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Akkermansia mono-colonization modulates microglia and astrocytes in a strain specific manner

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Abstract

Microglia and astrocytes are the primary glial cells in the central nervous system (CNS) and their function is shaped by multiple factors. Regulation of CNS glia by the microbiota have been reported, although the role of specific bacteria has not been identified. We colonized germ-free mice with the type strain *Akkermansia muciniphila* (*Am*^{T}) and a novel *A. muciniphila* strain BWH-H3 (*Am*-H3) isolated from a subject with multiple sclerosis and compared to mice colonized with *Bacteroides cellulosilyticus* strain BWH-E5 (*Bc*) isolated from a healthy control subject. We then investigated the effect of these bacteria on microglia and astrocyte gene expression by RNA sequencing. We found altered gene expression profiles in brain microglia, with *Akkermansia* downregulating genes related to antigen presentation and cell migration. Furthermore, we observed strain specific effects, with *Akkermansia* H3 upregulating histone and protein binding associated genes and downregulating channel and ion transport genes. Astrocyte pathways that were altered by *Akkermansia* H3 mono-colonization included upregulation of proliferation pathways and downregulation in cytoskeletal associated genes. Furthermore, animals colonized with type strain *Akkermansia* and strain H3 had effects on the immune system including elevated splenic γδ-T cells and increased IFNγ production in CD4+T cells. We also measured intestinal short chain fatty acids and found that both *A. muciniphila* strains produced proprionate while *B. cellulosilyticus* produced acetate, proprionate, and isovalerate. Taken together, our study shows that specific members of the intestinal microbiota influence both microglial and astroyctes which may be mediated by changes in short chain fatty acids and peripheral immune signaling.

Keywords Microglia, Astrocyte, Germ-free, Mono-colonization, Akkermansia, RNAseq

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Introduction

Microbes and their metabolic products are key regulators of the gut-brain axis. How gut resident bacterial species influence central nervous system glia including microglia and astrocytes is not well understood. Eluciating this relationship has important implications for developing microbiome based therapies to modulate nervous system disease. Germ free (GF) mice are a valuable tool for investigating microbes and their effect on both central and peripheral immunity. GF mice do not harbor a native microbiota and thus can be mono-colonized with individual bacterial strains [1, 2]. Mono-association studies have shown a protective capacity of microbes in inflammatory bowel disease models [3, 4] and immune effects including increased Foxp3 + Treg production in mice mono-colonized with *B. fragilis* [2].

In multiple sclerosis (MS), one of the most consistent findings is an elevation in *Akkermansia* [1, 5–11], which we have previously associated with improved clinical outcomes [6, 11]. These findings raise the question of whether *Akkermansia* has a beneficial or detrimental effect on the disease. In one study, investigators found that incubating *Akkermansia* with PBMCs increased IFNγ production by CD4 + T cells in vitro. However, mono-colonization with *Akkermansia* did not increase inflammatory cytokines in vivo [1]. In the autoimmune encephalomyelitis (EAE) model we and others have found that administering *Akkermansia* to specific pathogen free (SPF) mice, which harbor a full microbiome, ameliorated disease [6, 12]. There are 4 clades of *Akkermansia* which have differential metabolic and immunologic effects [13], and mono-colonization approachs have been used to define microbe-immune interactions in mice harboring > 50 bacterial species [14]. We identified novel MS-derived *Akkermansia* strains and found that *Am*-H3 had the greatest effect in EAE and reduced IL-17 producing γδ-T cell [15, 16]. Although it has been demonstrated that *Akkermansia* strains can affect immunity in mono-colonization models and CNS disease in EAE, their effect on microglia and astrocytes, the major glial cells in the CNS, is unknown.

The development of microglial and their homeostatic function is dependent on the gut microbiota [17]. Microglial in GF mice have an immature phenotype and are inefficient in clearing pathogens, which can be restored by reconstitution of the gut microbiota or treatment with several microbial metabolites including short chain fatty acids (SCFAs) [17]. In Alzheimer's and Parkinson's models, GF mice have showed decreased disease pathology and immature microglial. The addition of SCFAs increased microglial activation and disease pathology to the level of SPF models [18]. These data demonstrate a critical role of gut microbiota and their metabolites in maintaining CNS immune homeostasis in both health and disease.

In order to investigate the strain-specific role of *Akkermansia* on CNS glia, we mono-colonized germ free mice with type strain *Akkermansia muciniphila* (Am^T) or a strain of *Akkermansia* isolated from an MS subject at Brigham and Women's Hospital, *Akkermansia* strain H3 (*Am*-H3) [6], and analyzed their effects on microglia and astrocytes as measured by transcriptomics.

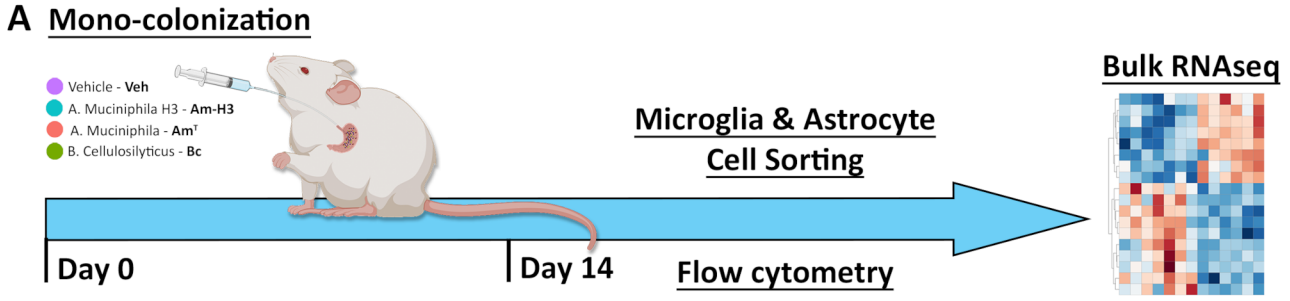
Materials and methods

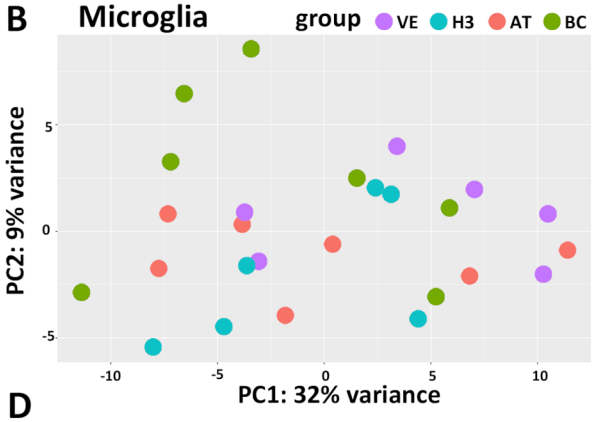
Animals

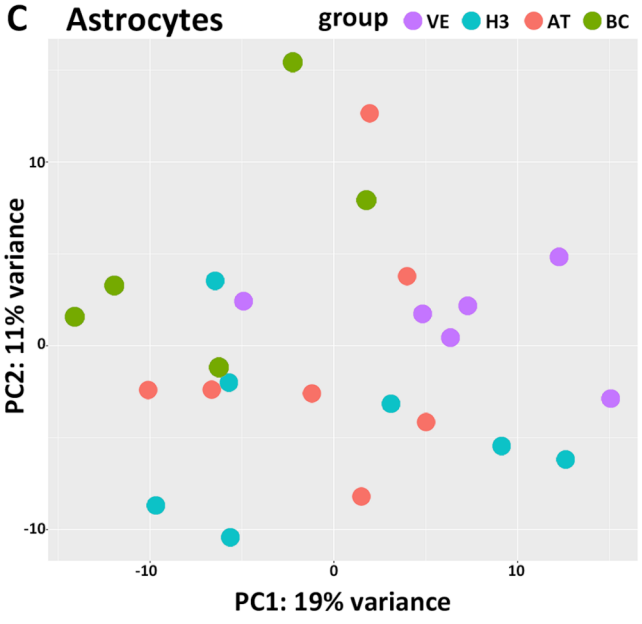
All animal procedures were approved by the Brigham and Women's Hospital IACUC. Germ-free mice on the C57BL/6 background (Jackson Laboratory, Bar Harbor, ME) were bred at the Massachusetts Host-Microbiome Center's gnotobiotic core and were maintained under a 12 h light-dark cycle and received water and chow ad libitum.

