Regulating colonic dendritic cells by commensal glycosylated large surface layer protein A to sustain gut homeostasis against pathogenic inflammation

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Microbial interaction with the host through sensing receptors, including SIGNR1, sustains intestinal homeostasis against pathogenic inflammation. The newly discovered commensal Propionibacterium strain, P. UF1, regulates the intestinal immunity against pathogen challenge. However, the molecular events driving intestinal phagocytic cell response, including colonic dendritic cells (DCs), by this bacterium are still elusive. Here, we demonstrate that the glycosylation of bacterial large surface layer protein A (LspA) by protein O-mannosyltransferase 1 (Pmt1) regulates the interaction with SIGNR1, resulting in the control of DC transcriptomic and metabolomic machineries. Programmed DCs promote protective T cell response to intestinal Listeria infection and resist chemically induced colitis in mice. Thus, our findings may highlight a novel molecular mechanism by which commensal surface glycosylation interacting with SIGNR1 directs the intestinal homeostasis to potentially protect the host against proinflammatory signals inducing colonic tissue damage.

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INTRODUCTION

Commensal bacteria, via their surface layer (S-layer) gene products, and the gastrointestinal phagocytic cells expressing sensing receptors (e.g., SIGNR1) synergistically interact to fine-tune the T cell signaling that is critical for protecting the host against pathogenic inflammation exerted by intestinal infections.1,2 In this process, bacterial S-layer macromolecules along with induced metabolites transduce critical signals via cognate receptors into these cells that profoundly control the host homeostasis to protect against tissue damage.3,4 Although the bacterial S-layer proteins display a similar architecture composed of a peptidoglycan layer decorated with proteins and polysaccharides, various modifications, particularly glycosylation, exhibit strain-specific properties that differentially modify the host immune physiology.5 Disruption of mutualistic interactions of the commensal’s S-layer with the host triggers deleterious signals that may manifest in pathogenic inflammation potentially impairing the intestinal barrier function.6 Thus, understanding how host intestinal immunity is regulated through the recognition of these well-structured bacterial gene products by their cognate receptors7 to coordinate protective immune responses is currently of particular therapeutic significance and requires further mechanistic investigations.

Propionibacterium strain, P. UF1, is a newly discovered commensal bacterium isolated from the gut microbiota of premature infants fed human breast milk.9 This bacterium increases the frequency of colonic Th17 and Treg cells involved in mucosal barrier repair and regulation of the intestinal inflammation.10 Induced bacteria-specific Th17 cell differentiation requires the bacterial dihydrolipoamide acetyltransferase (DlaT), an enzymatic component of the pyruvate dehydrogenase complex.9 Chromosomal deletion of dlaT gene impairs the regulation of protective Th17 cell response to intestinal and systemic Listeria monocytogenes (L. m) infection.9,11 Furthermore, P. UF1 regulates the neonatal T cells against necrotizing enterocolitis (NEC)-like injury in mice9 and enhances the neonatal protective T cells against intestinal pathogen infection over time.12 However, the bacterial effector mechanisms potentially instructing the function of colonic DCs to possibly control protective T cell immunity remain largely unknown. Here, we demonstrate that the glycosylation of bacterial LspA interacting with SIGNR1 is a pivotal factor, which transcriptionally and metabolically programs colonic DCs, leading to protective T cell activation in steady state and during intestinal infection. Further, glycosylated LspA-SIGNR1 interaction critically protects mice against colitis-induced intestinal barrier injury. Errors in the bacterial glycosylation significantly disrupt the intestinal homeostasis, manifesting in an inflammatory condition resulting in pathogen persistence and colonic tissue damage. Thus, this finding highlights the critical relevance of the glycosylated LspA in programming DC immunophysiology to mitigate pathogenic inflammation and the induced colitogenic potential in mice.
RESULTS
Glycosylation of LspA by Pmt1
Knowing the significance of bacterial S-layer complexes in communicating with host cells, we sought to investigate the functional relevance of P. UF1 S-layer proteins potentially involved in the regulation of colonic DC function. One of the S-layer proteins of P. UF1 is LspA, which contains six N-terminal LGFP repeats [L-G-X-P-(7-8)-D/N-G] involved in cell membrane anchoring and a C-terminal N-acetylglucosaminidase-like domain, potentially implicated in bacterial cell wall metabolism (Supplementary Fig. 1a). Phylogenetic analysis demonstrated that LspA was highly conserved in P. UF1 and closely related Propionibacterium strains. Moreover, LspA homologs were also found in evolutionarily distantly related bacterial species, including Bifidobacterium and Geodermatophilus (Supplementary Fig. 1b). Thus, to elucidate the functional significance of LspA within P. UF1 molecular machinery, the lspA gene was deleted from the bacterial chromosome, resulting in ∆lspA P. UF1 (Fig. 1a, b). ∆lspA P. UF1 demonstrated enhanced bacterial clusters and autoagglutination (Fig. 1c), suggesting the critical involvement of this protein in bacterial S-layer structures. Further, deletion of LspA significantly affected the bacterial transcriptomic and metabolomic signaling, including differential metabolic pathways involved in peptidoglycan biosynthesis, amino and nucleotide sugar metabolism, fructose and mannose metabolism (Supplementary Fig. 2a). The analyzed metabolites involved in protein glycosylation (e.g., GDP-mannose and mannose 1-phosphate), along with those important for cell wall metabolism (e.g., GlcNAc-6-phosphate and UDP-GlcNAc), were significantly deregulated within ∆lspA P. UF1 compared to P. UF1 (Supplementary Fig. 2b).

RNA-Seq analysis further documented differentially expressed genes implicated in bacterial mannosylation and nucleotide sugar metabolism, including phosphatidylinositol mannosyltransferase pimt1 and GDP-mannose-dependent alpha-mannosyltransferase mgts1 (Supplementary Fig. 2c). Thus, these data emphasize the importance of LspA in the regulation of glycans metabolism that may fundamentally impact the bacterial S-layer glycosylation.

The bacterial S-layer proteins are generally glycosylated for their noncovalent anchoring to the cell surface and interactions with environmental factors and host immune cells. Data demonstrated that the S-layer of P. UF1 reacted with concanavalin A (ConA), a mannose/glucose-binding lectin, while LspA deficiency resulted in the loss of ConA binding (Fig. 1d), suggesting that LspA may be glycosylated. Therefore, we investigated the glycosyltransferases responsible for adding glycan moieties to the bacterial S-layer using genome-wide bioinformatic analysis. Pmt1, a potential member of protein O-mannosyltransferase family responsible for mannose transfer to serine and threonine residues of proteins in yeast was identified in P. UF1 genome. Further analysis demonstrated that Pmt1 homologs fell into separate and loosely related groups of bacteria, including Actinobacteria (Fig. 1e). The pmt1 gene was then deleted in P. UF1 to assess the status of LspA glycosylation (Fig. 1f). Although Δpmt1 P. UF1 showed similar S-layer protein patterns when compared to P. UF1, no binding to ConA was observed for S-layer proteins isolated from Δpmt1 P. UF1 (Fig. 1g). To underscore the role of Pmt1 in the glycosylation of LspA, this protein was overproduced by ∆lspA P. UF1 and Δpmt1 P. UF1 strains. While the glycosylated LspA (G-LspA) and non-glycosylated LspA (NG-LspA), respectively, (Fig. 1h), while both G-LspA and NG-LspA were recognized by anti-LspA serum antibodies, only purified G-LspA bound to ConA and illustrated staining for glycoprotein (Fig. 1i). Thus, Pmt1 is critically required for the glycosylation of LspA.

