Identification of Commensal Species Positively Correlated with Early Stress Responses to a Compromised Mucus Barrier

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Background: Our aims were (1) to correlate changes in the microbiota to intestinal gene expression before and during the development of colitis in Muc2$^{-/-}$ mice and (2) to investigate whether the heterozygote Muc2$^{+/-}$ mouse would reveal host markers of gut barrier stress.

Methods: Colon histology, transcriptomics, and microbiota profiling of faecal samples was performed on wild type, Muc2$^{+/-}$, and Muc2$^{-/-}$ mice at 2, 4, and 8 weeks of age.

Results: Muc2$^{-/-}$ mice develop colitis in proximal colon after weaning, resulting in inflammatory and adaptive immune responses, and expression of genes associated with human inflammatory bowel disease. Muc2$^{+/-}$ mice do not develop colitis, but produce a thinner mucus layer. The transcriptome of Muc2$^{+/-}$ mice revealed differential expression of genes participating in mucosal stress responses and exacerbation of a transient inflammatory state around the time of weaning. Young wild type and Muc2$^{+/-}$ mice have a more constrained group of bacteria as compared with the Muc2$^{-/-}$ mice, but at 8 weeks the microbiota composition is more similar in all mice. At all ages, microbiota composition discriminated the groups of mice according to their genotype. Specific bacterial clusters correlated with altered gene expression responses to stress and bacteria, before colitis development, including colitogenic members of the genus Bacteroides.

Conclusions: The abundance of Bacteroides pathobionts increased before histological signs of pathology suggesting they may play a role in triggering the development of colitis. The Muc2$^{+/-}$ mouse produces a thinner mucus layer and can be used to study mucus barrier stress in the absence of colitis.

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Key Words: Muc2 deficiency, colitis, Bacteroidetes, stress markers

A key element of the mammalian strategy for maintaining a microbiota accommodating intestinal homeostasis is to minimize and regulate contact between luminal microorganisms and the intestinal epithelial cell surface. Physical separation of bacteria and the epithelium is largely accomplished by secretion of mucus, antimicrobial proteins, and IgA into the lumen.[1,2] Intestinal mucus is primarily composed of the highly *O*-glycosylated mucin 2 (Muc2), which is secreted by goblet cells in the epithelium. In the mouse colon, 2 distinct layers can be distinguished; a stratified inner layer, which is attached to the epithelium and largely devoid of bacteria, and a less dense outer mucus layer that is accessible to commensal microbes.[3] The protective properties of mucus are evident in Muc2$^{-/-}$ mice, which develop spontaneous colitis after weaning, when there is an expansion of the microbiota and loss of protective factors in the mother's milk.[4,5] This is associated with exacerbation of inflammatory gene expression and a decline in regulatory T cells.[6] In the ileum, the interleukin (IL)-22 regulated network

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of genes involved in antimicrobial and wound-healing functions plays a role in protecting the epithelium from damage.[7]

Mucosal barrier dysfunction is observed in inflammatory bowel diseases (IBDs), and different knockout mouse models of colitis have shown that mucus was more penetrable to fluorescent beads and bacteria than their healthy counterparts.[3,8–10] The reasons for these changes in mucus permeability are not fully understood, but may result from the structural changes in the glycoprotein core and/or the sulphation and sialylation of mucins oligosaccharide residues, as reported in patients with IBD.[11] Excessive and/or altered bacterial contact with the epithelium is known to trigger production of inflammatory cytokines, which increase epithelial permeability,[12] leading to an influx of bacteria and their products across the epithelium, thereby perpetuating the inflammatory response. In human IBD, clinical pathology is associated with the altered transcription of 32 common genes.[13] Many of which are also differentially expressed in mouse models of colitis.[13]

Mucosal inflammation may be a key driver for the abnormal composition and decreased diversity and richness of the microbiota that are common features in patients with IBD and mouse models of colitis.[14–16] Many studies on the microbiota of patients with IBD have shown that there is a lower proportion of Firmicutes, an increase in Gammaproteobacteria including the *Enterobacteriaceae*, and an overall decrease in microbiota diversity. Reduced complexity of the phylum Firmicutes is a signature of faecal microbiota of patients with Crohn's Disease with many studies describing decreased abundance of *Faecalibacterium prausnitzii*.[17] Decreased relative abundance of members of the families *Lachnospiraceae* and *Ruminococcaceae* has been described in some studies, an exception being *Ruminococcus gnavus,* which increases in abundance.[18] *Escherichia coli* pathobionts exhibiting pathogen-like behaviors such as adhesion and invasiveness are more frequently cultured from patients with IBD[19] because of their selective growth advantage in inflammatory conditions.[20–23] Functional tests of the ability of specific microbes to induce colitis in genetically susceptible mice has led to the identification of other pathobionts including members of the *Bacteroides*, with the 2 most potent disease inducing isolates belonging to the species *Bacteroides thetaiotaomicron* and *Bacteroides vulgatus*.[24,25] The studies performed in non–germ-free mice with genetic defects relevant to IBD pathways suggest that Bacteroides species may have colitogenic effects in at least a subset of patients with IBD.[25] However, changes in the abundance of species belonging to the Bacteroidetes are not consistently reported in patients with IBD.[14,26–29]

A detailed understanding of the temporal changes in microbiota outlined above and their relationship to intestinal gene expression before and during the development of colitis is currently lacking. Such knowledge might provide new insights into the dynamics of the interplay between the host and microbiota in IBD and have implications for future therapies, for example by manipulation of the microbiota. However, prospective studies requiring repeated biopsy sampling are difficult to perform in humans and the data will be complex to statistically analyze and interpret because of genetic diversity and variability in environmental exposures of the subjects. To address these problems, we took advantage of the $Muc2^{-/-}$ mouse

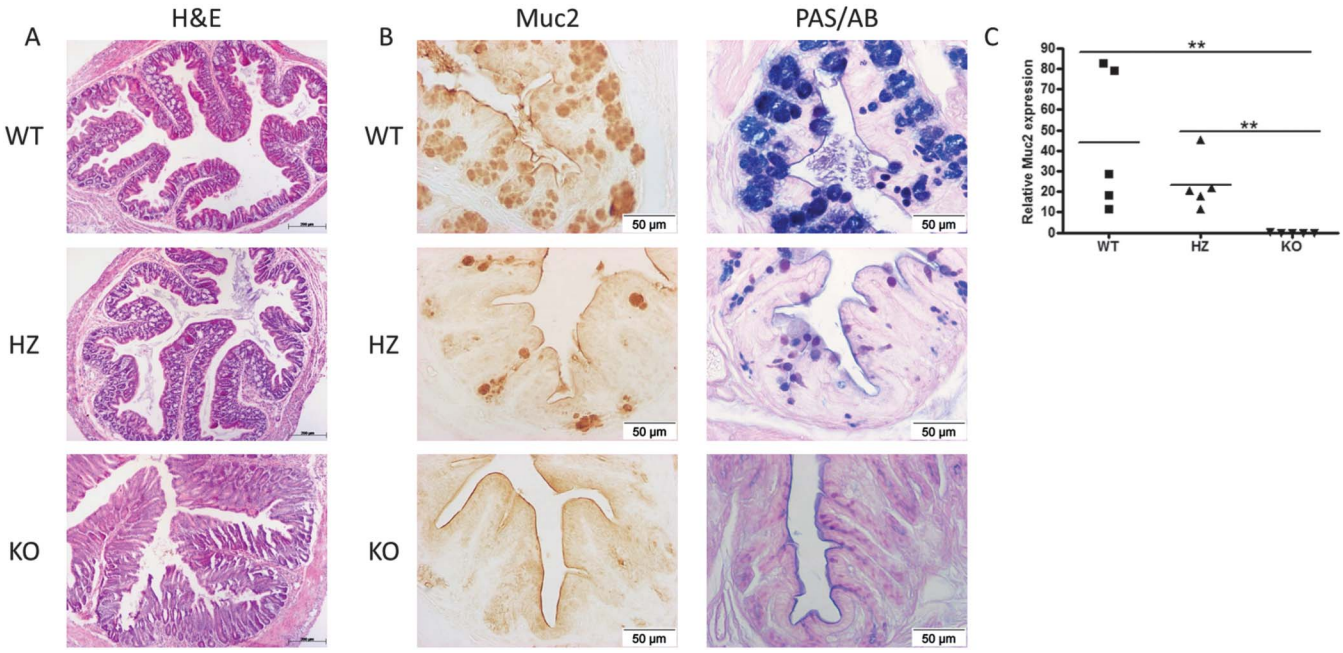

FIGURE 1. A, Representative pictures of hematoxylin and eosin staining of the proximal colon of 8-week-old WT, $Muc2^{+/-}$ (HZ), and $Muc2^{-/-}$ mice (KO). B, Representative pictures of Muc2-specific immune-histochemical staining and PAS/Alcian blue staining of the proximal colon of WT, $Muc2^{+/-}$, and $Muc2^{-/-}$ mice at 8 weeks of age. C, Relative Muc2 expression determined by quantitative polymerase chain reaction in samples of the proximal colon at 8 weeks of age (**$P < 0.01$).