Mono-colonization

At 8-weeks of age, mice received a single oral gavage with 200 µl of one of four treatments: vehicle (brain heart infusion broth; Veh), *Akkermansia muciniphila* type strain (Am^T; ATCC BAA-835), *Akkermansia muciniphila* strain BWH-H3 (*Am*-H3), or *Bacteroides cellulosyliticus* strain BWH-E5 (*Bc*; Fig. 1A). All bacterial cultures were diluted to 1×10^8 CFU/mL in brain heart infusion broth. Mice were maintained germ-free for 2-weeks with one cage change 7 days into the experiment. Confirmation of mono-association was performed at the end of experiment via microbiological culture of cecal samples at the Host-Microbiome Center. Cecal contents were plated onto tryptic soy agar (Veh samples) and incubated in ambient oxygen air, onto *Brucella* blood agar with menadione (*Bc* samples) or brain heart infusion plates (Am^T, *Am*-H3 samples) and incubated anaerobically. Aerobic and anaerobic microbial growth were viewed for each group as were Gram stains. Veh samples with growth or Gram stain reactivity in any oxygen condition were considered contaminated. Any aerobic growth or aerobic Gram stain reactivity in the *Bc*, Am^T, or *Am*-H3 groups was considered a contaminated sample. Anaerobic growth and a Gram stain with expected structure of microbe of interest was required to be considered mono-colonized (e.g., Gram negative rods consistent with *Akkermansia*). A total of $n = 68$ animals were inoculated with one of the 4 treatments, of which $n = 43$ were confirmed to be mono-associated giving a success rate of 63.2%. Successful mono-colonization rates by group were as follows: Veh treated = 53.9%, Am^T = 75%, *Am*-H3 = 47.1%, and *Bc* = 72.2%. Animals that were determined to not be mono-colonized were omitted from the study.







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PCA - PERMANOVA Results					
Astrocytes	R2	pval	Microglia	R2	pval
VE vs AT	0.17	0.12	VE vs AT	0.21	0.07
VE vs H3	0.25	**0.05**	VE vs H3	0.25	0.06
VE vs BC	0.47	**0.03**	VE vs BC	0.21	0.08
AT vs BC	0.20	0.21	AT vs BC	0.11	0.24
H3 vs BC	0.36	**0.01**	H3 vs BC	0.16	0.15
AT vs H3	0.08	0.46	AT vs H3	0.07	0.44

Fig. 1 Microbial mono-colonization schematic and plots of PCA scores. Schematic showing workflow for mono-colonization through to RNAseq (**A**). PCA plots for four mono-colonized groups show PCA scores for microglia (**B**) and astrocytes (**C**) by treatment. PERMANOVA of PCA scores (**D**). Individual group PCAs are in Figure S1

Tissue processing

After sacrifice via CO_2 inhalation, mice received thoracotomy and subsequent transcardial perfusion with ice cold HBSS. Brains were removed and the whole left hemisphere was placed into 7mL glass tissue homogonizer with cold HBSS. Tissue was homogenized prior to centrifugation in 4° C at 1200 g for 7 min and subsequent aspiration of supernatant. Pellets were resuspended in 5mL of 37% Percoll before centrifugation at 4° C at 800 xg for 10 min. Percoll was washed with 5 mL FACS buffer (v: v in HBSS: 2% FBS, 2.5% HEPES, 0.4% EDTA) before centrifugation in 4° C at 1200 g for 7 min and supernatant was aspirated until ~50 µl remained in the tube. Cells were stained for FACS using anti-CD45 FITC (1:400; BD, Franklin Lakes, NJ, USA), anti-CD11b PeCy7 (1:400; Biolegend), anti-ACSA-2 PE (1:200; Miltenyi Biotec, Bergisch Gladbach, Germany). Lineage negative markers were used including PerCP conjugated antibodies at 1:800 dilution to anti-CD3, anti-CD19, anti-Nk1.1, anti-B220, and anti-Ly6G (Biolegend). Cells were incubated with antibodies for 25 min at RT in the dark before being washed with 5 mL FACS buffer and subsequently centrifuged in 4° C at 1200 xg for 7 min. Supernatants were aspirated and pellets resuspended in 100 µl of FACS buffer. Cells were transferred to flow polystyrene tubes and washed with 50 µl FACS buffer. Finally, cells were transferred into clean Eppendorf tubes containing 5 µl of 7-AAD. Microglia and astrocytes were sorted on a BD FACSAria II cell sorter. After sorting, cells were spun at 3500 xg for 10 min at 4° C before being processed for RNAseq.

SCFA quantification

Cecal contents were removed and placed into a sterile Eppendorf tube prior to analysis of SCFAs via gas chromatography-mass spectrometry at the Massachusetts Host-Microbiome Center. Samples were analyzed for the following short-chain acids: acetate, propionate,

isobutyrate, butyrate, isovalerate, valerate, isocaproate, caproate, and heptanoate. Detectable levels were observed in a majority of samples for 4 SCFAs: acetate, propionate, isobutyrate, and isovalerate which were quantified and analyzed via one-way ANOVA across mono-colonization groups.

RNAseq

Microglia and astrocytes were processed on the same plate at the Broad Institute using their mRNA SMART-Seq2 Cell Population v1 sequencing pipeline. Transcriptome alignments were conducted using the Salmon tool with its standard parameters [19]. The R software (version 4.2.1) and DESeq2 package (version 1.36.0) [20] were employed to analyze differential gene expression. After transcript alignment with Salmon, genes were filtered based on adjusted p value < 0.1 and p value < 0.05 to yield a significant DEGene table. The genes used to generate heatmaps were further filtered and selected based on an adjusted p value < 0.05, a baseMean greater than 20, and a $\log_2$ fold change exceeding 1.5. We then used Ingenuity Pathway Analysis (IPA; Qiagen, Hilden, Germany) to identify pathways based on 626 and 909 input genes for microglia and astrocytes, respectively. Genes included in pathway analysis were chosen in IPA based on the number of genes remaining after filtering on unadjusted $p < 0.05$ [21–23] and fold change between − 1.5 and 1.5. Pathway outputs are presented based on p value < 0.05 across mono-colonization groups and presented according to biological relevance.