O-mannosylated LspA interaction with SIGNR1
To elaborate on the nature of LspA glycosylation, the purified G-LspA and NG-LspA proteins were treated with PNGase F to release any N-glycans, permethylated and analyzed by MALDI-MS. Here, no N-linked glycans were detected in either of the LspA proteins (Supplementary Fig. 1c). The O-linked glycans were released by β-elimination procedure and permethylated prior to MALDI-MS analysis. Signals corresponding to Hex-, Hexa-, were observed in the G-LspA protein (Fig. 2a), but not in the NG-LspA (Supplementary Fig. 1d). Furthermore, glycan compositional analysis demonstrated that mannose (Man) was the major monosaccharide of G-LspA, with a retention time of 10.9 min (Fig. 2b). In contrast, NG-LspA showed no traces of Man (Supplementary Fig. 1e). Note that a minor peak of glucose was also detected in both samples. However, glucose, as a very common contaminant, could be derived from reagents and detected as a free and minor glucose peak in HPAEC analysis. Moreover, glycomic analysis of released oligosaccharides demonstrated that Manα was the major glycan in the G-LspA, comprising 77% of the total glycans. While Manβ and Manα oligosaccharides were minor glycans, only traces of Manβ, Manα and Manβ were detected in the G-LspA (Fig. 2c).

The purified G-LspA protein was then digested with trypsin and elastase, resulting in peptides with >75% coverage (Supplementary Fig. 1a). LC-MS/MS analysis of the enriched peptides revealed seven O-glycopeptides at the N-terminus of LspA (Fig. 2d). In addition to the 41 threonine/serine residues involved in Man attachment, adjacent proline and alanine residues that may facilitate local conformational changes for protein O-glycosylation were also found in all the glycopeptides (Fig. 2d). Further, GC-MS analysis was performed to investigate the glycosyl linkages and positions of released O-glycans. Data demonstrated that Man oligosaccharides of LspA were short linear chains interconnected via (1→2)-linkage (Fig. 2e). Man(1→6)Man(1→2)Man comprised a small percentage (Fig. 2e). These data indicate that LspA is a mannosylated S-layer glycoprotein with linear short-chain O-glycans.

SIGNR1 expressed by myeloid DCs recognizes characteristic molecular patterns with complex mannos and fucose structures in bacteria and fungi. Recently, we observed the binding of P. UF1 to SIGNR1, but not SIGNR3. To precisely delineate the role of G-LspA binding to SIGNR1 in regulating DCs to subsequently initiate T cell commitment, the interaction of G-LspA with SIGNR1 was biochemically investigated. Here, G-LspA and NG-LspA proteins were first separated by SDS-PAGE, transferred to PVDF membrane, and then incubated with SIGNR1-hFc fusion protein. The protein binding complex was analyzed by subsequent incubation with anti-human Fc secondary antibody. Data demonstrated that the purified G-LspA bound specifically to SIGNR1-hFc, and this binding was abolished in the presence of EDTA (Fig. 2f).

Further, SIGNR1 interaction with G-LspA was assessed by ELISA showing G-LspA binding to SIGNR1-hFc (Fig. 2g), but not to Dectin-1-hFc used as a control fusion protein (Fig. 2h). This binding was completely blocked by pre-incubation of SIGNR1-hFc with anti-SIGNR1 antibody, or with the competitive ligand zymosan that is composed of β-glucan, α-mannan and mannosyl proteins (Fig. 2g). In contrast, no binding was observed for NG-LspA using similar assays (Fig. 2g). Furthermore, the G-LspA exhibited a dose-dependent binding with SIGNR1-hFc using protein concentrations ranging from 0.08 μg/ml to 10 μg/ml with a Kd value of 2.617 μg/ml G-LspA (Fig. 2i). In contrast, NG-LspA did not react with SIGNR1-hFc, even with higher protein concentrations up to 20 μg/ml (Fig. 2i), highlighting the specificity of SIGNR1 binding to glycosylated LspA.

Activation of colonic DCs by glycosylated LspA in steady state SIGNR1 was majorly expressed by colonic CD11c+ MHCIi+ DCs (Supplementary 3a, b). To shed light on the relevance of LspA glycosylation interacting with colonic DCs, CD11c+ MHCIi+ CD11b+ F4/80+ DCs were FACs sorted (Supplementary Fig. 4a, b) from mice gavaged with P. UF1 or ∆lspA P. UF1 to analyze their Mucosal Immunology (2020) 13:34 – 46
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transcriptome by RNA-Seq. Data demonstrated the modulation of costimulatory molecules (Cd40, Cd80, Cd86, and Tnfsf4) in colonic DCs by P. UF1 (Fig. 3a). NF-κB signaling (Casp4, Traf1, Tnfrsf1b, Mopk6, Nfkbia, and Nfkbia2), cytokine/chemokine transcripts (e.g., Il1b, Il12b, Cxcl1, and Cxcl2), and antigen presentation-related genes (e.g., Serpinb9, Rab8b) were also significantly augmented in DCs derived from mice gavaged with P. UF1 compared to ΔispA P. UF1. In contrast, DCs derived from ΔispA P. UF1-gavaged mice showed activation of Sod3, Rhoh and Klf2, which may instruct functional suppression in these cells. Migrating DCs constitutively express genes with regulatory functions. Accordingly, DCs derived from mice gavaged with P. UF1 had elevated expression of genes, such as Cd274, Spred1, Etv3, Tnfrnlp2, Stat3, Stat4, and Stat5a. These cells were also enriched with genes implicated in DC development (Edn1, Cish), migration (Nrp2, Ccr10, Eps8), and differentiation (Pdk1, Hilpda), while DCs of ΔispA P. UF1-gavaged mice exhibited increased quantities of genes suppressing cellular regulatory functions (e.g., Cyr61, Sdc1). In addition, transcription factor Ifi4 controlling Th17 cell cytokine machinery,20 cell cycle inhibitor Cdkn1a involved in Treg cell formation,21 and T cell-attracting chemokines Ccl17 and Ccl22, were all significantly activated in DCs of P. UF1-gavaged mice. In contrast, ΔispA P. UF1 enhanced the DC expression of Cd55 and GIlz genes associated with suppression of T cell function.22,23

