we do not know whether *R. gnavus* and *L. reuteri* are interacting with Myd88 on Paneth cells, other enterocytes, or immune cells (e.g., dendritic cells) in the lamina propria. We speculate these bacteria signal through Myd88 present on non-Paneth cells (potentially in addition to Paneth cell–associated Myd88) because of the increased levels of Reg3γ we observed in the villi and not just the crypts where Paneth cells are localized. We observed a difference between the induction of *Reg3g* in the villus, with *R. gnavus* having an effect in the midvillus and *L. reuteri* induction occurring at the villus tips. However, this distinction likely reflects the fact that these images were taken at different days after bacterial administration (day 3 for *R. gnavus* and day 5 for *L. reuteri*), which corresponds with the idea that enterocytes take ∼5 d to complete their migration to the top of the villus (26). Our data suggest that the induction of *Reg3g* persists for this entire time and overrides the normal zonation program present in the small intestine (27). Given that all our bacterial treatments were done in the context of mice with a complex microbiota, it is possible the requisite Myd88 or ILC3 signal comes from the endogenous microbiota, with *R. gnavus* or *L. reuteri* providing the second signal. Discriminating between these differences may require identifying the relevant bacterial factor that induces Reg3γ and determining whether it signals through Myd88, ILC3s, or both.

Interestingly, in the absence of either Myd88 or ILC3s, treatment of mice with either *R. gnavus* or *L. reuteri* led to suppression of *Reg3g* expression. This finding indicates that these bacteria target at least two different pathways that regulate *Reg3g* expression in discordant ways. Future work will determine whether this decreased expression results in a decreased level of protein. Given that *R. gnavus* and *L. reuteri* also lead to suppression of other AMPs in a Myd88- and ILC3-independent manner, it is likely the same pathway regulates multiple AMPs. One possibility is that *R. gnavus* and *L. reuteri* inhibit expression of peroxisome proliferative-activated receptor α, which is known to be negatively regulated by commensal bacteria and increase *Reg3g* expression (28, 29). Comparing transcriptional profiles in wild-type, Myd88$^{-/-}$, and ILC3-deficient animals may help identify this inhibitory pathway.

By comparing the colonization kinetics of *R. gnavus* and *L. reuteri* with the kinetics of *Reg3g* induction, we found that colonization was not required for activity. This delay between bacterial exposure and increased Reg3γ levels indicates that the process of *Reg3g* induction is relatively slow. Although some work has suggested that commensal organisms must colonize the host to be effective (30, 31), others have similarly shown that transient exposure to bacteria and/or bacterial products can have long-lasting effects (32, 33). With Reg3γ specifically, it has been shown that TLR ligands are sufficient for induction (12). It is likely that the bioactive molecule from *R. gnavus* and *L. reuteri* is either present on their surface or secreted into the culture media such that the long-term colonization by the organisms is not required.

In summary, we have detailed the mechanism by which two specific commensal bacteria induce *Reg3g* expression in the ileum. Moreover, our studies revealed that regulation of *Reg3g* expression by the microbiota is more complex than previously thought and involves both stimulatory and inhibitory pathways. These findings may aid the development of microbiome-derived treatments that protect against infection by increasing AMP expression.

DISCLOSURES

The authors have no financial conflicts of interest.

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