Flow cytometry

Spleens were homogeized and passed through a 70 µm mesh filter on a 96-well culture plate containing complete media composed of: v:v; Iscove's modified dulbecco's medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA, USA), 1% penicillin/streptomycin (Thermo Fisher Scientific, Waltham, MA, USA), 1% Non-essential amino acid solution (Millipore-sigma, St. Louis, MO, USA), and 0.1% 2-Mercaptoethanol (Gibco, Grand Island, NY, USA). Splenocytes were centrifuged at 2000 rcf for 3 min. Supernatant was aspirated and cells were incubated for 2 min at RT in 1 mL ACK lysing buffer (Gibco, Grand Island, NY, USA). Cell pellet then received 10 mL of FACS buffer before being centrifuged at 2000 rcf for 3 min. Supernant was then aspirated prior to resuspension in 1 mL of complete media. Cells were filtered a second time before being plated at 50 µL per well and subsequently centrifuged at 2000 rcf for 3 min and decanted to remove remaining FACS buffer. Cells were treated with Fc blocking antibody (BD) at 1:200 dilution in FACS buffer and incubated at 4 C for 5 min. Next, 50 µL of zombie fixable viability stain (BioLegend, San Diego, CA, USA) was added prior to incubation at 4 C for 20 min. Cells were then centrifuged and decanted prior to washing 1x in 150 µL FACS buffer before addition of 50 µL extracellular antibody solution or fluorescence minus one (FMO) controls. Antibodies for extracellular staining were as follows: BUV395-anti-CD62L (1:800; BD), BUV496-anti-CD4 (1:800; BD), BV785-anti-CD8 (1:800; Biolegend), AF700-anti-CD44 (1:400; Biolegend), APC-Cy7-anti-CD3 (1:400; Biolegend), BV605-anti-PD-1 (1:400; Biolegend), PE-anti-LAP (1:400; Biolegend), PE-Dazzle-594-anti-CXCR3 (1:400; Biolegend), APC-anti-γδTCR (1:400; Carlsbad, CA, USA), BV650-anti-CXCR5 (1:200; Biolegend), Per-Cp-anti-CD107 (1:100; Biolegend). Cells were incubated in antibody solution for 20 min at 4 C before washing with 150 µL FACS buffer, centrifugation, and decanting. Intracellular staining was performed by resuspending splenocytes in 100 µL of fixation and permeabilization solution from a Foxp3 / transcription factor staining buffer set (eBioscience, San Diego, CA, USA) for 20 min at 4 C. Cells were washed with 200 µL permeabilization buffer and centrifuged at 2000 rcf for 3 min. After decanting, 50 µL of intracellular antibody solution or FMO controls was added to appropriate wells. Splenocytes for cytokine analysis were stimulated at 37 °C for 4 h with PMA/ionomycin and GolgiStop containing monensin (1:1000; BD) prior to being centrifuged at 1500 rcf for 5 min and decanted. Cells were washed with 200 µL FACS buffer before centrifugation at 2000 rcf for 3 min and subsequently decanted. Antibodies for cytokine staining were as follows: APC-Cy7-anti-CD3, BUV496-anti-CD4, BV785-anti-CD8, and BV421-anti-IFNγ (1:400, Biolegend). Cells were incubated in antibody solution for 30 min at RT before being washed with 200 µL of permeabilization buffer, centrifuged, and decanted. Splenocytes were resuspended in 400 µL of FACS buffer and transferred to flow tubes. Cells were processed for flow cytometric acquisition on a Fortessa using DIVA software (BD Biosciences). Data was analyzed in FlowJo (v10.1, TreeStar Inc, Ashland, OR, USA).

Goblet cell analysis

Lectin labelling was performed on 50 µm sections of proximal colon from mono-colonized animals. Protocols were similar to those previously described [24]. Sections received 1.25 µg / mL of the lectin *Ulex europus* agglutinin– 1 (UEA-1; Vector Laboratories, Newark, CA) conjugated to fluorescein and diluted in PBS with 1% BSA, 5% $CaCl_2$, 2% polyvinylpyrolodine-40, and 0.125% Tween-20 to incubation for 72 h. After incubation with UEA-1, sections were washed in PBS before mounting (Aqua-Poly/Mount; Polysciences, Inc., Warrington, PA) and imaged on a Leica DMi8 Widefield microscope. Quantification of goblet cell counts and size was performed as previously

described [25], with z-stack images being acquired at 1 µm intervarls from 3 crypts / section, from 3 sections for each animal being averaged and reported with the $n = 3$ animals / group. Image analysis was performed on max intensity Z-projections using FIJI (v2.14, 1.54f; NIH).

Data and code availability

All raw sequencing data is accessible at the NCBI Sequence Read Archive: SUB14686820 and BioProject PRJNA1152580. We used standard bioinformatic workflows throughout this study which are available on a GitHub repository (https://github.com/tobylanser/akk_monocolonization).

Results

Mono-colonization with *Akkermansia* modulates microglia gene expression

We previously found a differential effect of *Akkermansia* strains and *B. cellulosyliticus* on CNS inflammation in the experimental autoimmune encephalomyelitis (EAE) model [6]. In order to identify strain-specific effects on glia, we mono-colonized mice with these strains and investigated transcriptional profiles of microglia and astrocytes. Principal component analysis (PCA) plots based on the mono-colonization group vs. vehicle controls (Fig. 1B-C, Figure S1A). Results of PERMANOVA on PCAs show R2 and *p* values for microglia and astrocytes with Veh vs. *Am*-H3, Veh vs. *Bc*, and *Am*-H3 vs. *Bc* being significantly different for astrocytes, while only Veh vs. *Am*-H3 trended towards significant ($p = 0.06$) in microglia (Fig. 1D). Microglia gene expression demonstrated a downregulation of *Ndufaf7*, *Mmgt2*, and *Lpar6* in Am^T treated mice vs. vehicle GF animals and an upregulation of *Dgkq* and Nhsl2 among others (Fig. 2A-B). Furthermore, *Am*-H3 mono-colonized mice had 18 downregulated and 14 upregulated genes vs. Veh (Fig. 2C-D). When we compared Am^T treated mice vs. *Am*-H3, we found 1 significantly altered gene, *Tbc1d24*, and when comparing Veh vs. a gram negative control bacteria, *Bc*, we found 3 genes significantly altered, *Pcdh17*, *Dennd1a*, and *Gprin2*. In microglia from mice mono-colonized with *Am*-H3 vs. *Bc*, 8 genes were downregulated and 4 upregulated (Fig. 2E-F). Analysis of all significant ($p < 0.05$) DEGs (Additional file 1) using IPA showed that mono-colonization with any microbe altered pathways involved in cellular immunity, extracellular matrix organization, and nervous system signaling (Fig. 2G). When comparing Am^T vs. *Am*-H3 mono-colonized animals we found altered pathways involved in cellular immune response, nervous system signaling, and extracellular matrix related alterations (Fig. 2H). Genes contributing to identified IPA pathways are available in Additional file 3.

Akkermansia mono-colonization modulates astrocyte gene expression

We then investigated the impact of mono-colonization with MS associated microbes on astrocyte gene expression and found altered gene expressions profiles in both Am^T and *Am*-H3 treated mice vs. Veh controls, and plotted PCA scores (Fig. 1C, Figure S1B). *Tmpo*, *Ppp1r7*, *Foxg1*, and *Ccdc22* were upregulated and *Sec24a*, *Gpt*, and *B3galt5* were downregulated in Am^T vs. Veh treated mice (Fig. 3A-B**).** When comparing *Am*-H3 astrocytes vs. Veh mono-colonized, we found 10 genes downregulated and 21 upregulated (Fig. 3C-D) including *Top2a* and *Ermn* (Fig. 3D). Analysis of astrocytes isolated from mice mono-colonized with *Am*-H3 vs. *Bc*, identified 14 downregulated and 60 upregulated genes (Fig. 3E-F). Pathway analysis on all significant ($p < 0.05$) DEGs (Additional file 2) using IPA and found differential pathways across mono-colonization groups for patwhays related to cellular immune response, myelination related signaling, and nervous system signaling (Fig. 3G). When comparing Am^T vs. *Am*-H3, numerous pathways associated with myelination were upregulated in *Am*-H3, as were multiple cellular immune and nervous system signaling related pathways (Fig. 3H). Genes contributing to identified IPA pathways are available in Additional file 3.