Gene set enrichment analysis (GSEA) demonstrated that gene sets for metabolic activities (e.g., glycolysis and oxidative phosphorylation (OXPHOS)) and DC activation, including activation of innate responses, regulation of i-kB/NF-κB signaling, cytokine receptor activity and cell migration, were enriched in DCs derived from mice gavaged with P. UF1 (Fig. 3b). In contrast,
Fig. 2  Recognition of glycosylated LspA by SIGNR1. a O-linked glycan analysis of β-eliminated and permethylated G-LspA protein. Asterisk (*) indicates the contamination peak derived from reagents. b Glycosyl composition analysis of monosaccharides in the G-LspA sample. Trace levels of glucose were detected as a common contaminant derived from reagents. c Summary table showing the relative percentage of O-linked glycans from G-LspA. d LspA glycopeptides identified by glycoproteomics. The glycan composition and potential glycosylation sites (bolded) are shown. e Glycosyl linkage analysis of the O-glycans. Asterisks (•) indicate non-carbohydrate peaks. RT, retention time. f Binding of G-LspA to SIGNR1-hFc. Equal amounts of G-LspA and NG-LspA proteins were separated by SDS-PAGE and the specific interactions with SIGNR1-hFc were demonstrated, as no binding was detected in the presence of EDTA. g ELISA binding assays demonstrating G-LspA binding specificity with SIGNR1-hFc. The binding was abolished in the presence of EDTA, competitive zymosan, or blocking antibody to SIGNR1. h ELISA showing G-LspA did not bind to Dectin-1-hFc, as a control fusion protein. Zymosan served as a positive control. i Binding kinetics between G-LspA and SIGNR1-hFc. Various amounts of LspA proteins were coated on ELISA plates and incubated with SIGNR1-hFc (0.5 μg/ml). Binding was detected using HRP-conjugated anti-human IgG antibody. $K_d$, LspA concentration required to achieve a half-maximum binding with SIGNR1-hFc.
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Fig. 3 Modulation of DC activation by glycosylated LspA in steady state. a-b C57BL/6 (Signr1+/+) mice were gavaged with P. UF1 or ΔlspA P. UF1, and colonic CD11c+ MHCIIdCD11b+ F4/80+ DCs were isolated for RNA-Seq analysis. Heatmap showing a selection of top differentially expressed genes (FDR P < 0.05, fold change ≥ 1.5). Gene set enrichment analysis (GSEA) showing differentially enriched gene sets. Number signs (#) indicate gene sets without FDR correction (P < 0.05).

c Colonic CD11c+ MHCIIdCD11b+ F4/80+ DCs were FACS sorted from Signr1−/− and Signr1+/+ mice gavaged with P. UF1 or ΔlspA P. UF1 and transcripts of indicated genes in these groups of DCs were analyzed by qRT-PCR. Data are from 1 experiment (n = 3–4 mice/group). Error bars indicate SEM. *P < 0.05, **P < 0.01, ***P < 0.001, two-tailed unpaired t test.

Glycosylated LspA deficiency abrogated cellular protein responses in DCs, resulting in enhanced endoplasmic reticulum (ER) stress-associated activities (Fig. 3b), which were associated with increased transcripts of unfolded protein response (UPR)–related genes Xbp1, Derl1, and Edem2 in these cells (Fig. 3a). Further, P. UF1-induced DC activation (e.g., Cd80, Cd86, Il1b, and Il12b) was abrogated due to Signr1 deficiency (Fig. 3c). Moreover, loss of LspA glycosylation (ΔlspA P. UF1-gavaged Signr1−/− mice) mirrored the deficiency of SIGNR1 (P. UF1-gavaged Signr1−/− mice), suggesting a role of glycosylated LspA-SIGNR1 interaction in DC activation.

Having shown that LspA deficiency impacted the transcriptome of DCs, we asked whether LspA deficiency would also influence the regulation of T cell polarization in steady state. Thus, C57BL/6 mice were gavaged with P. UF1 or ΔlspA P. UF1, and then analyze colonic DC and T cell responses. Data demonstrated that ΔlspA P. UF1 compared to P. UF1 significantly decreased DC and T cell responses, including IL-10− Th17 cells and 10− Tregs (Supplementary Fig. 5a, b). Notably, no difference in intestinal bacterial colonization was observed in mice gavaged with P. UF1 or ΔlspA P. UF1, as both strains transiently colonized conventional mice (Supplementary Fig. 5c), suggesting that glycosylated LspA is not involved in bacterial colonization, but instructs DC activation to polarize T cells.

Programming transcriptomic and metabolomic DC-machiiners during intestinal infection

Intestinal DCs rapidly respond to invading pathogens.24 However, these cells may be functionally conditioned by pathological surrounding signals induced by intestinal infections.25 To elaborate on the bacterial glycosylated LspA influencing DC transcriptome, colonic MHCIIdCD11c+CD11b+ F4/80− DCs were FACS sorted from mice gavaged with P. UF1 or ΔlspA P. UF1 and then orally infected with ΔlspA P. UF1, respectively (Fig. 4a).

Further, transcripts of Tnips regulating NF-κB activation and Dok-2 suppressing Ras-Erk signaling were stimulated in colonic DCs isolated from mice gavaged with P. UF1. Importantly, Cd209b (Signr1), whose activation highly depends on the mannosylation of glycans, was upregulated in DCs derived from mice gavaged with P. UF1. In contrast, Toll-like receptors (Tlr3, Tlr4, and Tlr12) associated with proinflammatory response were upregulated in DCs of mice gavaged with ΔlspA P. UF1 (Fig. 4a, b). Mx1 and Ifit1, selectively activated during TLR-induced DC stimulation,26 were also enriched in DCs derived from ΔlspA P. UF1-gavaged mice. Consistently, a proinflammatory status was readily observed in DCs of mice gavaged with ΔlspA P. UF1. Accordingly, a set of proinflammatory...
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Fig. 4 Transcriptional programming of colonic DCs by P. UF1 expressing glycosylated LpsA during Listeria infection. C57BL/6 mice were gavaged with P. UF1 or ΔlspA P. UF1 and then orally infected with ΔactA L. m. Colonic CD11c<sup>+</sup> MHCII<sup>+</sup> CD11b<sup>+</sup> F4/80<sup>+</sup> DCs were FACS sorted for transcriptomic analysis seven days after infection. a Heatmap showing significantly differentially expressed genes (FDR < 0.05, fold change ≥ 1.5) in DCs derived from mice gavaged with P. UF1 compared to ΔlspA P. UF1. b qRT-PCR analysis of a selection of differentially expressed genes. Results are presented as fold change over DCs of P. UF1-gavaged mice. c GSEA showing differentially enriched pathways. Number signs (#) indicate gene sets without FDR correction (P < 0.05). Data are from 1 experiment (n = 4 mice/group). Error bars indicate SEM. *P < 0.05, **P < 0.01, two-tailed unpaired t test.

genes (e.g., Ifng, Tnfsf15, and Ifnb2), several genes associated with cell apoptosis (e.g., Daxx, Nab2, and Fbxw7), and stress-associated activities were activated in DCs derived from mice gavaged with ΔlspA P. UF1 (Fig. 4a, b). In addition, GSEA demonstrated enhanced mitochondrial biogenesis in DCs isolated from P. UF1-gavaged mice, as indicated by enriched gene sets for OXPHOS, TCA cycle and respiratory chain, and NADH dehydrogenase complex (Fig. 4c). Moreover, pathways regulating apoptosis and protein localization to ER were also enhanced in these cells. In contrast, DCs from ΔlspA P. UF1-gavaged mice exhibited dysregulated protein folding and ER stress-associated activities (Fig. 4c), consistent with increased transcripts of the UPR-related genes Xbp1 and Deri3 in these cells (Fig. 4a, b).