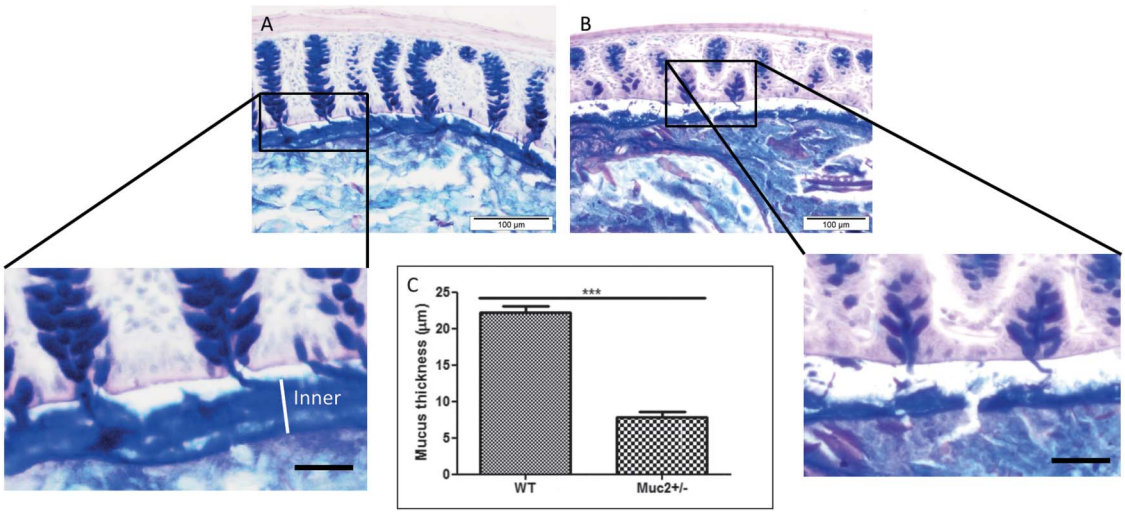

FIGURE 2. A, Representative pictures of PAS/Alcian blue staining of the proximal colon of 8-week WT (A) and Muc2$^{+/-}$ mice (B). The inner mucus layer thickness (white bar) has been measured using ImageJ software (***$P < 0.001$) (C). Scales: 100 and 20 µm.

experimental model of colitis, which provides an opportunity to identify microbiota changes and host gene expression before and after the onset of colitis. Although Muc2$^{+/-}$ mice do not develop colitis, we hypothesized that there would be decreased mucus production and a mild mucus barrier dysfunction, which might lead to altered microbiota-host interactions.

MATERIALS AND METHODS

Animals

Muc2$^{-/-}$ mice with a 129SV background were bred as previously described.[30] Mice were generated from interbreeding Muc2$^{+/-}$ mice and genotyped.[30] Mice were housed in a specific pathogen-free environment with ad libitum access to AIN93 diet (Special Diets Services, Witham, Essex, England), and acidified tap water in a 12-hours light/dark cycle. The Erasmus MC Animal Ethics Committee (Rotterdam, the Netherlands) approved the animal experiments.

Experimental Set Up

Groups of wild type (WT), Muc2$^{+/-}$, and Muc2$^{-/-}$ (n = 5 for each age and genotype) littermates were housed together with their respective birth mothers until weaning at 21 days, and were sacrificed at 14, 28, and 56 days postnatal. Proximal colons were excised and fixed in 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS), stored in RNAlater (Qiagen, Venlo, the Netherlands) at −20°C, or frozen in liquid nitrogen and stored at −80°C. In addition, colonic tissue was collected, fixed in 4% paraformaldehyde in PBS or Carnoy's fixative, and embedded in paraffin.

Histology

Paraffin sections (5 µm) of proximal colon were attached to poly-L-lysine-coated glass slides (Thermo Fisher Scientific, Dreieich, Germany). After overnight incubation at 37°C, slides were dewaxed and hydrated stepwise using 100% xylene followed by several solutions of distilled water containing decreasing amounts of ethanol. Sections were stained with hematoxylin and eosin or Periodic Acid–Schiff (PAS)/Alcian blue.[31]

Immunohistochemistry

Cell proliferation marker Ki67 was detected by incubating the sections with anti-Ki67 antibody (Abcam, Cambridge,

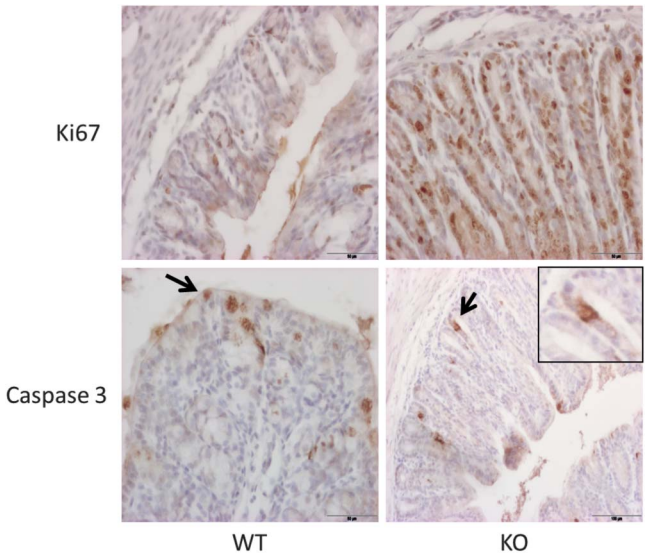

FIGURE 3. Immune-histochemical Ki67- and caspase-3–staining of sections of the proximal colon of WT and Muc2$^{-/-}$ (KO) mice. All scale bars are 50 µm. The arrows indicate apoptotic cells, which are located primarily in the surface epithelium in WT, but predominantly near the bottom of the crypts in the Muc2 knockout animals.

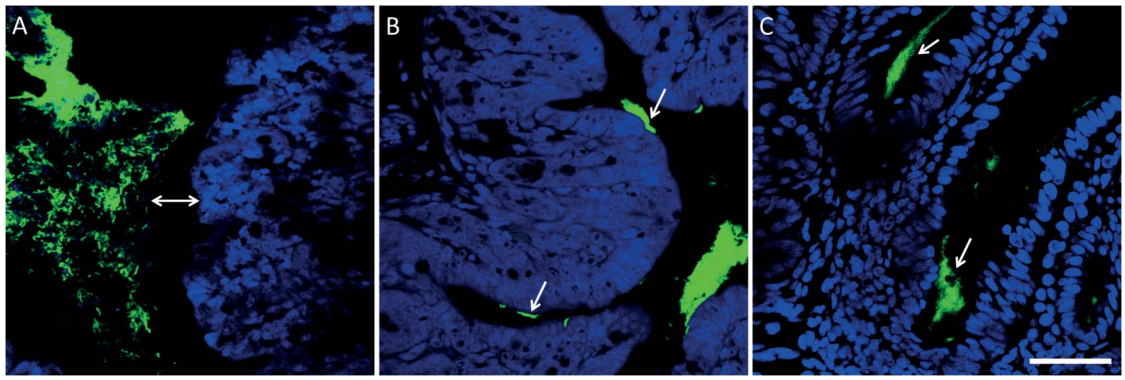

FIGURE 4. Fluorescence in situ hybridization analysis of the proximal colon of WT (A), Muc2$^{+/-}$ (B), and Muc2$^{-/-}$ (C) using the general bacterial probe EUB338-Alexa Fluor 488 (green) and nuclei staining DRAQ5 (blue). Scale for all panels identical, bar: 50 μm. Double arrow indicates the usually observed "gap" between the microbiota and the epithelium in the WT (panel A), whereas the gap is in fact the unstained mucus layer. The single arrows point to bacteria on epithelium (panel B) and deep within the crypt (panel C).

United Kingdom) diluted 1:200 in PBS, 90 minutes at room temperature. Apoptotic cells were identified by staining for cleaved caspase-3 expression, using an anticaspase-3 antibody (Abcam) diluted 1:200 in PBS, overnight at 4°C, after manufacturer's instructions.

Detection of Bacteria Using Fluorescence In Situ Hybridization

Paraffin sections were deparaffinized with xylene and hybridized with a general bacterial probe, EUB338 conjugated to Alexa 488 as described previously.[7] Nuclei were stained with