Am^T and *Am*-H3 modulate T cells

Because the microbiome may also influence glia by modulating peripheral immunity, we investigated with effect of mono-colonization of Am^T and *Am*-H3 on splenic T cells. Total CD3 + T cells were increased in Am^T ($p < 0.01$) and *Am-H3* ($p < 0.05$) mono-colonized compared to Veh treated animals (Fig. 4A). Effector CD4 + T cells ($p < 0.01$) and CD44(Hi) CD62L(lo) activated T cells ($p < 0.05$) were increased in Am^T compared to Veh (Fig. 4B-C). We observed an increase in γδ-TCR T cells in Am^T ($p < 0.05$) and *Am*-H3 ($p < 0.001$) vs. vehicle, whereas *Am*-H3 was also increased compared to *Bc* ($p < 0.05$; Fig. 4D, E). Total CD4 + and CD8 + frequency were not altered (Figure S2A, C, E). However, we did find increased IFNγ production by CD4 + T cells in mice colonized with *Am-H3* and *Bc* (Figure S2B) an effect not observed in CD8 + T cells (Figure S2D).

Differential production of SCFAs in mono-colonized mice and gut mucosal integrity

Studies have shown that SCFAs modulate microglia [18]. We found that specific SCFAs were elevated in the cecum from mono-colonized animals vs. Veh treated GF mice. *Bc* mono-colonized animals had increased acetate ($p < 0.0001$) propionate ($p < 0.0001$) and isovalerate ($p < 0.0001$) compared to Veh, Am^T, and *Am*-H3 mono-colonized animals (Fig. 5A-C). Both Am^T and *Am*-H3 produced propionate as the primary SCFA ($p < 0.001$;

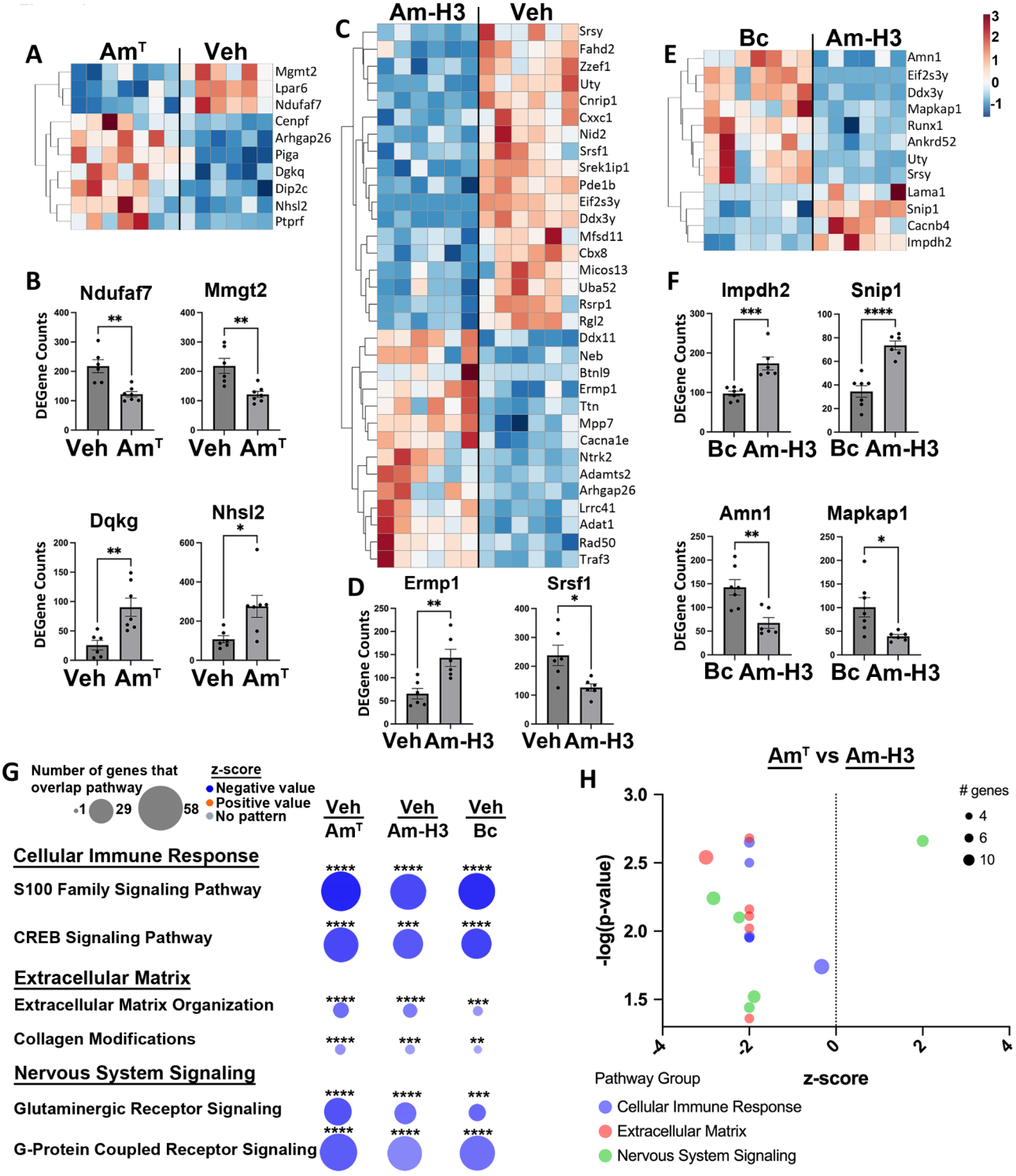

Fig. 2 Mono-colonization with *Akkermansia* alters microglia gene expression. Altered gene expression in *Am*^{T} mono-colonized animal microglia compared to Veh (**A**) and representative DEGene count graphs (**B**). Genes altered between *Am*-H3 vs. Veh (**C**) and representative bar graphs of DEGene counts (**D**). Significantly altered genes between Bc and *Am*-H3 (**E**) and representative graphs of up and downregulated DEGene counts (**F**). Ingenuity Pathway Analysis (IPA) top pathways altered in animals mono-colonized with microbes vs. vehicle treated for relevant pathway families (**G**). Pathways identified in IPA significantly (-log(p-val) > 1.3) for *Am*^{T} vs. *Am*-H3 groups (**H**). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. $n = 6$ Veh, *Am*-H3, $n = 7$ for *Am*^{T} and *Bc*