Inflammatory DC response is associated with declined mitochondrial activity promoted by TLR signaling. Having demonstrated that glycosylated LspA controlled TLR-activation and enriched gene sets for OXPHOS (Fig. 4), we further assessed whether P. UF1 expressing glycosylated LspA would maintain mitochondrial respiration during intestinal infection. Thus, colonic DCs were enriched by magnetic beads from mice gavaged with P. UF1 or ΔlspA P. UF1 and orally infected with ΔactA L. m to analyze the real-time changes in the rate of extracellular acidification (ECAR), a measurement of glycolysis, and the mitochondrial rate of oxygen consumption (OCR). While no difference in the ECAR was observed, OCR was significantly decreased in enriched DCs derived from mice gavaged with ΔlspA P. UF1 compared to DCs derived from P. UF1-gavaged mice (Fig. 5a). These data support the notion that glycosylated LspA may regulate DC metabolic function during intestinal infection.

It was recently demonstrated that DC function can be impacted by cellular metabolic factors that facilitate the biosynthetic and bioenergetic needs of these cells. To elaborate on this notion, colonic MHCII<sup>+</sup> CD11c<sup>+</sup> CD11b<sup>+</sup> F4/80<sup>+</sup> DCs were FACS sorted from mice gavaged with P. UF1 or ΔlspA P. UF1 and then infected with ΔactA L. m to analyze the metabolomic activity of these cells. Here, a distinct metabolome was identified in DCs derived from P. UF1-gavaged mice compared to those derived from ΔlspA P. UF1-gavaged mice (Fig. 5b). Metabolic pathways, including arginine and proline metabolism, purine metabolism and de novo fatty acid biosynthesis, were differentially activated in DCs isolated from the aforementioned groups of mice (Fig. 5c). Putatively annotated eicosatrienoic acid, known as an anti-inflammatory metabolite, 4-aminobutanal indicative of anti-inflammatory putescine metabolism, and proline involved in suppressing reactive oxygen species (ROS), were markedly accumulated in DCs from P. UF1-gavaged mice compared to the other group (Fig. 5d). In contrast, putative metabolites associated with
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![Diagram](image_url)

**Fig. 5** Metabolomic programming of colonic DCs by P. UF1 expressing glycosylated LspA during *Listeria* infection. a) Colonic DCs were enriched from mice gavaged with P. UF1 or ΔsspA P. UF1 and orally infected with ΔactA L. m. The mitochondrial respiration of enriched DCs by magnetic beads was evaluated by measuring their real-time change in the ECAR and OCR in response to sequential addition of oligomycin (Oligo), FCCP, rotenone (Rot), and antimycin A (Ant). Bar graphs show the basal ECAR and OCR. b) Metabolomic analysis of metabolites with intensities significantly altered by comparing FACS sorted DCs derived from P. UF1- and ΔsspA P. UF1-gavaged mice. Data from 1 experiment (n = 4–5 samples/group). Error bars indicate SEM. *P < 0.05, **P < 0.01, ***P < 0.001, two-tailed paired t test

Proinflammation and energy starvation [e.g., methylimidazoleacetic acid and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR)] and cell apoptosis-inducing deoxyadenosine were significantly enriched in DCs derived from ΔsspA P. UF1-gavaged mice (Fig. 5d). Thus, these data specifically demonstrate the requirement of glycosylated LspA expressed by P. UF1 that programs the regulation of DC function during *Listeria*-induced inflammation.

Regulating protective T cell response by P. UF1 expressing glycosylated LspA

To further elucidate the functional relevance of glycosylated LspA in modulating colonic homeostasis during intestinal infection, C57BL/6 mice were gavaged with P. UF1, ΔsspA P. UF1 or PBS, and then orally infected with ΔactA L. m. Notably, P. UF1, compared to ΔsspA P. UF1, regulated proinflammatory responses by controlling the frequencies and cell counts of IL-17A, IL-6, and IL-12/23p40+ DCs (Fig. 6a). However, such induced DC regulation was abolished in *Signr1+/-* mice gavaged with P. UF1, ΔsspA P. UF1 or PBS and subsequently infected with ΔactA L. m (Fig. 6a), denoting a potential role of glycosylated LspA-SIGNR1 interaction in controlling inflammatory DC response. Further, tuned DC response was associated with T cell regulation, leading to an increased percentage and number of Th17 and IL-10+ Treg cells in mice gavaged with P. UF1 compared to ΔsspA P. UF1 or PBS (Fig. 6b). Loss of protective immunity in *Signr1+/-* mice gavaged with ΔsspA P. UF1 resulted in delayed ΔactA L. m clearance compared to mice gavaged with P. UF1 (Fig. 6c). In addition, transcripts of proinflammatory molecules such as *Il6*, *Il12b*, *Ifng*, *Ccl5*, and *Cxc12* were significantly decreased in colonic tissues of *Signr1+/-* mice gavaged with P. UF1 compared to those gavaged with ΔsspA P. UF1 or PBS (Fig. 6d). In contrast, the protective T cell immunity was blunted in *Signr1+/-* mice gavaged with P. UF1, ΔsspA P. UF1 or PBS and then infected with ΔactA L. m (Fig. 6b), and no difference in pathogen clearance was observed in these groups of mice (Fig. 6c). Furthermore, data demonstrated that deficiency in bacterial glycosylated LspA did not induce any changes in the gut microbial composition of mice gavaged with ΔsspA P. UF1 compared to P. UF1 during ΔactA L. m infection (Supplementary Fig. 6a, b). Together, these results indicate the crucial role of glycosylated LspA-SIGNR1 interaction in regulating colonic DCs to orchestrate protective T cell response to intestinal *Listeria* infection.