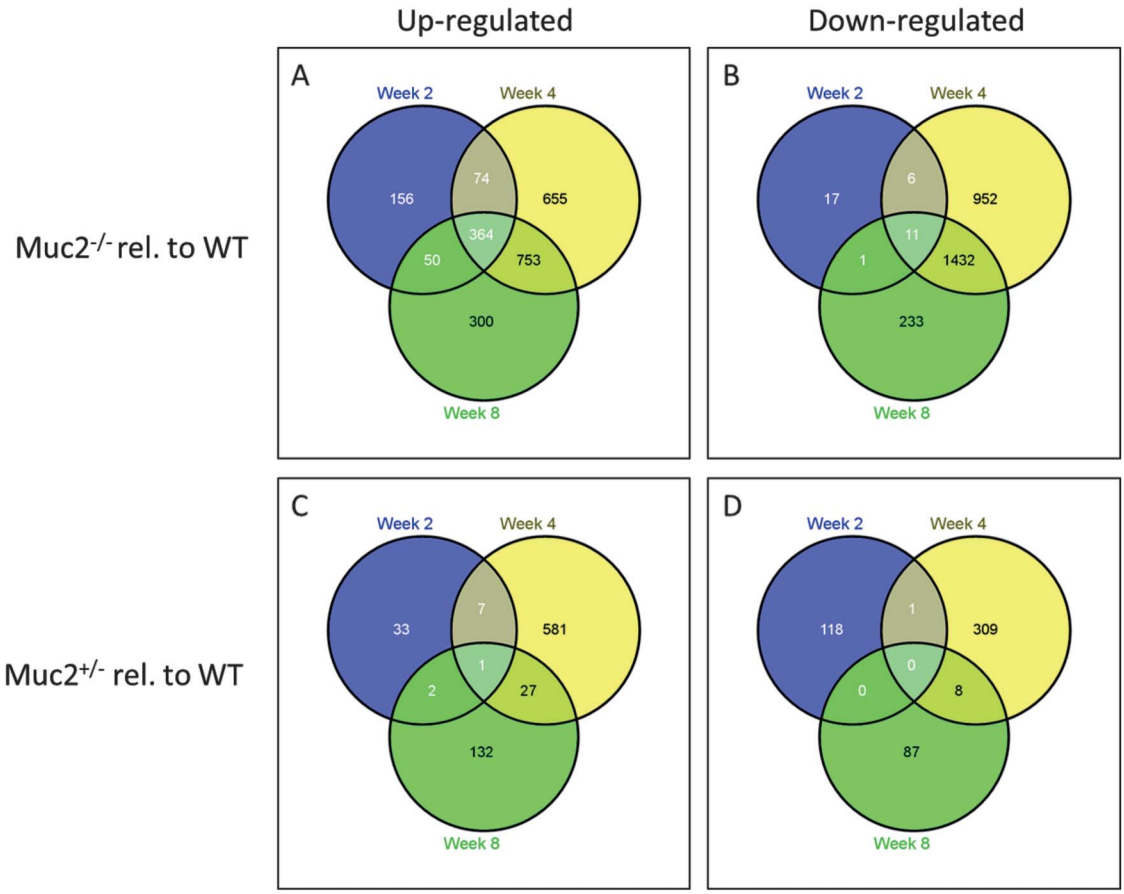

FIGURE 5. Venn diagram of the number of genes upregulated (A, C) and downregulated (B, D) in the proximal colon of Muc2$^{-/-}$ (A, B) and Muc2$^{+/-}$ (C, D) mice, compared with WT mice (P value < 0.05) at weeks 2, 4, and 8.

DRAQ5 (Invitrogen, Life Technologies Ltd., Paisley, United Kingdom), mounted in Fluoromount G (SouthernBiotec, Birmingham, Alabama), and stored at 4°C.

Transcriptome Analysis

Total RNA was extracted from the colon as described in supplementary methods (see Supplemental Digital Content 1, http://links.lww.com/IBD/B187) and hybridized to the Mouse Gene 1.1 ST array (Affymetrix, Santa Clara, CA). The labelling and hybridization methods, quality control procedures on RNA, and data sets generated were performed as described in supplementary methods (see Supplemental Digital Content 1, http://links.lww.com/IBD/B187).

Microbiota Profiling and Multivariate Integration and Correlation Analysis

Microbiota composition was determined using the Mouse Intestinal Tract Chip (MITChip) as previously described,[32] and analyzed as described in supplementary methods (see Supplemental Digital Content 1, http://links.lww.com/IBD/B187). To get insight into the interactions between changes in gene expression and microbiota composition, the data sets per time point were combined using the linear multivariate method partial least squares,[33] as described by Lange et al.[34] By this integration, the different genotypes in the study are not taken into account but the 2 data sets were integrated per individual mouse. For 14 mice,

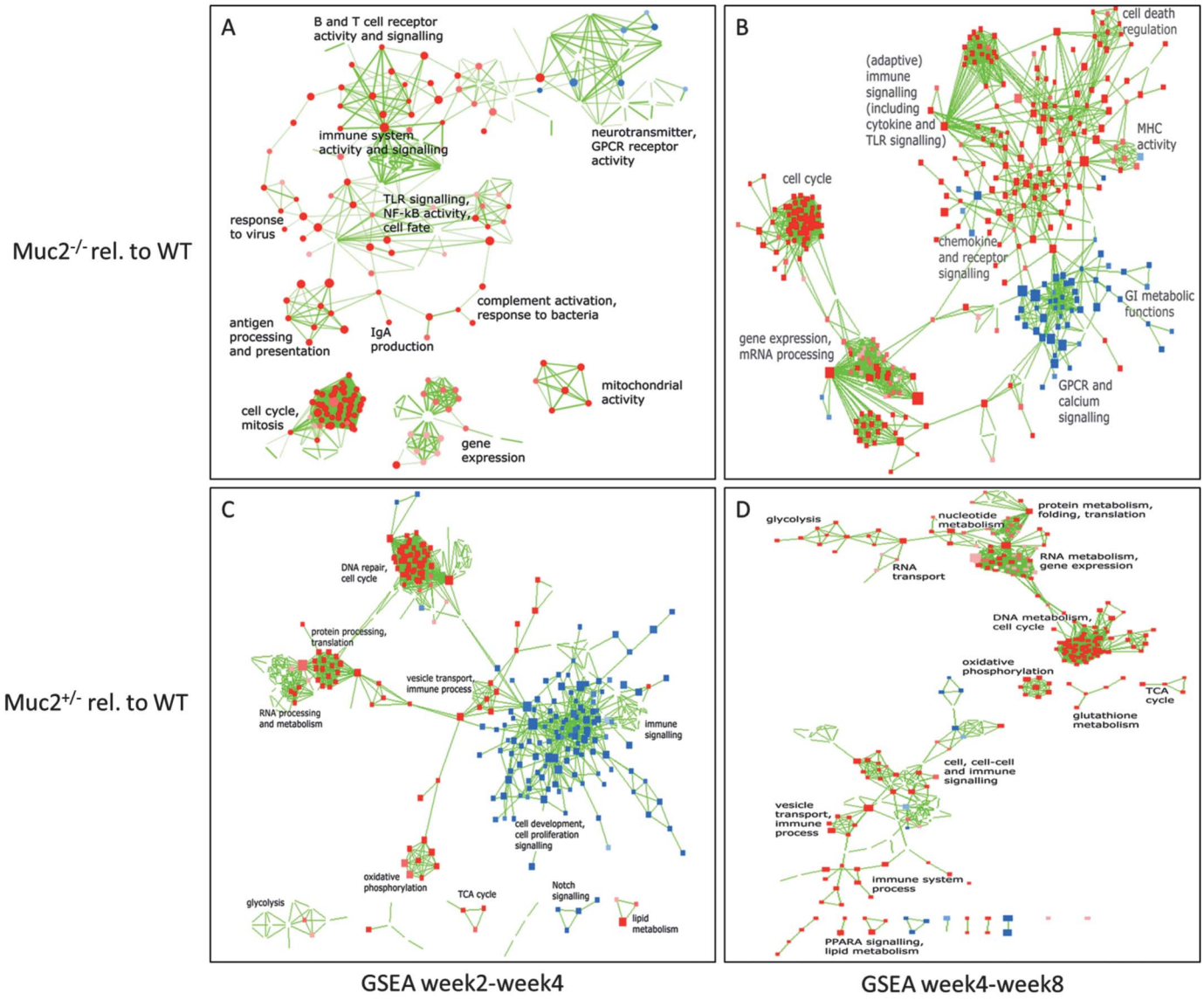

FIGURE 6. Network representation of gene set enrichment analysis profile of upregulated (red) or downregulated (blue) pathways in the proximal colon of Muc2 knockout mice compared with WT mice at (A) week 2 versus week 4 and (B) week 4 versus week 8. Network representation of gene set enrichment analysis profile of upregulated (red) or downregulated (blue) pathways in the proximal colon of $Muc2^{+/-}$ mice compared with WT mice at (C) week 2 versus week 4, and (D) week 4 versus week 8. The "geography" of these representations has no implicit meaning. Nodes are colored in more intense shades of red depending on strength of induction; or nodes are colored in more intense shades of blue depending on strength of repression.

TABLE 1. A Comparison of the Differentially Expressed Genes, Involved in Human IBD,[13] in the Colon of Muc2$^{-/-}$ and Muc2$^{+/-}$ Mice

		Genes Regulated in IBD					
		Week 2		Week 4		Week 8	
Gene	Name	Muc 2$^{+/-}$	Muc 2$^{-/-}$	Muc 2$^{+/-}$	Muc 2$^{-/-}$	Muc 2$^{+/-}$	Muc 2$^{-/-}$
Cytokine and cytokine receptor genes							
Tnf	Tumor necrosis factor	NS	NS	NS	2.028773	NS	1.840602
Infr1	Interferon-γ receptor 1	NS	NS	NS	NS	NS	NS
Ltb	Lymphotoxin β	NS	1.382878	NS	1.333015	1.243062	1.462893
IL6ra	Interleukin 6 receptor	NS	NS	NS	NS	NS	NS
IL16	Interleukin 16	NS	NS	NS	NS	NS	NS
IL18r1	Interleukin 18 receptor 1	NS	NS	NS	NS	NS	NS
IL22ra1	Interleukin 22 receptor 1	NS	NS	NS	1.256704	NS	NS
IL22ra2	Interleukin 22 receptor 2	1.265914	2.190521	1.300013	NS	NS	−1.43333
Chemokine and chemokine receptor genes							
Ccr2	CCR2	NS	NS	NS	1.746213	NS	1.378777
Ccr7	CCR7	NS	NS	NS	NS	NS	NS
Ccl2	JE (hu:MCP-1)						
Ccl3	MIP-1α	NS	NS	1.37451	NS	NS	1.893286
Ccl5	RANTES	NS	1.277747	NS	NS	NS	NS
Ccl7	MARC (hu:MCP-3)	NS	NS	1.365612	NS	NS	NS
Ccl11	Eotaxin	NS	NS	NS	−1.56851	NS	NS
Ccl17	TARC	NS	NS	NS	NS	NS	NS
Ccl20	MIP-3	NS	−1.42516	NS	NS	NS	NS
Cxcr3	CXCR3	NS	NS	NS	NS	NS	NS
Cxcl1	Chemokine (C-X-C motif) ligand 1	NS	NS	NS	NS	NS	NS
Cxcl5	Chemokine (C-X-C motif) ligand 5						
Cxcl10	IP-10	NS	NS	NS	NS	NS	1.617166
Gene involved in tissue remodeling							
Mmp3	Stromelysin 1	NS	NS	NS	NS	1.250031	1.554525
Mmp7	Matrilysin	1.510885	2.19082	NS	1.276596	1.295367	1.254885
Mmp9	Gelatinase B	NS	NS	NS	1.454466	NS	NS
Mmp14	Membrane type 1-matrix metalloproteinases	NS	NS	NS	1.312401	NS	1.2019
Timp1	Tissue inhibitor of metalloproteinase 1	NS	1.126899	1.340559	1.323982	NS	1.740643
Regenerating islet-derived genes							
Reg3g	Regenerating islet-derived 3 gamma	NS	2.410785	1.719448	2.246373	NS	1.822651
Pap	Pancreatitis-associated protein	NS	NS	NS	NS	NS	NS
S110 family genes							
S100-a8	S100 calcium-binding protein a8	NS	NS	NS	NS	NS	NS
S100-a9	S100 calcium-binding protein a9	NS	NS	NS	NS	NS	NS