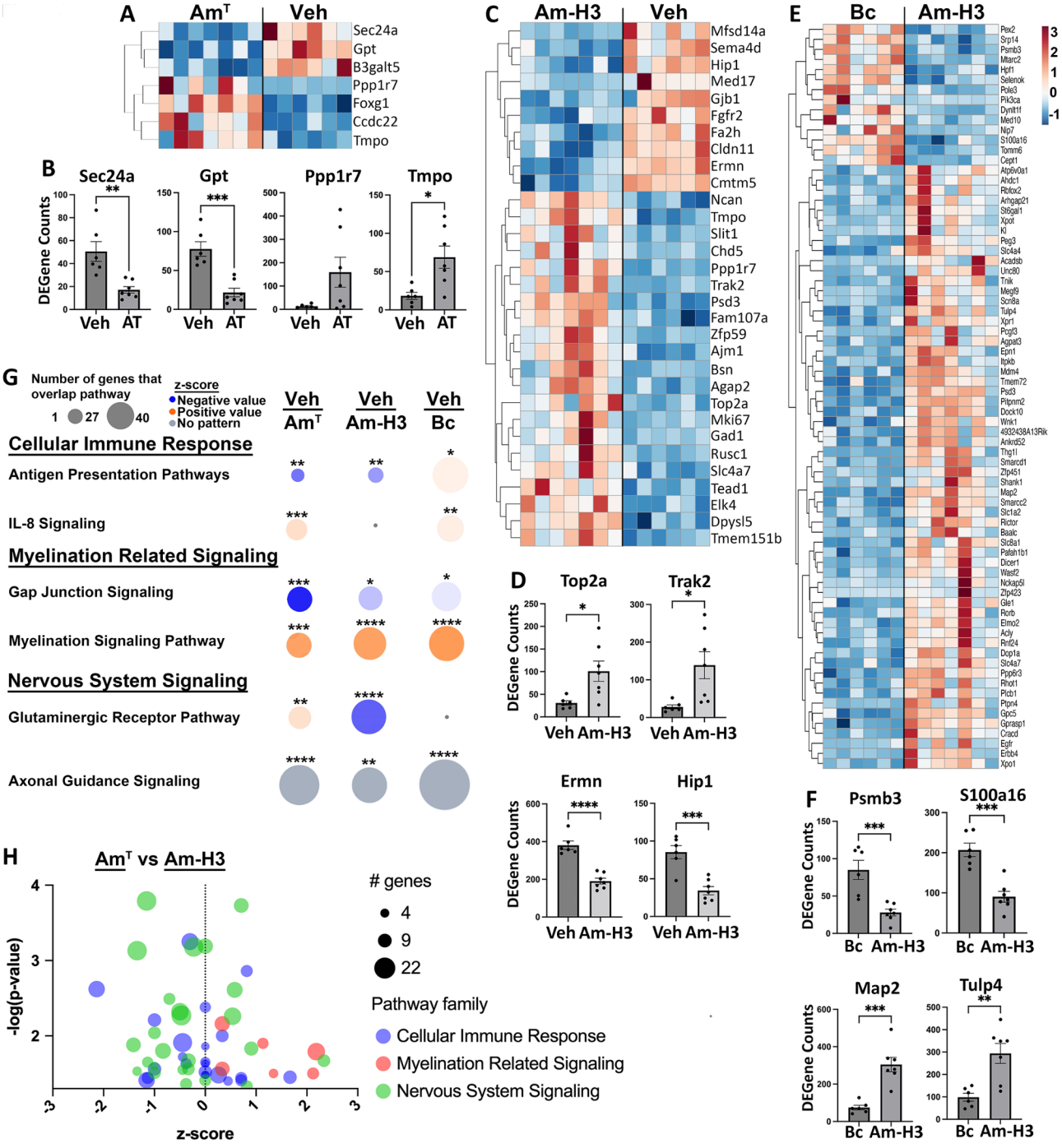

Fig. 3 Akkermansia mono-colonization alters astrocyte gene expression. Mono-colonization with *Am*^{T} altered gene expression compared to Veh (**A**) with DEGene counts showing genes highly altered (**B**). *Am*-H3 treatment altered gene expression profiles compared to Veh treated animals (**C**) with DEGene counts (**D**) showing important genes significantly altered by *Am*-H3 mono-colonization. In mice mono-colonized with *Bc* vs. *Am*-H3, gene expression alterations are heatmapped (**E**) and DEGene counts of interest plotted (**F**). Ingenuity Pathway Analysis (IPA) top pathways altered across groups for relevant pathway families (**G**). Pathways identified in IPA with cellular immunology relevance in *Am*^{T} vs. *Am*-H3 mono-colonized animals (**H**). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. $n = 6$ Veh and Bc, $n = 7$ for *Am*^{T} and *Am*-H3

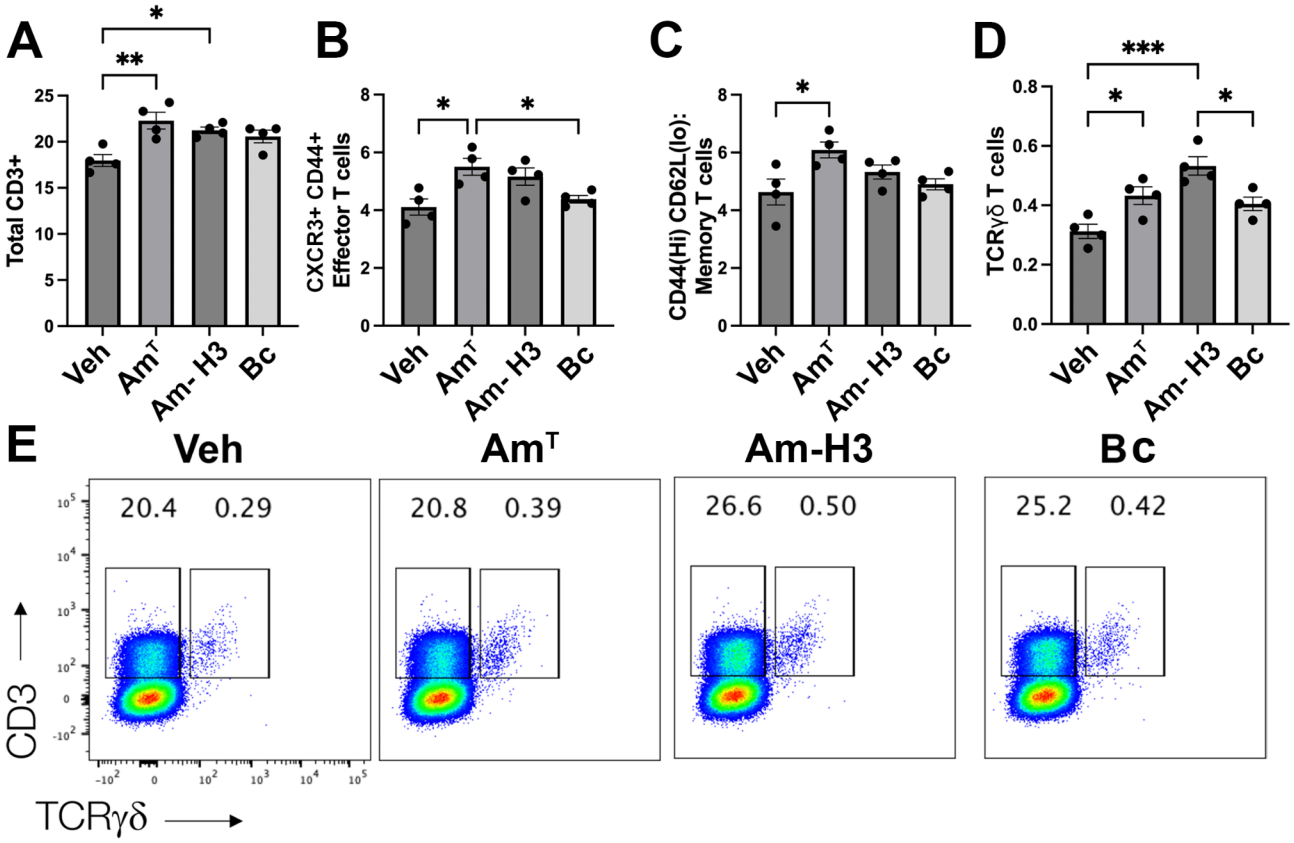

Fig. 4 Effect of *Am*^{T} and *Am*-H3 on T cells. (**A**) Total CD3 + T cells, (**B**) CXCR3 + CD44 + Effector T cells, (**C**) CD44(Hi) CD62(lo) activated T cells, and (**D**) γδ-TCG T cells in mice colonized with *Akkermansia* and *Bacteroides* strains. Representative dot plot of CD3 by γδ-TCR across mono-colonization groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. $n = 4$ per group