Ameliorating dextran sulfate sodium (DSS)-induced colitis by P. UF1 expressing glycosylated LspA

IL-17A and IL-10+ Tregs contribute to protection against acute intestinal colitis. 30–32 To further investigate the protective...
Fig. 6 Requirement of glycosylated LspA for protective T cell regulation. Sign1+/− and Sign1−/− mice were gavaged with P. UF1, ΔlspA P. UF1 or PBS and then orally infected with ΔactA Lm. Seven days post infection, colonic DC and T cell responses were analyzed. a Percentages and total counts of IL-1β+, IL-6, and IL-12/23p40+ DCs in Sign1+/− (top panel) and Sign1−/− mice (bottom panel). Flow plots are from Sign1+/− mice. b Percentages and total counts of Th17, IL-10+ Th17, and IL-10+ FoxP3+ Treg cells in Sign1+/− (top panel) and Sign1−/− mice (bottom panel). Flow plots are from Sign1+/− mice. c Box and whisker plots of fecal ΔactA L. m burdens measured in Sign1+/− (left panel) and Sign1−/− mice (right panel) on days 1–4 after infection. Dashed lines represent the limit of pathogen detection. d Box and whisker plot showing transcript levels of proinflammatory molecules in the colonic tissues of Sign1+/− mice. Data are representative of 2 (Sign1+/− mice) or 3 (Sign1−/− mice) independent experiments (n = 5 mice/group, a, b) or pooled from 2 independent experiments (n = 9–10 mice/group, c, d). Error bars indicate SEM. *P < 0.05, **P < 0.01, ANOVA plus Tukey’s post-test (a, b) or Kruskal-Wallis plus Dunn’s post-test (c, d)

role of glycosylated LspA expressed by P. UF1 in chemically induced colitis, groups of mice were gavaged with P. UF1, ΔlspA P. UF1 or PBS, and the experimental colitis/tissue damage was induced by 3% DSS. Data demonstrated that P. UF1, compared to other groups of mice, significantly reduced the disease severity, as indicated by the reduction of weight loss, diarrhea and fecal blood scores (Fig. 7a, b), and increased colon length (Fig. 7c). Furthermore, severe signs of inflammation-induced thickening of the bowel wall and loss of regular blood vessel structures were observed in the colons of PBS- and ΔlspA P. UF1-gavaged mice but not in P. UF1-gavaged mice (Fig. 7d). Histological analysis demonstrated severe tissue destruction, characterized by extensive segmental to diffuse mucosal epithelium and crypt loss with associated mucosal and submucosal inflammation in the colonic tissues of mice gavaged with PBS or ΔlspA P. UF1, while DSS-induced colitis was substantially mitigated in mice gavaged with P. UF1 (Fig. 7e, f). Furthermore, obtained data also demonstrated that DSS-treated mice gavaged with P. UF1 were markedly protected from leaky gut when compared with other groups, as documented by FITC-dextran assay (Fig. 7g). Consistently, enhanced transcripts of tight junction proteins (Cldn2, Cldn3, Cldn7, Cldn8, and ZO-3) in colonic tissues of P. UF1-gavaged mice were observed compared to the other groups (Fig. 6h). Thus, glycosylated LspA expressed by P. UF1 contributes to the maintenance of intestinal barrier integrity.
To further elucidate the protective role of glycosylated LspA-SIGNR1 signaling in DSS-induced colitis, Signr1−/− mice were gavaged with P. UF1, ΔlspA P. UF1 or PBS and treated with DSS. Once again, lack of SIGNR1 interaction with glycosylated LspA resulted in no improvement of colitogenic disease progression in any of the groups of mice (Fig. 7a–f). Here, the disruption of glycosylated LspA-SIGNR1 interaction abolished the regulation of intestinal permeability (Fig. 7g) and blunted the expression of...
tight junction proteins in Signr1Δ−/− mice (Fig. 7). Collectively, these data suggest that glycosylated LspA interacting with SIGNR1 sustains gut homeostasis to protect against detrimental signals inducing tissue damage.

**DISCUSSION**

The maintenance of gut homeostasis requires a rigidly balanced dialog between the resident microbes and the host. This can be established through the interaction of a variety of bacterial gene products with their cognate receptors expressed by phagocytic cells. Disruption of such elegantly induced communication may result in pathogenic inflammation and intestinal tissue damage. In this study, we demonstrate a protective mechanism at the intestinal interface whereupon glycosylated LspA interacting with SIGNR1 dictates DC response that in turn regulates protective T cells against intestinal pathogen infection and chemically induced colitis.

There has recently been a growing interest in the bacterial S-layer proteins with multiple regulatory properties. Accordingly, the composition of the S-layer proteins of Propionibacterium species is remarkably variable, and the functions of bacterial S-layer proteins are strain-dependent. For instance, surface layer protein B (SlpB), highly expressed by some of Propionibacterium strains, facilitates bacterial adhesion to epithelial cells. While no SlpB homolog is found in P. UF1, LspA constitutes the major extractable S-layer protein in this bacterium. The high protein expression may also suggest that LspA, particularly in P. UF1, is a valuable factor for not only supporting the S-layer structure of this bacterium but could also be important for fine-tuning intestinal immunity and may serve as an elegant vehicle for mucosal vaccine and therapeutic approaches. Although LspA is conserved, the levels of protein expression vary dramatically in different Propionibacterium strains, indicating potentially differential gene regulation in these bacterial strains. With this notion in mind and the unique expression pattern of LspA by P. UF1, we were prompted to further elaborate on its physiological characteristics and its potential implication in regulating intestinal immunity. Here, we demonstrate that deletion of lspA leads to fundamental changes in pathways associated with S-layer carbohydrate metabolisms, including mannosylation, suggesting the critical role of LspA in maintaining the S-layer glycosylation profile. Indeed, ConA binding assays comparing S-layer proteins isolated from P. UF1 and ΔlspA P. UF1 show that glycosylation of other potential glycoproteins is also likely impaired by LspA deficiency. Further, changes in S-layer glycosylation may directly impact the bacterial cell-cell interactions, leading to enhanced autoagglutination due to LspA deficiency in P. UF1, possibly as a result of altered cell surface hydrophobicity. This is consistent with the observations that surface glycosylation, such as flagella glycosylation, is highly associated with autoagglutination, which is an important step for microcolony formation on the intestinal epithelial cells that contributes to intestinal colonization.

In this study, we also clearly demonstrate that LspA is an O-mannosylated glycoprotein, uniquely representing the first glycoprotein characterized in Propionibacteria. Furthermore, the glycosylation of LspA critically requires Pmt1 activity. This feature is reminiscent of protein glycosylation in Streptomyces and Mycobacterium, whereupon the O-glycosylation of surface lipoproteins or phosphate-binding protein PstS, is highly dependent on the membrane-associated lipoprotein Pmt. Thus, the Pmt-mediated protein O-mannosylation seems to be a general pathway in actinomycetes. In fungi and yeasts, up to seven Pmt family members have been identified thus far, and the homomer and/or heteromeric interactions among Pmt members cooperatively initiate the protein glycosylation, resulting in the biosynthesis of diversified glycan structures. However, Pmt1 seems to be the only protein glycosyltransferase in P. UF1, and no other protein glycosylation is observed in the S-layer proteins isolated from Δpmt1 P. UF1. Furthermore, the linear short-chain mannose is found to be the major glycan structure of bacterial LspA; thus, concluding that Pmt1 is the crucial enzyme responsible for modifying S-layer proteins, particularly LspA, with simple mannoses in P. UF1.