TABLE 1 (*Continued*)

Gene	Name	Genes Regulated in IBD					
		Week 2		Week 4		Week 8	
		Muc $2^{+/-}$	Muc $2^{-/-}$	Muc $2^{+/-}$	Muc $2^{-/-}$	Muc $2^{+/-}$	Muc $2^{-/-}$
Multidrug resistance gene							
Abcb1a	ATP-binding cassette, subfamily B (MDR/TAP), member 1A	NS	NS	NS	−1.56233	NS	−1.52592
Gene involved in epithelial metabolism and biosynthesis							
Ptgs2	Prostaglandin-endoperoxide synthase 2 (COX-2)	NS	NS	NS	1.201594	NS	1.490934

Up or downregulated genes, compared with WT, at different time points as indicated.
NS, nonsignificant.

both gene expression and microbiota composition data were available at 2 weeks. Both data sets were log2 transformed before analysis and the canonical correlation framework of partial least squares was used.[35] The correlation matrices were visualized in clustered image maps.[36] Analyses were performed in R using the library mixOmics.[37]

RESULTS

Muc2$^{+/-}$ Mice Have Altered Mucus Properties Compared with WT Mice, but Do Not Develop Histopathological Features of Colitis

Alcian blue staining was used to identify acidic carbohydrates and PAS for neutral carbohydrates, both of which occur on the Muc2 glycoprotein. PAS-positive and combined PAS–Alcian blue-positive goblet cells were observed in the colon of WT and Muc2$^{+/-}$ mice. In contrast, goblet cells in Muc2$^{-/-}$ mice only stained slightly positive for PAS (Fig. 1B). Moreover, these PAS-positive cells did not stain positive for Muc2 and lacked the swollen morphology typical of goblet cells. As expected, a secreted mucus layer was absent Muc2$^{-/-}$ mice (not shown).

Muc2$^{+/-}$ mice had fewer Muc2-positive goblet cells than WT mice (Fig. 1B). The relative amount of Muc2 transcript was not significantly different between WT and Muc2$^{+/-}$ because of large biological variation (Fig. 1C). Nevertheless, the mucus was significantly thinner in Muc2$^{+/-}$ compared with WT mice, supporting the hypothesis that this genotype produces less mucus (Fig. 2).

From 4 weeks of age, colitis was observed in the proximal colon of Muc2$^{-/-}$ mice coinciding with altered crypt architecture, increased crypt length, and mild infiltration of lymphocytes in the lamina propria (Fig. 1A). In Muc2$^{-/-}$, there were increased numbers of proliferative enterocytes (Ki67-positive), and capase-3–positive apoptotic cells present within the crypt epithelium, compared with WT colon tissue (Fig. 3). In contrast, no distinct morphological differences were observed between WT and Muc2$^{+/-}$ at 2, 4, and 8 weeks (Fig. 1A).

In WT mice, we observed a clear spatial separation of the microbiota and the epithelium (Fig. 4A). At many locations in the colon of Muc2$^{-/-}$ mice, the microbiota was observed in direct contact with epithelial surfaces and deep in the crypts, which was never observed in the WT (Fig. 4C). Bacteria were also occasionally seen in direct contact with epithelium of Muc2$^{+/-}$ mice (Fig. 4B), presumably due to the thinner colonic mucus layer (Fig. 2).

Weaning Leads to a Transient Peak in the Number Differentially Induced or Repressed Gene Transcripts in Both Muc2$^{-/-}$ and Muc2$^{+/-}$ Mice

At all ages, both the Muc2$^{-/-}$ and Muc2$^{+/-}$ mice showed altered gene expression in the colon, compared with WT (Fig. 5). At week 4, there were substantially larger numbers of differentially induced or repressed gene transcripts in the colon of Muc2$^{-/-}$ and Muc2$^{+/-}$ mice than at week 2 or 8 (Fig. 5). Many differentially expressed genes in Muc2$^{-/-}$ and Muc2$^{+/-}$ mice were associated with induction of immune pathways and epithelial remodelling (see below and Fig. 6). At week 4, histopathological signs of colitis were evident in the Muc2$^{-/-}$ mice and inflammatory pathway responses persisted to the end of the experiment (week 8). However, in Muc2$^{+/-}$ mice, some differentially expressed genes encoding cytokines, chemokines, and cytokine receptors induced at week 4 were not altered at week 8 compared with WT mice.

Pathways Related to Inflammation, Cell Death, Extracellular Matrix Remodelling, and Cell Cycle are Upregulated in the Colon of Muc2$^{-/-}$ Mice

To gain insight into the pathways affected in Muc2$^{-/-}$ and Muc2$^{+/-}$ mice, a gene set enrichment analysis[38] was performed, comparing changes in gene expression in Muc2$^{+/-}$ and Muc2$^{-/-}$ mice to WT at the intervals from week 2 to 4 and week 4 to 8. From week 2 to week 4, comparing Muc2$^{-/-}$ with WT mice, the major cellular pathways differentially induced involve innate signalling, B-cell and T-cell activity, antigen processing, antiviral response, and IgA production; compatible with immune activation and inflammatory responses in the colonic mucosa. The strong induction of pathways involved in cell cycle and mitosis most likely indicate increased immune and/or epithelial cell proliferation and/or turnover (Fig. 6A, B). Several nodes in the gene networks for neurotransmitter and G protein-coupled receptor activity were downregulated at week 4 in colon of Muc2$^{-/-}$ mice (Fig. 6A). At week 8, similar gene sets were increased in the colon of Muc2$^{-/-}$ mice as in week 4, with the addition of induced expression of genes linked to chemotaxis of granulocytes, IL-12 signalling, and glutathione metabolism (redox homeostasis) (Fig. 6). Seven of the 32 IBD-related genes significantly altered in expression in human IBD[13] were differentially expressed in the colon of Muc2$^{-/-}$ mice at week 2 compared with WT mice (Table 1), including 2 matrix metalloproteinases that contribute to epithelial and endothelial barrier disruption and enable immune cells to infiltrate into the tissue.

At week 4, 13 IBD-related genes were upregulated, and 14 were upregulated at week 8, concomitant with major tissue damage. IBD-related genes differentially expressed in Muc2$^{-/-}$ mice at weeks 4 and 8 were matrix metalloproteases (Mmp) *Mmp3, Mmp7*, *Mmp9*, *Cxcl10*, *IL22ra2*, and *IL22ra1*, which were all upregulated except for the IL-22 receptor (*IL22ra1*) at week 8. Relative expression of the IL-22 receptor (*IL22ra1*) was increased at week 4 and week 8 compared with week 2, whereas, *IL22ra2*,