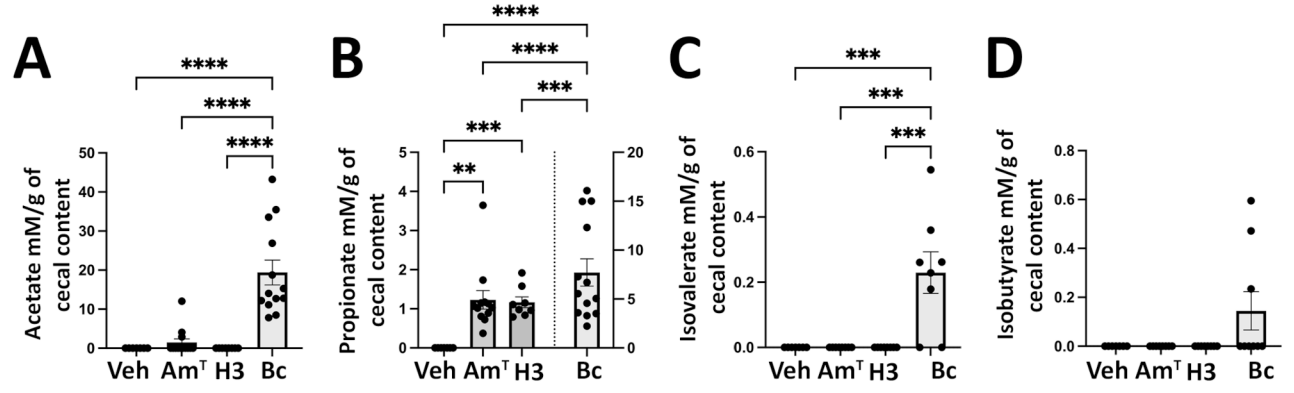

Fig. 5 Mono-colonization promotes SCFA production. Acetate (A), propionate (B), and isovalerate (C) were significantly elevated in *Bc* mono-colonized mice while *Am*^{T} and *Am*-H3 increased propionate levels. Isobutyrate was not detected in any group besides *Bc* mono-colonized mice (D). **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$. $n = 8$–13 per group

Fig. 5B). Since *Akkermansia and Bc* are known to degrade mucus [26, 27], we investigated the gut mucus barrier and epithelial integrity by measuring goblet cells labelled for UEA-1 + and analyzed cell quantity and size. Colonic goblet cells were labeled with UEA-1 lectin and we found similar quantities (Figure S3A-B) and sizes (Figure S3C) in Veh, *Bc*, *Am*^{T}, and *Am*-H3 mono-colonized mice.

Discussion

The gut microbiota influences peripheral and central immune function in health and disease [28–30]. It is not known whether strain-specific effects of single bacteria or their metabolites can augment protective pathways involved in CNS homeostasis, or conversely promote pathogenic processes in the CNS. To address

these questions, we colonized GF mice with MS associated microbes [6] and investigated their effects on gene expression in brain microglia and astrocytes and linked these effects to changes in peripheral immunity and intestinal microbial metabolites.

Multiple studies have reported microbiota alterations in neurologic diseases [1, 5–9, 31–35]. *Akkermansia* has been shown to ameliorate disease in animal models of MS, AD, ALS, and epilepsy [6, 36–39], whereas disease related pathways are worsened when *Akkermansia* is cultured with PBMCs from MS subjects [1] and when cultured with an enteroendocrine cell line [40]. There are 4 clades of *Akkermansia* with distinct metabolic and immunologic properties [13]. We previously found that treatment of SPF mice with *Akkermansia* strain BWH-H3 had a greater protective effect in EAE than the 3 other Akkermansia strains [6]. In addition, strain specific associations of *Akkermansia* with MS and MS clinical measures have been described [5, 6], and increased abundance of *Akkermansia* is associated with lower measures of neuroinflammation in mice with a complex microbiome [41].

To investigate the properties of these Akkermansia strains, we mono-colonized mice with either *Akkermansia muciniphila* type strain, or our recently isolated *Akkermansia* BWH-H3 strain [6]. We observed strain specific effects on microglial gene expression after mono-colonization. Minimal effects were observed with the Am^T strain compared to vehicle treated animals. We found that *Am*-H3 had a greater effect on microglia gene expression including those involved in cell adhesion (e.g., Nid2), none of which were altered by Am^T mono-colonization. Srsf1, a nuclear export adapter whose depletion prevents neurodegeneration in the drosophila model of C9ORF72 related diseases [42, 43] was downregulated in *Am*-H3 treated animals. *Eif2s3y*, a Y-chromosomal gene that causes autism-like behaviors in male mice [44] was also downregulated in *Am*-H3 mono-colonized microglia. *Bc*, a Gram-negative anaerobic microbe that we used as a non-Akkermansia control elevated Eif2s3y compared to *Am*-H3, supporting *Am*-H3's beneficial influence on brain microglia. Our study used germ free, non-immunized mice, as opposed to other studies that have shown *Akkermansia* to lower inflammation in EAE [6]. Due to the mostly absent inflammatory microglial signatures in these mice, the changes we observed in our non-immunized mice were not dramatic. This was compounded by use of commensal bacteria which we would not expect to result in overt inflammation. These methodological considerations make it possible that the effect of *Akkermansia* on microglia may differ during inflammatory states (e.g., in MS) compared to homeostasis. Taken together, our data provide evidence of strain specific effects on microglial gene expression, however, future investigations with multi-strain and multi-species colonizations would provide further evidence of *Akkermansia*'s role in MS.

Microbial mono-colonization also influenced astrocyte gene expression in a strain specific manner. We observed an increase in *Tmpo* in mice mono-colonized with Am^T. *Tmpo* codes for thymopoietin which downregulates inflammatory responses in EAE [45]. *Am*-H3 altered astrocyte gene expression pathways involved in extracellular matrix organization, myelination, and cellular immune regulation, and our observed downregulation of *Ermn* in *Am*-H3 mono-colonized animals is consistent with the altered astrocyte cytoskeletal rearrangement we observed in our pathway analysis. *Ermn* is downregulated in RRMS [46], and knocking-out *Ermn* leads to inflammation, microgliosis and increased astrocyte numbers [47]. This suggests that downregulation of *Ermn* in *Am*-H3 treated animals is associated with astrocyte restructuring. We also observed elevation of actin and cytoskeletal associated genes in *Am*-H3 vs. vehicle mono-colonized animals, including *Fam107a*, an actin associated gene that's protein product functions to maintain astrocytes in a quisescent, nonproliferative state [48]. Together our findings identify the structural and functional regulation of astrocytes by *Akkermansia* strain *Am*-H3.

Alterations in immune function during mono-colonization are likely driven by a combination of factors. We observed strain specific alterations in peripheral immune populations from mono-colonized mice, with *Am*-H3 being associated with elevated splenic γδ-T cells. In MS, γδ-T cells have two distinct phenotypes, one characterized by IL-17 production and one driven by IFNγ producing cells [16]. We previously found that *Am-H3* reduces IL-17 producing γδ-T cells in EAE [6]. We found an elevation in γδ-T cells in *Am*-H3 mono-colonized animals, and an elevation of IFNγ producing T cells in our *Am*-H3 and *Bc* mono-colonized animals but not type strain Am^T. Our data point towards *Akkermansia* mono-colonization, and specifically *Am*-H3, shifting peripheral T populations towards a more protective phenotype. How this T cell phenotypic shift relates to central immunity still needs to be investigated.

Gut epithelial and mucus barrier integrity are important components of intestinal homeostasis and SCFAs have an important physiologic role including modulating barrier function [49]. SCFAs have also been shown to promote microglial homestasis and contribute to microglial maturation in germ free animals [17]. *Bc* produced multiple SCFAs [50] and both strains of *Akkermansia* we investigated produced propionate, which has been shown to be beneficial in MS [51]. It is well recognized that the microbiota is responsible for SCFAs in the gut and as expected we did not detect SCFA in vehicle treated mice.

While SCFAs are potentially influencing our observed gene alterations in CNS glia, they are likely only one of many microbial metabolites involved. *Akkermansia* is a mucin degrader [52] raising the question of whether mono-colonization would impair gut barrier function. We found no differences in gut wall mucus in goblet cells in mono-colonized groups.

In this study, we present important data on the impact of mono-colonization with MS derived microbes on microglia and astrocyte gene expression profiles, however, there are limitations. Mono-colonization studies in germ-free animals are inherently limited due to physiologic and developmental issues of germ-free mice [53]. Furthermore, the differences in gene and immune profiles between *Akkermansia* strains in our study were subtle, likely due to our study being conducted in wild-type, non-immunized mice as opposed to immunized / challenged models like EAE, in which we have previously shown that *Akkermansia* lowers inflammation [6]. While protein level validation for our gene expression alterations would be valuable, we were limited by tissue availability for these investigations. Influence of multi-species and multi-strain colonizations on microglia and astrocytes in homeostatic vs. immunized (e.g., EAE) models at the gene and protein level should be further investigated.