Recognition of microbial gene products by sensing receptors, including C-type lectins, is essential for translating the nature of microbes into gene-transcriptional and metabolic programs that may initiate the regulation of DC function to prime T cell polarization. SIGNR1, a murine homolog of human DC-SIGN, conditions intestinal DCs for the induction of oral tolerance and plays a key role in host defense against pathogen infection. However, further rigid molecular studies are still required to mechanistically elucidate the gene and metabolic programs that are modulated by the interactions of this sensing receptor with the bacterial surface glycosylation. Here, we demonstrate that LspA glycosylation is required to be recognized by its receptor, SIGNR1, leading to the regulation of DC activation in steady state and during pathogen-induced inflammation. Accordingly, in steady state P. UF1, via its glycosylated LspA, seems to optimally induce the activation of colonic DCs when compared with its counterpart, ΔlspA P. UF1. Mainly, P. UF1 regulates the expression of costimulatory, cytokine and antigen presentation molecules (e.g., C440, Iitb, Serpinb9, Rab8b, and Bcl3) in colonic DCs while glycosylated LspA deficiency results in the activation of suppressive molecules (e.g., Sod3, Rhoh and Ifit2, Cry61, Sdc1) that may dysfunction colonic DCs to properly induce T cell differentiation. More importantly, P. UF1 decorated with glycosylated LspA regulates DCs that control T cell response to pathogen infections. Here, regulatory signaling appears to be activated by LspA glycosylation, as Tgfβ1, 9Tc2, and Cited2 genes associated with suppression of TGF-β signaling are tuned down in DCs derived from mice gavaged with P. UF1 compared to ΔlspA P. UF1. Further, proinflammatory genes (e.g., Il1α and Il6) are also downregulated in DCs from mice gavaged with P. UF1. Collectively, anti-inflammatory polyunsaturated fatty acid eicosa-trienoic acid is highly enriched in DCs of P. UF1-gavaged mice, while proinflammatory metabolites, such as methylimidazolacetic acid and AICAR, are significantly elevated in DCs of ΔlspA P. UF1-gavaged mice during Listeria infection. In addition, DCs derived from ΔlspA P. UF1-gavaged mice exhibit significant quantities of genes associated with cell apoptosis and stress response, consistent with decreased cellular proline levels that are important for protecting against various cellular stresses. This may emphasize the impaired DC metabolism induced by...
glicosylated LspA deficiency, which is further supported by reduced mitochondrial respiration and enriched metabolite AICAR that serves as a metabolic activator responding to energy starvation of the cells. Collectively, these data suggest that LspA glicosylation may be an important factor that modulates colonic DC response via transcriptomic and metabolic reprogramming.

Proinflammatory DC regulation may impact T cell polarization. Indeed, dysregulated cellular pathways in DCs induced by ΔlspA P. UF1 significantly reduce the protective T cell immunity and correspondingly manifest in uncontrolled intestinal pathogen infection, resulting in delayed pathogen clearance and enhanced intestinal inflammation. In addition, no protection is seen in mice when either SIGNR1 or its ligand, glycosylated LspA, is deficient, indicating the critical role of the glicosylated LspA-SIGNR1 axis in regulating colonic DC response that dictates protective T cell response against pathogen infection. We have previously shown that P. UF1 induces DlaT-specific Th17 cells, which are indispensable for protection against Listeria infection. While DlaT expression is not impacted by lspA deletion (Supplementary Fig. 2c), induced Th17 cells notably require a regulated cytokine environment initiated by DCs through glicosylated LspA interacting with SIGNR1.

Interestingly, glicosylated LspA deficiency does not impact the phylum of gut microbiota in bacterially-gavaged groups of mice and then infected with Listeria, suggesting that glicosylated LspA may not contribute to any changes in the intestinal bacterial community. Yet, do changes in bacterial metabolome due to glicosylated LspA deficiency contribute to reduced regulation of DCs during intestinal infection? Although critical metabolic changes are observed within ΔlspA P. UF1, we posit that these metabolites may be mostly restricted to intracellular bacterial metabolic networks in response to altered S-layer glicosylation and may not directly impact the immune cells. Nonetheless, this important notion still requires further rigid investigations.

Ample data demonstrate that IL-17A serves as a protective factor against DSS-induced colitis by maintaining intestinal tight junctions and promoting epithelial repair. Here, Th17 cells, together with IL-10+ Tregs, regulated by colonic SIGNR1+ DCs interacting with glicosylated LspA, may be required for ameliorating DSS-induced colitis, all of which potentially result in the maintenance of tight junction expression that controls the intestinal permeability and the mitigation of chemically induced inflammation and intestinal tissue damage.

In summary, our data demonstrate a molecular mechanism through which DC functions can be fine-tuned via bacterial glicosylated LspA interacting with SIGNR1. Such a regulated DC response is pivotal in priming protective T cell response to intestinal infection and plays a critical role in mitigating DSS-induced colitis in mice. Induced immune regulatory processes involving the fine-tuned receptor-ligand interaction, mainly glicosylated LspA-SIGNR1, elicits functional gut homeostasis during the intestinal inflammatory condition. Thus, consistent with this finding, glicosylation of a bacterial gene product such as LspA may be an important feature for a future formulated prebiotic, conjugated vaccines and therapeutic targets that not only induce the regulation of innate and protective T cells against intestinal pathogen challenges, but may also prevent induced pathogenic inflammation that triggers tissue damage and the progression of intestinal proinflammatory diseases (e.g., colitis) in affected patients. Thus, a deeper understanding of beneficial bacterial gene products influencing the regulation of gut homeostasis may pave the way for the development of new pre or probiotic therapeutic strategies to potentially treat inflammatory bowel disease (IBD) or colon cancer. Finally, our work uniquely illustrates the significance of the mannosylated ligand, bacterial LspA, and its critical binding to SIGNR1 that sustains the optimal activation and regulation of colonic DCs in intestinal steady state and during inflammatory condition. Conclusively, shedding light on the relevance of a sensing receptor, SIGNR1 and its human homolog, DC-SIGN, may reveal the critical innate factor in resisting detrimental signals inducing tissue damage that manifest in IBD. Thus, activating critical signals involving SIGNR1 (DC-SIGN) in health and human diseases may advance our vision to improve and develop therapeutic platforms mitigating IBD, which increasingly affects more than 3.5 million worldwide.