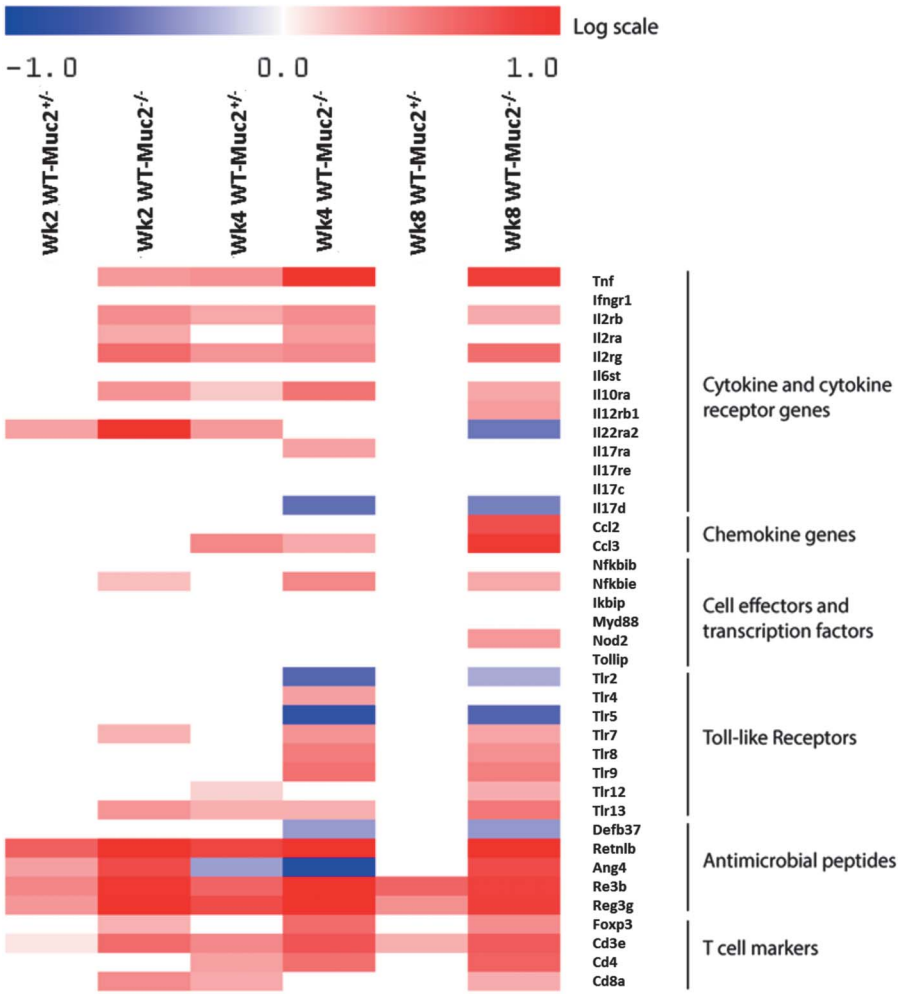

FIGURE 7. Heat maps of immunity related genes differentially expressed in proximal colon, using genes differentially expressed in Muc2$^{-/-}$ versus WT, or Muc2$^{+/-}$ versus WT mice with week 8 as a reference. In red, genes are represented, which are upregulated and in blue the genes that are downregulated. The intensity of the color is related to the level of expression.

the soluble antagonist of *IL22ra1*, was significantly downregulated at these times. The most strongly downregulated gene at week 4 and 8 was *Abcb1a*, a gene expressing an epithelial cell surface-located transporter that is proposed to export toxins from the mucosa into the lumen.

Interestingly, 7 other of these IBD-related genes such as *Ltb*, *IL22ra2*, *Ccl5*, *Ccl20*, *Mmp7*, *Timp1*, and *Reg3γ* were already altered before the onset of histopathology. These genes are related to mucosal healing and defenses and chemotactic responses. A heat map was generated for immunity genes including those that encode pattern recognition receptors, cytokines, antimicrobial (poly)peptides, components of the NF-κB pathway, T-cell markers (CD3e) and T-helper (CD4), Tregs (Foxp3), and Th17 (RORgamma t) subsets that were differentially expressed in the proximal colon of Muc2$^{-/-}$ versus WT mice at weeks 2, 4, and 8 (Fig. 7). In Muc2$^{-/-}$ mice, transcription of several TLR genes was upregulated (*Tlr4, TlR7, Tlr8, Tlr9, Tlr12,* and *Tlr13*), whereas *Tlr2* and *Tlr5*, which were strongly repressed at weeks 4 and 8. The genes encoding antimicrobial stress proteins *Reg3β* and *Reg3γ* were strongly upregulated, but the beta-defensin (Defb) 37 encoding gene was downregulated. The upregulated *Tnf-α*, *Cd3ε*, *Cd4*, and *Cd8* suggest an increased infiltration and/or activity of T cells. At 2 weeks, even before histological signs of colitis were apparent, many immune-related genes were upregulated in the colon. This included inflammatory cytokines, T-cell markers, innate defense factors and regulators of inflammatory processes such as inhibitor of NF-κB epsilon and soluble antagonist of IL-22 receptor signalling.

The Transcriptome of Muc2$^{+/-}$ Mice Reveals a Homeostatic Response to Decreased Mucus Production

A large number of differentially expressed genes are identical in Muc2$^{+/-}$ and Muc2$^{-/-}$ mice when compared with WT at each age, suggesting that these changes are brought about by similar mechanisms in both genotypes. For example, of the genes differentially regulated at week 2, 19 were upregulated and 36 downregulated in both Muc2$^{+/-}$ and Muc2$^{-/-}$ mice (data not shown). Gene set enrichment analysis of the networks affected in the proximal colon of Muc2$^{+/-}$ revealed that DNA repair, energy metabolism, and cell-cycle pathways were strongly upregulated at weeks 2, 4, and 8 (Fig. 6C, D). Expression of these pathways was also altered in Muc2$^{-/-}$ mice compared with WT, although the magnitude of the changes was greater than in Muc2$^{+/-}$ mice. Even though Muc2$^{+/-}$ mice do not develop colitis, IBD-related genes *IL22ra2* and *Mmp3* were differentially expressed in Muc2$^{+/-}$ mice compared with WT at week 2; this increased to 5 genes (*IL22ra2*, *Ccl3*, *Ccl7*, *Timp1,* and *Reg3γ*) at week 4, and 3 genes (*Ltb*, *Mmp3,* and *Mmp7*) at week 8 (Table 1). Genes upregulated in both Muc2$^{-/-}$ and Muc2$^{+/-}$ mice at either weeks 4 or 8 encode cytokines *(Ccl3*, *Ccl22)*, antimicrobial protein *(Reg3γ)*, or tissue-remodeling enzymes *(Mmp3*, *Mmp7*, and *Timp1),* and lymphotoxin B. These genes are involved in remodeling of the extracellular matrix, mucosal healing, and innate defense including chemotactic functions.

Muc2 Deficiency Affects Colon Microbiota Composition, Diversity, and Richness in Early Life

16S ribosomal RNA (rRNA) gene-derived microbiota profiles were obtained from faeces of 2-, 4-, and 8-week-old WT, Muc2$^{+/-}$, and Muc2$^{-/-}$ mice. At 2 weeks, the faecal content of Muc2$^{-/-}$ mice displayed a significantly higher microbial diversity than WT and Muc2$^{+/-}$ mice ($P < 0.05$) because of increased richness of the profiles as measured at the probe level (Fig. 8A, B). Furthermore, redundancy analysis (RDA) established that at weeks 2, 4, and 8 the microbiota composition could be discriminated on basis of the host's genotype (Fig. 9A–C). The

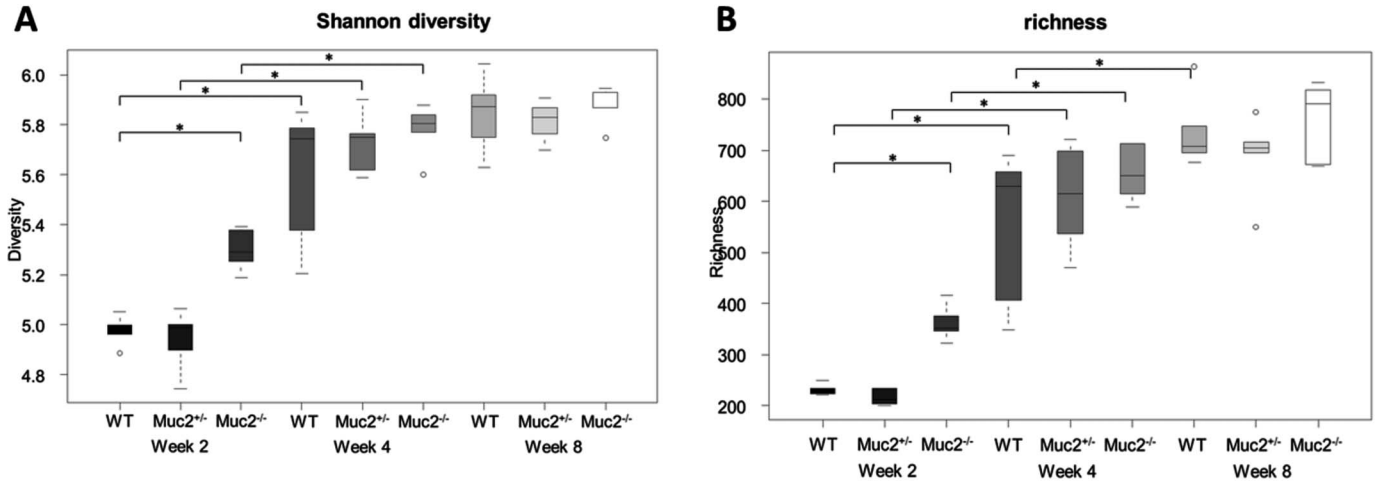

FIGURE 8. Box-and-whiskers plot showing the diversity (Shannon index) of microbiota in colon of WT, Muc2$^{+/-}$, and Muc2$^{-/-}$ mice (A). Box-and-whiskers plot showing the richness of microbiota in colon of WT, Muc2$^{+/-}$, and Muc2$^{-/-}$ mice (B). Statistically significant differences among groups and time points were indicated (*$P < 0.05$).