In conclusion, we found that *Akkermansia* mono-colonization modulates microglia and astrocyte gene expression, which may be mediated by changes in short chain fatty acids and peripheral immune signaling.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12974-025-03417-3.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

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Author contributions
LAS conceived the study, performed the experiments, analyzed and interpreted the data and wrote the manuscript. TBL and FM, analyzed RNAseq data and edited the manuscript. DSL performed experiments and edited the manuscript. TM analyzed and interpreted immunology data and edited the manuscript. LMC and HLW conceived the study and oversaw the entirety of the project. All authors reviewed the manuscript.

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Data availability
All raw sequencing data is accessible at the NCBI Sequence Read Archive: SUB14686820 and BioProject PRJNA1152580. We used standard bioinformatic workflows throughout this study which are available on a GitHub repository (https://github.com/tobylanser/akk_monocolonization).

Declarations

Conflict of interest
None to declare.

Ethics declaration
All animal procedures were approved by the Brigham and Women's Hospital IACUC under protocol# 2016N000230.

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References

1. Cekanaviciute E, Yoo BB, Runia TF, Debelius JW, Singh S, Nelson CA, Kanner R, Bencosme Y, Lee YK, Hauser SL, et al. Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. Proc Natl Acad Sci U S A. 2017;114:10713–8.
2. Round JL, Mazmanian SK. Inducible Foxp3 + regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci U S A. 2010;107:12204–9.
3. Chiu CC, Ching YH, Wang YC, Liu JY, Li YP, Huang YT, Chuang HL. Monocolonization of germ-free mice with bacteroides fragilis protects against dextran sulfate sodium-induced acute colitis. Biomed Res Int. 2014;2014:675786.
4. Hudcovic T, Stepankova R, Kozakova H, Hrncir T, Tlaskalova-Hogenova H. Effects of monocolonization with Escherichia coli strains O6K13 and Nissle 1917 on the development of experimentally induced acute and chronic intestinal inflammation in germ-free immunocompetent and immunodeficient mice. Folia Microbiol (Praha). 2007;52:618–26.
5. iMSMSConsortium. Gut Microbiome of multiple sclerosis patients and paired household healthy controls reveal associations with disease risk and course. Cell. 2022;185:3467–e34863416.
6. Cox LM, Maghzi AH, Liu S, Tankou SK, Dhang FH, Willocq V, Song A, Wasen C, Tauhid S, Chu R, et al. Gut Microbiome in progressive multiple sclerosis. Ann Neurol. 2021;89:1195–211.
7. Berer K, Gerdes LA, Cekanaviciute E, Jia X, Xiao L, Xia Z, Liu C, Klotz L, Stauffer U, Baranzini SE, et al. Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. Proc Natl Acad Sci U S A. 2017;114:10719–24.
8. Jangi S, Gandhi R, Cox LM, Li N, von Glehn F, Yan R, Patel B, Mazzola MA, Liu S, Glanz BL, et al. Alterations of the human gut Microbiome in multiple sclerosis. Nat Commun. 2016;7:12015.
9. Tankou SK, Regev K, Healy BC, Tjon E, Laghi L, Cox LM, Kivisakk P, Pierre IV, Hrishikesh L, Gandhi R, et al. A probiotic modulates the Microbiome and immunity in multiple sclerosis. Ann Neurol. 2018;83:1147–61.
10. Ventura RE, Iizumi T, Battaglia T, Liu M, Perez-Perez GI, Herbert J, Blaser MJ. Gut Microbiome of treatment-naive MS patients of different ethnicities early in disease course. Sci Rep. 2019;9:16396.
11. Schwerdtfeger LA, Montini F, Lanser TB, Ekwudo MN, Zurawski J, Tauhid S, Glanz BI, Chu R, Bakshi R, Chitnis T et al. Gut microbiota and metabolites are linked to disease progression in multiple sclerosis. *Cell Rep Med* 2025, In Press.
12. Liu S, Rezende RM, Moreira TG, Tankou SK, Cox LM, Wu M, Song A, Dhang FH, Wei Z, Costamagna G, Weiner HL. Oral administration of miR-30d from feces of MS patients suppresses MS-like symptoms in mice by expanding Akkermansia muciniphila. Cell Host Microbe. 2019;26:779–e794778.
13. Becken B, Davey L, Middleton DR, Mueller KD, Sharma A, Holmes ZC, Dallow E, Remick B, Barton GM, David LA et al. Genotypic and phenotypic diversity among human isolates of Akkermansia muciniphila. mBio 2021, 12.