**MATERIALS AND METHODS**

**Mice**

C57BL/6 mice (6–9 weeks old) were obtained from Jackson Laboratory and maintained under specific pathogen-free, Helicobacter-free conditions. Signr1+/− and Signr1−/− mice were provided by Huang Shau-Ku (Johns Hopkins University School of Medicine, Baltimore, Maryland, USA). All animal studies were approved by the Animal Care and Use Committee of the University of Florida under the protocol number 201708484. Mice were maintained in accordance with the Animal Welfare Act and the Public Health Policy on Humane Care.

Bacterial administration

In steady state, Signr1+/− and Signr1−/− mice were gavaged with P. UF1 or ΔlspA P. UF1 (10⁷ CFU/mouse/100 μl) every 3 days over the course of 12 days, and mice were euthanized on day 14 to isolate colonic immune cells. During ΔactA L. m. infection, Signr1−/− and Signr1+/− mice were gavaged with P. UF1, ΔlspA P. UF1 (10⁹ CFU/mouse/100 μl) or PBS on days −7, −4, −1, and 2. Mice were denied food for 4 h on day 0 and then orally infected with 100 μl PBS containing 50 mg/ml CaCO₃ and approximately 10⁹ CU of ΔactA L. m. Fecal samples were collected on days 1–4, and mice were sacrificed on day 7. Pathogen loads were determined by plating serial dilutions of fecal samples on BHI agar supplemented with 200 μg/ml streptomycin.

Cell isolation and flow cytometry

Density gradient centrifugation using Percoll was performed to isolate lamina propria cells from mouse colon. Briefly, tissues were collected and fecal contents in the colon were carefully removed. Colon tissues were opened longitudinally and cleaned with cold PBS and then shaken in PBS containing 20 mM Hepes and 10 mM EDTA for 30 min at 37 °C. Tissues were cut into small pieces and incubated with digestion solution [RPMI 1640 containing 10% FBS (Thermo Fisher Scientific, Waltham, MA), 0.4% β-mercaptoethanol, 400 U/ml collagenase VIII (Sigma Aldrich, St. Louis, MO) and 100 μg/ml DNase I (Sigma Aldrich, St. Louis, MO)] for 1.5 h at 37 °C in a 5% CO₂ incubator. Digested tissues were filtered through 100 μm cell strainer (Gensese Scientific, San Diego, CA), and cells were resuspended in 5 ml of 40% Percoll (Sigma Aldrich, St. Louis, MO) and overlaid on 5 ml of 80% Percoll. Cells in the interphase were collected after gradient centrifugation (1258 g, 25 min, 25 °C).

Flow cytometry was performed as described previously with some modifications. Isolated cells were stimulated with 50 ng/ml PMA (Sigma Aldrich, St. Louis, MO) and 500 ng/ml ionomycin calcium salt (Sigma Aldrich, St. Louis, MO) for 4 h and 3 μg/ml brefeldin A (BioLegend, San Diego, CA) was added 2 h before cells were harvested. Stimulation was performed in IMDM medium (Sigma Aldrich, St. Louis, MO) containing 10% FBS, 1% penicillin/streptomycin, 0.4% β-mercaptoethanol, 400 U/ml collagenase VIII (Sigma Aldrich, St. Louis, MO) and 100 μg/ml DNase I (Sigma Aldrich, St. Louis, MO) for 1.5 h at 37 °C in a 5% CO₂ incubator. Digested tissues were filtered through 100 μm cell strainer (Gensese Scientific, San Diego, CA), and cells were resuspended in 5 ml of 40% Percoll (Sigma Aldrich, St. Louis, MO) and overlaid on 5 ml of 80% Percoll. Cells in the interphase were collected after gradient centrifugation (1258 g, 25 min, 25 °C).

Flow cytometry was performed as described previously with some modifications. Isolated cells were stimulated with 50 ng/ml MPA (Sigma Aldrich, St. Louis, MO) and 500 ng/ml ionomycin calcium salt (Sigma Aldrich, St. Louis, MO) for 4 h and 3 μg/ml brefeldin A (BioLegend, San Diego, CA) was added 2 h before cells were harvested. Stimulation was performed in IMDM medium (Sigma Aldrich, St. Louis, MO) containing 10% FBS, 1% penicillin/streptomycin, 0.4% β-mercaptoethanol, 400 U/ml collagenase VIII (Sigma Aldrich, St. Louis, MO) and 100 μg/ml DNase I (Sigma Aldrich, St. Louis, MO) for 1.5 h at 37 °C in a 5% CO₂ incubator. Digested tissues were filtered through 100 μm cell strainer (Gensese Scientific, San Diego, CA), and cells were resuspended in 5 ml of 40% Percoll (Sigma Aldrich, St. Louis, MO) and overlaid on 5 ml of 80% Percoll. Cells in the interphase were collected after gradient centrifugation (1258 g, 25 min, 25 °C).

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with following fluorescent antibodies: eVolva 655-CD45 (catalog 86-0451-42), eFlour560-CD11c (catalog 69-0114-80), PE-MHCII (catalog 12-5321-82), PE/Cy5-F4/80 (catalog 15-4801-80), APC-SIGNR1 (catalog 17-2093-82), PE/Cy5-CD8 (catalog 15-0081-83), PE/Cy7-Pro-IL-1β (catalog 25-7114-82)/rat IgG1 κ, FITC-IL-6 (catalog 11-7061-82)/rat IgG1 κ, eFlour 450-IL-12/23p40 (catalog 48-7123-82)/mouse IgG1 κ, and eFlour450-FoxP3 (catalog 48-5773-82)/rat IgG2a κ from Thermo Fisher Scientific; APC/Cy7-CD11b (catalog 101226), PerCP/Cy5.5-CD64 (catalog 139308), APC/Cy7-CD3 (catalog 100330), Brilliant Violet 605-CD4 (catalog 100548), PE-IL-17A (catalog 506904)/rat IgG1 κ, and FITC-IL-10 (catalog 505006)/rat IgG2b κ from BioLegend. Data were collected by an LSR II Fortessa (BD Biosciences, San Jose, CA) and analyzed with FlowJo software (version 10) (TreeStar, Ashland, OR). After dead and doublet cell exclusion, and the subsequent CD45+ selection, DCs were defined as CD11c+ MHCIIhi CD11b+ F4/80− (Supplementary Fig. 7a) and T cells as CD3+ CD8− CD4− (Supplementary Fig. 7b).

DC sorting
Colonic cell suspensions were prepared from mice gavaged with P. UF1 or ΔlspA P. UF1 in steady state or during intestinal ΔactA L. m infection. Cells were labeled with a cocktail of fluorescent antibodies specific for: PE/Cy7-CD11c (BioLegend, catalog 117318), PE-MHCII (Thermo Fisher Scientific, catalog 12-5321-82), APC-CD11b (BioLegend, catalog 101212), FITC-CD4/F80 (Thermo Fisher Scientific, catalog 11-4801-82). Dead cells were identified and excluded using LIVE/DEAD Fixable Violet Dead Cell Stain (Thermo Fisher Scientific, Waltham, MA). CD11c+ MHCII+ CD11b+ F4/80− DCs (Supplementary Fig. 4a) were isolated using a SONY SH800S Cell Sorter (Sony, Tokyo, Japan). The purity of sorted cells analyzed by flow cytometry was determined to be >98% (Supplementary 4b). Subsequently, cell pellets were resuspended in RLT plus buffer (Qiagen, Germantown, MD) for RNA extraction or snap-frozen in liquid nitrogen for metabolomic analysis.