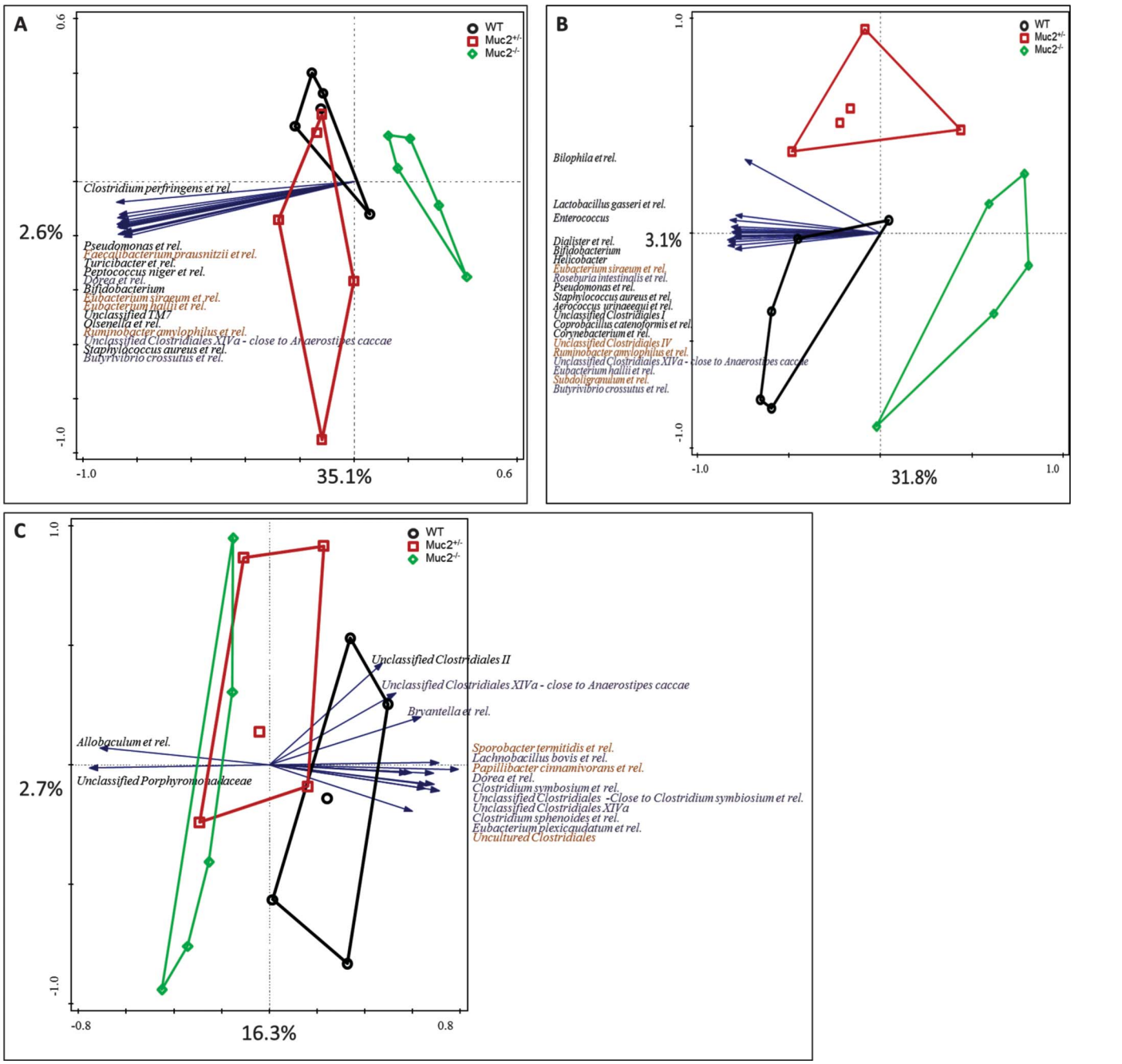

FIGURE 9. Redundancy analysis representing microbial community of Muc2$^{-/-}$ (green cluster), Muc2$^{+/-}$ (red cluster), and WT (black cluster) in the proximal colon at weeks 2 (A), 4 (B), and 8 (C). Scores of all individual mice are indicated in each of panels (A–C); the clusters include all mice within a given genotype.

differences in colonic microbiota composition of Muc2$^{-/-}$ mice and the other groups of mice were largest at week 2 and decreased over time (Fig. 9C). The WT mice are characterized by higher relative abundances of members of the *Clostridium* clusters IV and XIVa compared with the Muc2$^{-/-}$ mice at each time point. *Bifidobacterium*, a well-known early life microbe in humans, had also higher relative abundances in the WT relative to the Muc2$^{-/-}$ mice at weeks 2 and 4, but not at 8 weeks. Remarkably, the microbiota composition of Muc2$^{+/-}$ mice displays an intermediate situation between the WT and Muc2$^{-/-}$ mice at all time points (Fig. 9A–C).

Taking the diversity, richness, and redundancy analysis plots into account, there are indications that in WT and Muc2$^{+/-}$ mice there is an initial colonization by a more constrained group of bacteria (2 weeks), which reached higher relative abundance in

the WT and Muc2$^{+/-}$ mice, compared with the Muc2$^{-/-}$ mice that are colonized by a more diverse microbial community. During further development of the colonic ecology at 4 and 8 weeks, the microbiota composition becomes more similar among mice with different genotypes, both with respect to diversity and richness, as well as in composition.

Specific Bacterial Clusters Correlate with Altered Gene Expression of Mucosal Responses to Stress and Bacteria, Before Colitis Development

To identify bacteria that might be correlated with early changes in colon gene expression, we focused on the data from week 2, which was before the onset of any histopathology in the Muc2$^{-/-}$ mice. Microbiota and transcriptomics data from week 2 were combined for individual mice of all genotypes to investigate direct correlations between gene expression and microbiota composition in these samples. Age and genotype were the major drivers of changes in gene expression and microbial composition (Figs. 6 and 8), and thus in this analysis, any correlations found between gene expression and microbiota will be the implicit result of variation in genotype. Four bacterial clusters strongly correlated positively (red) or negatively (blue) with 6 clusters of genes (about 100 genes per cluster; Fig. 10).

Strongest correlations between microbiota members (orange in the vertical bar) and specific changes in mucosal gene expression (orange and blue in horizontal bar) were detected for *Bacteroides plebeius* et rel., unclassified *Prevotella*, *Alistipes* et rel., *Bacteroides fragilis* et rel., and *B. vulgatus* et rel. (Fig. 10). These bacteria had higher relative abundances (data not shown) in the Muc2$^{-/-}$ mice compared with WT and displayed a positive correlation with immune response genes. A positive correlation was also found with stress response genes involved in apoptosis and cell proliferation (Table 2). Conversely, other microbial groups (pink in the vertical bar) displayed opposite correlations with the same mucosal gene sets, including *Pseudomonas* et rel., *Fusobacterium vibrio* et rel., *Solobacterium moorei* et rel., and *Sutterella wadsworthia* et rel.

DISCUSSION

Previously, microarray analysis and quantitative polymerase chain reaction of gene transcripts from colon of Muc2$^{-/-}$ and

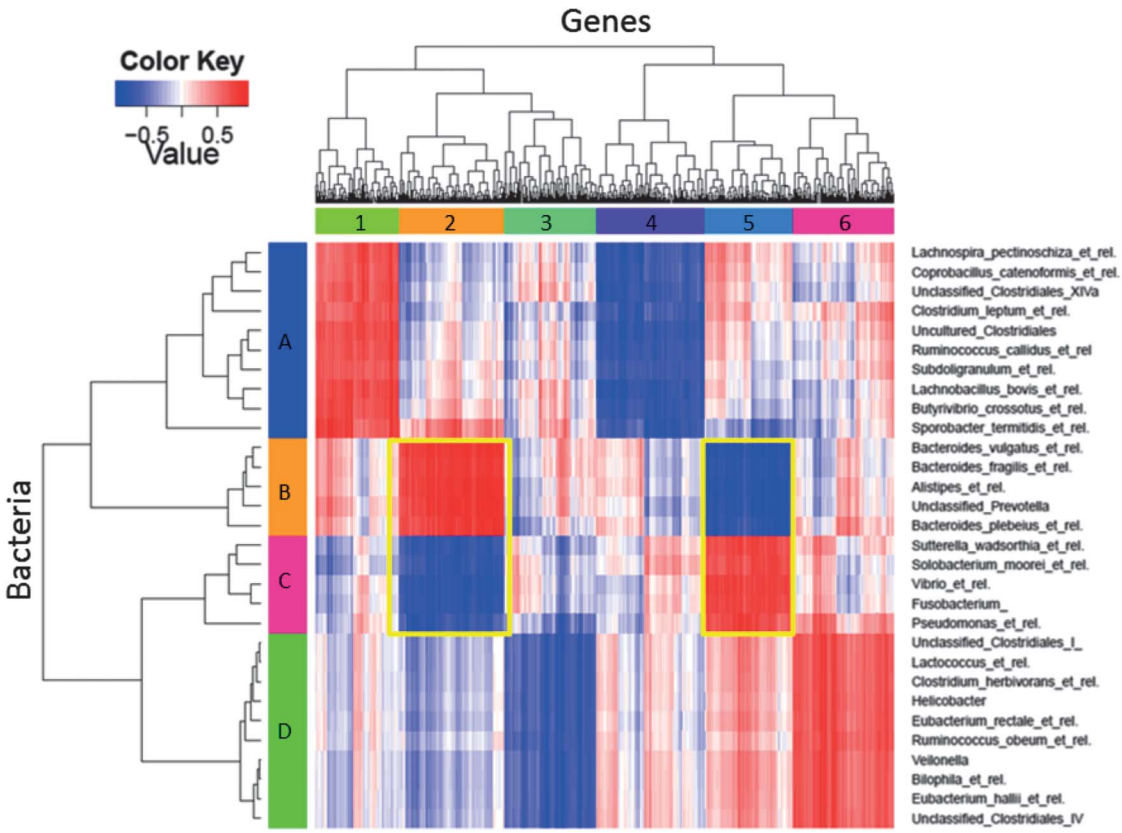

FIGURE 10. Heat map of correlation analysis of MITChip (vertical) and transcriptome (horizontal) data sets at week 2. The integration of data sets was performed per individual and gives direct correlation between gene expression and microbiota composition over these samples. The genotypes are the largest driver for differences in gene expression and microbiota composition, which is reflected in the highly correlated clusters in the plot. In deep red, the cluster of genes that most positively correlated with 1 group of bacteria. In deep blue, the cluster of genes that most negatively correlated with a respective group of bacteria. Framed in yellow; the clusters discussed in more detail in the article. Framed in black; the gene clusters 2 and 5 discussed in more detail in the article. Six main gene clusters (1–6) and 4 main bacterial clusters (A–D) were identified.