14. Geva-Zatorsky N, Sefik E, Kua L, Pasman L, Tan TG, Ortiz-Lopez A, Yanortsang TB, Yang L, Jupp R, Mathis D, et al. Mining the human gut microbiota for Immunomodulatory organisms. Cell. 2017;168:928–e943911.
15. Rezende RM, Cox LM, Moreira TG, Liu S, Boulenouar S, Dhang F, LeServe DS, Nakagaki BN, Lopes JR, Tatematsu BK, et al. Gamma-delta T cells modulate the microbiota and fecal micro-RNAs to maintain mucosal tolerance. Microbiome. 2023;11:32.
16. Zarobkiewicz MK, Kowalska W, Rolinski J, Bojarska-Junak AA. Gammadelta T lymphocytes in the pathogenesis of multiple sclerosis and experimental autoimmune encephalomyelitis. J Neuroimmunol. 2019;330:67–73.
17. Erny D, Hrabe de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, Keren-Shaul H, Mahlakoiv T, Jakobshagen K, Buch T, et al. Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci. 2015;18:965–77.
18. Colombo AV, Sadler RK, Llovera G, Singh V, Roth S, Heindl S, Sebastian Monasor L, Verhoeven A, Peters F, Parhizkar S et al. Microbiota-derived short chain fatty acids modulate microglia and promote Abeta plaque deposition. Elife 2021, 10.
19. Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. Salmon provides fast and bias-aware quantification of transcript expression. Nat Methods. 2017;14:417–9.
20. Love MI, Huber W, Anders S. Moderated Estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550.
21. Kalantar K, LaHue SC, DeRisi JL, Sample HA, Contag CA, Josephson SA, Wilson MR, Douglas VC. Whole-Genome mRNA gene expression differs between patients with and without delirium. J Geriatr Psychiatry Neurol. 2018;31:203–10.
22. Casden N, Belzer V, El Khayari A, El Fatimy R, Behar O. Astrocyte-to-microglia communication via Sema4B-Plexin-B2 modulates injury-induced reactivity of microglia. Proc Natl Acad Sci U S A. 2024;121:e2400648121.
23. Ortiz C, Pearson A, McCartan R, Roche S, Carothers N, Browning M, Perez S, He B, Ginsberg SD, Mullan M, et al. Overexpression of pathogenic Tau in astrocytes causes a reduction in AQP4 and GLT1, an immunosuppressed phenotype and unique transcriptional responses to repetitive mild TBI without appreciable changes in Tauopathy. J Neuroinflammation. 2024;21:130.
24. Schwerdtfeger LA, Ryan EP, Tobet SA. An organotypic slice model for ex vivo study of neural, immune, and microbial interactions of mouse intestine. Am J Physiol Gastrointest Liver Physiol. 2016;310:G240–248.
25. Schwerdtfeger LA, Tobet SA. Vasoactive intestinal peptide regulates ileal goblet cell production in mice. Physiol Rep. 2020;8:e14363.
26. Qu S, Zheng Y, Huang Y, Feng Y, Xu K, Zhang W, Wang Y, Nie K, Qin M. Excessive consumption of mucin by over-colonized Akkermansia muciniphila promotes intestinal barrier damage during malignant intestinal environment. Front Microbiol. 2023;14:1111911.
27. Glover JS, Ticer TD, Engevik MA. Characterizing the mucin-degrading capacity of the human gut microbiota. Sci Rep. 2022;12:8456.
28. Ghezzi L, Cantoni C, Pinget GV, Zhou Y, Piccio L. Targeting the gut to treat multiple sclerosis. J Clin Invest 2021, 131.
29. Jank L, Bhargava P. Relationship between multiple sclerosis, gut dysbiosis, and inflammation: considerations for treatment. Neurol Clin. 2024;42:55–76.
30. Stolzer I, Scherer E, Suss P, Rothhammer V, Winner B, Neurath MF, Gunther C. Impact of Microbiome-Brain communication on neuroinflammation and neurodegeneration. Int J Mol Sci 2023, 24.
31. Cantarel BL, Waubant E, Chehoud C, Kuczynski J, DeSantis TZ, Warrington J, Venkatesan A, Fraser CM, Mowry EM. Gut microbiota in multiple sclerosis: possible influence of immunomodulators. J Investig Med. 2015;63:729–34.
32. Chen J, Chia N, Kalari KR, Yao JZ, Novotna M, Paz Soldan MM, Luckey DH, Marietta EV, Jeraldo PR, Chen X, et al. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. Sci Rep. 2016;6:28484.
33. Cosorich I, Dalla-Costa G, Sorini C, Ferrarese R, Messina MJ, Dolpady J, Radice E, Mariani A, Testoni PA, Canducci F et al. High frequency of intestinal T 17 cells correlates with microbiota alterations and disease activity in multiple sclerosis. Sci Adv 2017, 3.
34. Miyake S, Kim S, Suda W, Oshima K, Nakamura M, Matsuoka T, Chihara N, Tomita A, Sato W, Kim SW, et al. Dysbiosis in the gut microbiota of patients with multiple sclerosis, with a striking depletion of species belonging to clostridia XIVa and IV clusters. PLoS ONE. 2015;10:e0137429.
35. Tremlett H, Fadrosh DW, Faruqi AA, Hart J, Roalstad S, Graves J, Spencer CM, Lynch SV, Zamvil SS, Waubant E. Centers USNoPM: associations between the gut microbiota and host immune markers in pediatric multiple sclerosis and controls. BMC Neurol. 2016;16:182.
36. Blacher E, Bashiardes S, Shapiro H, Rothschild D, Mor U, Dori-Bachash M, Kleimeyer C, Moresi C, Harnik Y, Zur M, et al. Potential roles of gut Microbiome and metabolites in modulating ALS in mice. Nature. 2019;572:474–80.
37. Olson CA, Vuong HE, Yano JM, Liang QY, Nusbaum DJ, Hsiao EY. The gut microbiota mediates the Anti-Seizure effects of the ketogenic diet. Cell. 2018;173:1728–e17411713.
38. Ou Z, Deng L, Lu Z, Wu F, Liu W, Huang D, Peng Y. Protective effects of Akkermansia muciniphila on cognitive deficits and amyloid pathology in a mouse model of Alzheimer's disease. Nutr Diabetes. 2020;10:12.
39. Qiao CM, Huang WY, Zhou Y, Quan W, Niu GY, Li T, Zhang MX, Wu J, Zhao LP, Zhao WJ et al. Akkermansia muciniphila Is Beneficial to a Mouse Model of Parkinson's Disease, via Alleviated Neuroinflammation and Promoted Neurogenesis, with Involvement of SCFAs. Brain Sci 2024, 14.
40. Amorim Neto DP, Bosque BP, Pereira de Godoy JV, Rodrigues PV, Meneses DD, Tostes K, Costa Tonoli CC, de Faustino H, de Gonzalez-Billault C. Castro Fonseca M: Akkermansia muciniphila induces mitochondrial calcium overload and alpha -synuclein aggregation in an enteroendocrine cell line. iScience. 2022;25:103908.
41. Steimle A, Neumann M, Grant ET, Willieme S, De Sciscio A, Parrish A, Ollert M, Miyauchi E, Soga T, Fukuda S, et al. Gut microbial factors predict disease severity in a mouse model of multiple sclerosis. Nat Microbiol. 2024;9:2244–61.
42. Castelli LM, Cutillo L, Souza CDS, Sanchez-Martinez A, Granata I, Lin YH, Myszczynska MA, Heath PR, Livesey MR, Ning K, et al. SRSF1-dependent Inhibition of C9ORF72-repeat RNA nuclear export: genome-wide mechanisms for neuroprotection in amyotrophic lateral sclerosis. Mol Neurodegener. 2021;16:53.
43. Hautbergue GM, Castelli LM, Ferraiuolo L, Sanchez-Martinez A, Cooper-Knock J, Higginbottom A, Lin YH, Bauer CS, Dodd JE, Myszczynska MA, et al. SRSF1-dependent nuclear export Inhibition of C9ORF72 repeat transcripts prevents neurodegeneration and associated motor deficits. Nat Commun. 2017;8:16063.
44. Zhang M, Zhou Y, Jiang Y, Lu Z, Xiao X, Ning J, Sun H, Zhang X, Luo H, Can D, et al. Profiling of sexually dimorphic genes in neural cells to identify Eif2s3y, whose overexpression causes Autism-Like behaviors in male mice. Front Cell Dev Biol. 2021;9:669798.
45. Lunin SM, Glushkova OV, Khrenov MO, Novoselova TV, Parfenyuk SB, Fesenko EE, Novoselova EG. Thymic peptides restrain the inflammatory response in mice with experimental autoimmune encephalomyelitis. Immunobiology. 2013;218:402–7.
46. Salek Esfahani B, Gharesouran J, Ghafouri-Fard S, Talebian S, Arsang-Jang S, Omrani MD, Taheri M, Rezazadeh M. Down-regulation of ERMN expression in relapsing remitting multiple sclerosis. Metab Brain Dis. 2019;34:1261–6.
47. Ziaei A, Garcia-Miralles M, Radulescu CI, Sidik H, Silvin A, Bae HG, Bonnard C, Yusof N, Ferrari Bardile C, Tan LJ, et al. Ermin deficiency leads to compromised Myelin, inflammatory milieu, and susceptibility to demyelinating insult. Brain Pathol. 2022;32:e13064.
48. Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, Xing Y, Lubischer JL, Krieg PA, Krupenko SA, et al. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for Understanding brain development and function. J Neurosci. 2008;28:264–78.
49. Kelly CJ, Zheng L, Campbell EL, Saeedi B, Scholz CC, Bayless AJ, Wilson KE, Glover LE, Kominsky DJ, Magnuson A, et al. Crosstalk between Microbiota-Derived Short-Chain fatty acids and intestinal epithelial HIF augments tissue barrier function. Cell Host Microbe. 2015;17:662–71.
50. Robert C, Chassard C, Lawson PA, Bernalier-Donadille A. Bacteroides cellulosilyticus Sp. nov., a cellulolytic bacterium from the human gut microbial community. Int J Syst Evol Microbiol. 2007;57:1516–20.
51. Duscha A, Gisevius B, Hirschberg S, Yissachar N, Stangl GI, Dawin E, Bader V, Haase S, Kaisler J, David C, et al. Propionic acid shapes the multiple sclerosis disease course by an Immunomodulatory mechanism. Cell. 2020;180:1067–e10801016.
52. Kim S, Shin YC, Kim TY, Kim Y, Lee YS, Lee SH, Kim MN, Kim OE, Kweon KS. Mucin degrader Akkermansia muciniphila accelerates intestinal stem cell-mediated epithelial development. Gut Microbes. 2021;13:1–20.

53. Diaz Heijtz R, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, Hibberd ML, Forssberg H, Pettersson S. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci U S A. 2011;108:3047–52.

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