RNA-Seq analysis
Total RNA was extracted from about 1 x 10⁶ CD11chi MHCIIhi CD11b+ F4/80− DCs isolated from each individual mouse using an RNeasy Plus Micro Kit (Qiagen, Germantown, MD). cDNA was generated using a SMART-Seq HT kit (Takara Bio Inc., Mountain View, CA) and RNA-Seq libraries were constructed using a Nextera XT DNA Library Preparation Kit (Illumina, Inc, San Diego, CA). Barcoded sequences were sampled on an Illumina HiSeq instrument (Illumina Inc, San Diego, CA) at the University of Florida ICBR NextGen DNA Sequencing Core Facility. The sequencing reads were mapped to the Mus musculus genome (NCBI GRCh38/mm10) using STAR aligner (v2.6.0), and count table was generated using SubReads featureCounts (v1.6.0). Significantly altered genes (RPKM ≥ 1, FDR P < 0.05, fold change ≥ 1.5) were identified by DESeq2. Regularized-log-transformation of count data was performed for heatmap plotting. Gene set enrichment analysis (GSEA) was performed in the javaGSEA (v3.0) using GO, Hallmark, KEGG and REACTOME database.

Extracellular flux analysis
Colonic DCs were isolated from mice gavaged with P. UF or ΔlspA P. UF1 and orally infected with ΔactA L. m using a Pan-DC Enrichment Kit (StemCell Technologies, Vancouver, Canada). For real-time analysis of extracellular acidification rate (ECAR) and oxygen consumption rate (OCR), DCs were analyzed using an XF96 Extracellular Flux Analyzer (Seahorse Bioscience, North Billerica, MA) as described. Briefly, enriched colonic DCs (3 x 10⁵ cells/well, pooled from 3 mice) were analyzed in non-buffered RPMI medium supplemented with 2.5 μM dextrose, 2 mM glutamine, and 1 μM sodium pyruvate. ECAR and OCR were analyzed in response to 1 mM oligomycin, 1.25 μM fluoro-carbonyl cyanide phenylhydrazone (FCCP), 1 μM rotenone and 1 μM antimycin A.

High-resolution metabolomics analysis
FACS sorted colonic CD11c+MHCII+CD11b+ F4/80− DCs were isolated from mice gavaged with P. UF1 or ΔlspA P. UF1 and orally infected with ΔactA L. m. Note: colonic cells of 2 mice were combined to obtain 60,000–80,000 DCs/sample. After vortex and incubation with acetonitrile-water (2:1) at 4 °C for 30 min, DC samples were centrifuged and the supernatants were analyzed by LC-MS. Each sample was run in triplicates on an Orbitrap Fusion Tribrid Mass spectrometer with the resolution of 120,000 (Thermo Fisher, San Diego, CA), with dual chromatography using a 5 min C18 reversed-phase chromatography in negative electrospray ionization (ESI) mode and a 5 min HILIC chromatography in positive ESI mode over a mass-to-charge ratio (m/z) range of 85–1250. Student’s t-test was performed between treatment groups. Subsequently, metabolite pathway analysis was performed by Mumichog software (v2.0) with default parameters. 786 significant metabolite features in negative mode and 1234 metabolite features in positive mode were used as input to Mumichog. The pathways represented by at least two significant metabolites and enriched at P < 0.05 in positive mode (HILIC column) are presented.

DSS-induced colitis
Sign1+/+ and Sign1−/− mice were treated with 3% DSS in drinking water for 5 consecutive days (made fresh every 2–3 days) to induce colitis. Mice were monitored for disease progression through day 12 (Sign1+/+ mice) or day 10 (Sign1−/− mice as they are more susceptible to induced colitis) after DSS treatment. For bacterial administration, mice were orally gavaged with P. UF1, ΔlspA P. UF1 or PBS on days −7, −4, −1, 2, 5, and 8 for a total of 6 gavages. Colitis severity was determined by histopathology. Tissues were fixed, sectioned, and stained with hematoxylin and eosin (Histology Tech Services, Gainesville, FL). Stained sections were evaluated by a boarded veterinary pathologist. Macroscopic damage in the colons of DSS-treated mice gavaged with P. UF1, ΔlspA P. UF1 or PBS was visualized with a Multi-Purpose Rigide Telescope attached to a TELE PACK X (Karl Storz-Endoscope, Germany), as described previously.

FITC-dextran gut permeability assay
DSS-treated mice were orally gavaged with FITC-dextran 4000 (Sigma-Aldrich, St. Louis, MO), a nonmetabolizable macromolecule that is used as a permeability probe. All mice were orally gavaged with FITC-dextran (0.6 mg/g mouse weight) and sacrificed 4 h later for serum harvest. Fluorescent intensity in the serum was measured using a microplate reader (BioTek, Winooski, VT) with an excitation wavelength of 485 nm and an emission wavelength of 519 nm. FITC-dextran concentrations in the mouse sera were determined from standard curves generated by serial dilution of FITC-dextran. Serum from mice that were not gavaged with the permeability tracer was used as a control and the results from the tested samples.

Statistical analysis
Statistical analyses were performed using GraphPad Prism v7.0. Prior to statistical analysis, normality was tested using the Shapiro-Wilk normality test. Where the groups follow a Gaussian distribution, parametric analyses were performed (2-tailed unpaired t test for 2 variables or one-way ANOVA followed by Tukey’s post-test for 3 variables). Where the groups did not follow a Gaussian distribution, nonparametric analyses were performed (Mann–Whitney U test for 2 variables or Kruskal–Wallis test followed by Dunn’s post-test for 3 variables). P values lower than 0.05 were considered as significant: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

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AUTHOR CONTRIBUTIONS
M.M. directed the experiments, which were executed by Y.G., M.G., M.Z., and J.L. Y.G. and M.Z. performed animal experiments; Y.G. performed flow cytometry analysis, genetic and biochemical assays. Y.G. and J.L. constructed RNA-Seq libraries. M.G. analyzed RNA-Seq data. J.R.A. evaluated and scored all colonic tissue sections. W.L. and L.M. performed extracellular flux analysis, R.S., N.T.S., and P.A. analyzed glycomics and glycoproteomics. M.G., Y.W., D.P.J., and S.L. performed, analyzed, and directed metabolomic studies. Y.G. and M.M. wrote the paper.

ADDITIONAL INFORMATION
The online version of this article (https://doi.org/10.1038/s41385-019-0210-0) contains supplementary material, which is available to authorized users.

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