TABLE 2. Gene Ontology Terms from the Gene Cluster 2 (Orange in the Horizontal bar) Correlated with Microbiota (Orange and Pink Vertical Bars) As Shown in Figure 10

GO ID	GO Term	*P*	Gene Count	Gene Name
GO:0043170	Macromolecule metabolic process	0.003191	53	
GO:0044260	Cellular macromolecule metabolic process	0.006806	47	
GO:0019222	Regulation of metabolic process	0.009964	39	
GO:0060255	Regulation of macromolecule metabolic process	0.003132	35	
GO:0006950	Response to stress	8.84E-05	28	S100a14[a], Fos[a], Hbegf[a], Il1rn[a], Apobec1[a], Krt20[a], Dsc2, Anxa3[a], Casp8, Pdia5[a], Proc, Anxa2, Tlr7[a], Pik3cg, Txndc5, Mapk13, Cd55, Hmgcl, Foxa3, Ruvbl2, Optn, Tnfrsf14, Nudt16, Os9, Eno3, Clca3[a], Ang4[a], Pnliprp2
GO:0009605	Response to external stimulus	2.62E-05	22	Itlt1, S100a14[a], Fos[a], Il1rn[a], Apobec1[a], Ccl9[a], Dsc2, Anxa3, Lgals3, Casp8, Proc, Anxa2, Pla2g10[a], Tlr7[a], Pik3cg, Cd55, Hmgcl, Foxa3, Optn, Tnfrsf14, Ccl6[a], And4[a]
GO:0051246	Regulation of protein metabolic process	0.00188	17	
GO:0002376	Immune system process	0.005061	17	S110a14[a], Fos[a], Ilrn[a], Apobec1[a], Ccl9[a], Anxa3, Lgals3, Casp8, Anxa2, Pla2g10, Tlr7, Pik3cg, Klf10, Cd55, Tnfrsf14, Ccl6[a], Ang4[a]
GO:0043207	Response to external biotic stimulus	0.000484	11	
GO:0051707	Response to other organism	0.000484	11	Itln1, S100a14[a], Fos[a], Ilrn[a], Apobec1[a], Anxa3, Casp8, Tlr7[a], Optn, Tnfrsf14[a], Ang4[a]
GO:0009607	Response to biotic stimulus	0.000754	11	
GO:0044092	Negative regulation of molecular function	0.006828	10	
GO:0001944	Vasculature development	0.001773	9	
GO:0001525	Angiogenesis	0.000516	8	
GO:0009617	Response to bacterium	0.001775	8	S100a14[a], Fos[a], Il1rn[a], Anxa3[a], CVasp8, Tnfrsf14[a], Ang4[a]
GO:0048514	Blood vessel morphogenesis	0.002008	8	
GO:0001568	Blood vessel development	0.004588	8	
GO:0051270	Regulation of cellular component movement	0.007115	8	
GO:0098542	Defense response to other organism	0.002368	7	S100a14[a], Apobec1[a], Anxa3, Tlr7[a], Optn, Tnfrsf14[a], Ang4[a]
GO:0071496	Cellular response to external stimulus	0.001336	6	Fos[a], Dsc2, Casp8, Tlr7[a], Foxa3, Optn
GO:0030335	Positive regulation of cell migration	0.002529	6	
GO:2000147	Positive regulation of cell motility	0.002813	6	
GO:0051272	Positive regulation of cellular component movement	0.003172	6	
GO:0040017	Positive regulation of locomotion	0.003868	6	
GO:0042594	Response to starvation	0.000947	5	
GO:0042742	Defense response to bacterium	0.003503	5	S100a14[a], Anxa3[a], Optn, Tnfrsf14[a], Ang4[a]

TABLE 2 (*Continued*)

GO ID	GO Term	*P*	Gene Count	Gene Name
GO:0070613	Regulation of protein processing	0.009256	5	
GO:0002573	Myeloid leukocyte differentiation	0.009472	4	Fos[a], Casp8, Anxa2, Klf10
GO:0002763	Positive regulation of myeloid leukocyte differentiation	0.001249	3	Fos[a], Casp8, Klf10
GO:0007173	Epidermal growth factor receptor signaling pathway	0.003741	3	
GO:0045639	Positive regulation of myeloid cell differentiation	0.004638	3	
GO:0038127	ERBB signaling pathway	0.005234	3	
GO:0030316	Osteoclast differentiation	0.005873	3	
GO:0002687	Positive regulation of leukocyte migration	0.006558	3	S100a14[a], Lgals3, Tnfrsf14[a]

[a]Genes significantly upregulated (fold-change > 1.2; $P < 0.05$).

WT mice at 2 and 4 weeks of age revealed distinct phases in colitis development pre and postweaning, which were presumed the result of changes in microbiota diversity and/or density.[5] Here, we extended these comparisons to 8 weeks of age, because the first several days after weaning are known to be characterized by a relatively chaotic pattern of microbial colonization leading toward an adult-like microbiota, and driving transient host-microbiota responses that lead to an adult-microbiota accommodating homeostatic state.[39] As predicted, the largest number of differentially expressed genes in Muc2$^{-/-}$ compared with WT mice was found at weaning (1607 genes) and subsequently reduced to 533 genes in week 8. The pathways differentially expressed from week 2 to week 8 in Muc2$^{-/-}$ compared with WT mice were diverse immune pathways consistent with immune activation and inflammatory responses in the colonic mucosa. There was also a strong induction of pathways involved in cell death, cell cycle, and mitosis, most likely as a response to the mucosal damage observed by histology. As in other mouse models of colitis a substantial number of the 32 genes known to be upregulated or downregulated in human IBD (Table 1)[13] were differentially expressed in the colon of Muc2$^{-/-}$ mice compared with WT mice.[13,38] The most strongly upregulated genes include genes encoding matrix metalloproteinases, an important family of metal-dependent enzymes that are responsible for the breakdown and reconstitution of extracellular matrix in normal physiological processes, like tissue remodelling, during development, growth and wound repair, and in pathological conditions, including destructive diseases, such as IBD.

In contrast to Muc2$^{-/-}$, the Muc2$^{+/-}$ mice did not develop colitis during the 8-week period. However, the colonic mucus layer was significantly thinner in Muc2$^{+/-}$ than in WT mice, which was also associated with altered mucosal gene expression. At week 2 and week 8 the affected gene networks in Muc2$^{+/-}$ mice were mainly associated with mucosal healing and innate defense including chemotactic functions, although there was transient increase in inflammatory genes (e.g., *Tnf* and cytokine receptors for *IFNγ*, *IL-2*) at week 4 resembling the transient inflammatory state observed in colonization studies with germ-free mice.[39] Thus, the Muc2$^{+/-}$ genotype seemed to exacerbate the transient inflammatory response to the changing microbial ecology of the colon around weaning. Several of the genes differentially expressed in the Muc2$^{+/-}$ mice were IBD-related, for example, the intestinal Reg3 secreted stress proteins and matrix metalloproteinases.

As pathobionts are known to play a role in the pathophysiology IBD, we investigated the impact of the Muc2 genotype on the temporal development of the microbiota at 2, 4, and 8 weeks. Initial colonization of microbiota in WT and Muc2$^{+/-}$ mice involved a more constrained group of bacteria as compared with the Muc2$^{-/-}$ mice, although during prolonged colonization the microbiota composition became more similar in all mice, but still consistently discriminated the groups of mice according to their genotype. This suggests that Muc2 shapes the microbiota colonizing the colon, especially in the 2 weeks postnatal, preweaning period, although a role of the antimicrobial factors that were more highly expressed in Muc2$^{-/-}$ mice compared with WT or Muc2$^{+/-}$ mice can also play a role. In the case of the Muc2$^{+/-}$ genotype mice, which still produce a mucus layer, the altered microbiota might be due to the mucosal responses identified by transcriptomics.

To identify bacteria that might be correlated with early changes in colon gene expression, we used the linear multivariate method partial least squares method[33] for each time point, as previously described (Lange et al, in revision). Interestingly, these analyses revealed that even at 2 weeks, therefore before the onset of colitis in the Muc2$^{-/-}$ mice, *B. plebeius* et rel., unclassified *Prevotella*, *Alistipes* et rel., *B. fragilis* et rel., and *B. vulgatus* et rel. were positively correlated with the induction of mucosal gene expression associated with immune and stress responses

(Table 2). These bacterial groups were more abundant in Muc2$^{-/-}$ mice than in WT littermates. An increased relative abundance of Bacteroidetes has been observed in only some animal models of colitis.[40,41] Nevertheless, *B. thetaiotaomicron* and *B. vulgatus* have been demonstrated to induce colitis in experimental rodent models, in which other commensal species do not induce colitis.[25,42–44] Nod2 mutations that disrupt bacterial recognition are one of the highest risk factors for CD. Recently, expansion of *B. vulgatus*, was shown to mediate exacerbated inflammation in Nod2$^{-/-}$ mice on small-intestinal injury, providing further evidence for its colitogenic potential in IBD.[45] A subset of intestinal *B. fragilis* strains produces an exotoxin (Enterotoxigenic *B. fragilis* strains) associated with diarrheal disease and a number of studies have shown associations of this species with IBD and colorectal cancer.[46]

Recently, inflammation was shown to select for modifications of the Bacteroidetes lipopolysaccharide, which increased their resistance to inflammation-associated antimicrobial peptides by 4 orders of magnitude.[47] These findings support the idea that in Muc2$^{-/-}$ mice, the innate inflammatory responses driven by increased contact of commensals with the epithelium lead to increased abundance of colitogenic members of the Bacteroidetes phylum, which contribute to the onset of colitis. Models such as Muc2$^{-/-}$ mice can be useful to isolate strains from genera correlated with early stages of colitis development and investigate the genetic and molecular basis for their increased abundance and their interactions with the host.

We showed that even a reduction in mucus production (i.e., in Muc2$^{+/-}$ mice) altered microbiota composition and diversity and induced mucosal stress response genes in the colon, highlighting the importance of the barrier function of mucus in maintaining intestinal homeostasis. Muc2$^{+/-}$ mice therefore represent an interesting model of a compromised barrier function and could be used to investigate the long-term effects on host physiology and other intestinal diseases including colon cancer and infection with pathogenic microbes. In addition, Muc2$^{+/-}$ mice could be used to screen for nutritional interventions that might improve barrier function and reduce expression of stress response proteins such as Reg3γ and Reg3β.

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REFERENCES

1. Macpherson AJ, Gatto D, Sainsbury E, et al. A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. *Science*. 2000;288:2222.
2. Macpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. *Nat Rev Immunol.* 2004;4:478–485.
3. Johansson ME, Phillipson M, Petersson J, et al. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc Natl Acad Sci U S A.* 2008;105:15064–15069.
4. Van der Sluis M, De Koning BA, De Bruijn AC, et al. Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. *Gastroenterology*. 2006;131:117–129.
5. Burger-van Paassen N, van der Sluis M, Bouma J, et al. Colitis development during the suckling-weaning transition in mucin Muc2-deficient mice. *Am J Physiol Gastrointest Liver Physiol.* 2011;301:G667–G678.
6. Lu P, Burger-van Paassen N, van der Sluis M, et al. Colonic gene expression patterns of mucin Muc2 knockout mice reveal various phases in colitis development. *Inflamm Bowel Dis.* 2011;17:2047–2057.
7. Sovran B, Loonen LM, Lu P, et al. IL-22-STAT3 pathway plays a key role in the maintenance of ileal homeostasis in mice lacking secreted mucus barrier. *Inflamm Bowel Dis.* 2015;21:531–542.
8. Johansson MEV, Hansson GC. The goblet cell: a key player in ischaemia-reperfusion injury. *Gut*. 2013;62:188–189.
9. Abraham C, Cho JH. Mechanisms of disease inflammatory bowel disease. *N Engl J Med.* 2009;361:2066–2078.
10. Swidsinski A, Loening-Baucke V, Theissig F, et al. Comparative study of the intestinal mucus barrier in normal and inflamed colon. *Gut*. 2007;56:343–350.
11. Boltin D, Perets TT, Vilkin A, et al. Mucin function in inflammatory bowel disease an update. *J Clin Gastroenterol.* 2013;47:106–111.
12. Wells JM, Rossi O, Meijerink M, et al. Epithelial crosstalk at the microbiota-mucosal interface. *Proc Natl Acad Sci U S A.* 2011;108: 4607–4614.
13. te Velde AA, de Kort F, Sterrenburg E, et al. Comparative analysis of colonic gene expression of three experimental colitis models mimicking inflammatory bowel disease. *Inflamm Bowel Dis.* 2007;13:325–330.
14. Frank DN, Amand ALS, Feldman RA, et al. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A.* 2007;104:13780–13785.
15. Ott SJ, Musfeldt M, Wenderoth DF, et al. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut*. 2004;53:685–693.
16. Rooks MG, Veiga P, Wardwell-Scott LH, et al. Gut microbiome composition and function in experimental colitis during active disease and treatment-induced remission. *ISME J.* 2014;8:1403–1417.
17. Miquel S, Martin R, Rossi O, et al. Faecalibacterium prausnitzii and human intestinal health. *Curr Opin Microbiol.* 2013;16:255–261.
18. Joossens M, Huys G, Cnockaert M, et al. Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives. *Gut*. 2011;60:631–637.
19. Chassaing B, Darfeuille-Michaud A. The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. *Gastroenterology*. 2011;140:1720–1728.
20. Fleckenstein JM, Hardwidge PR, Munson GP, et al. Molecular mechanisms of enterotoxigenic Escherichia coli infection. *Microbes Infect.* 2010;12:89–98.
21. Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. *Nat Rev Immunol.* 2013;13:790–801.
22. Winter SE, Winter MG, Xavier MN, et al. Host-derived nitrate boosts growth of E. coli in the inflamed gut. *Science*. 2013;339:708–711.
23. Chow J, Tang H, Mazmanian SK. Pathobionts of the gastrointestinal microbiota and inflammatory disease. *Curr Opin Immunol.* 2011;23: 473–480.
24. Hansen JJ, Huang Y, Peterson DA, et al. The colitis-associated transcriptional profile of commensal Bacteroides thetaiotaomicron enhances adaptive immune responses to a bacterial antigen. *PLoS One*. 2012;7:e42645.
25. Bloom SM, Bijanki VN, Nava GM, et al. Commensal Bacteroides species induce colitis in host-genotype-specific fashion in a mouse model of inflammatory bowel disease. *Cell Host Microbe.* 2011;9:390–403.
26. Takaishi H, Matsuki T, Nakazawa A, et al. Imbalance in intestinal microflora constitution could be involved in the pathogenesis of inflammatory bowel disease. *Int J Med Microbiol.* 2008;298:463–472.
27. Gophna U, Sommerfeld K, Gophna S, et al. Differences between tissue-associated intestinal microfloras of patients with Crohn's disease and ulcerative colitis. *J Clin Microbiol.* 2006;44:4136–4141.
28. Bibiloni R, Mangold M, Madsen KL, et al. The bacteriology of biopsies differs between newly diagnosed, untreated, Crohn's disease and ulcerative colitis patients. *J Med Microbiol.* 2006;55:1141–1149.

29. Andoh A, Tsujikawa T, Sasaki M, et al. Faecal microbiota profile of Crohn's disease determined by terminal restriction fragment length polymorphism analysis. *Aliment Pharmacol Ther.* 2009;29:75–82.
30. Velcich A, Yang W, Heyer J, et al. Colorectal Cancer in mice genetically deficient in the mucin Muc2. *Science.* 2002;295:1726–1729.
31. Yamabayashi S. Periodic acid-Schiff-alcian blue: a method for the differential staining of glycoproteins. *Histochem J.* 1987;19:565–571.
32. Geurts L, Lazarevic V, Derrien M, et al. Altered gut microbiota and endocannabinoid system tone in obese and diabetic leptin-resistant mice: impact on apelin regulation in adipose tissue. *Front Microbiol.* 2011;2:149.
33. Boulesteix AL, Strimmer K. Partial least squares: a versatile tool for the analysis of high-dimensional genomic data. *Brief Bioinform.* 2007;8:32–44.
34. Lange K, Hugenholtz F, Jonathan MC, et al. Comparison of the effects of five dietary fibers on mucosal transcriptional profiles, and luminal microbiota composition and SCFA concentrations in murine colon. *Mol Nutr Food Res*. 2015;59:1590–1602.
35. Le Cao KA, Martin PG, Robert-Granie C, et al. Sparse canonical methods for biological data integration: application to a cross-platform study. *BMC Bioinformatics.* 2009;10:34.
36. Gonzalez I, Cao KA, Davis MJ, et al. Visualising associations between paired "omics" data sets. *BioData Min.* 2012;5:19.
37. Le Cao KA, Gonzalez I, Dejean S. integrOmics: an R package to unravel relationships between two omics datasets. *Bioinformatics*. 2009;25: 2855–2856.
38. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A.* 2005;102:15545–15550.
39. El Aidy S, Derrien M, Merrifield CA, et al. Gut bacteria-host metabolic interplay during conventionalisation of the mouse germfree colon. *ISME J.* 2013;7:743–755.
40. Hansen J, Gulati A, Sartor RB. The role of mucosal immunity and host genetics in defining intestinal commensal bacteria. *Curr Opin Gastroenterol.* 2010;26:564–571.
41. Schwab C, Berry D, Rauch I, et al. Longitudinal study of murine microbiota activity and interactions with the host during acute inflammation and recovery. *ISME J.* 2014;8:1101–1114.
42. Hammer RE, Maika SD, Richardson JA, et al. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human beta 2m: an animal model of HLA-B27-associated human disorders. *Cell*. 1990;63: 1099–1112.
43. Rath HC, Herfarth HH, Ikeda JS, et al. Normal luminal bacteria, especially Bacteroides species, mediate chronic colitis, gastritis, and arthritis in HLA-B27/human beta2 microglobulin transgenic rats. *J Clin Invest.* 1996; 98:945–953.
44. Rath HC, Wilson KH, Sartor RB. Differential induction of colitis and gastritis in HLA-B27 transgenic rats selectively colonized with Bacteroides vulgatus or Escherichia coli. *Infect Immun.* 1999;67:2969–2974.
45. Ramanan D, Tang MS, Bowcutt R, et al. Bacterial sensor Nod2 prevents inflammation of the small intestine by restricting the expansion of the commensal Bacteroides vulgatus. *Immunity*. 2014;41:311–324.
46. Chow J, Mazmanian SK. A pathobiont of the microbiota balances host colonization and intestinal inflammation. *Cell Host Microbe.* 2010;7: 265–276.
47. Cullen TW, Schofield WB, Barry NA, et al. Antimicrobial peptide resistance mediates resilience of prominent gut commensals during inflammation. *Science*. 2015;347:170–175.