Reprogrammed and transmissible intestinal microbiota confer diminished susceptibility to induced colitis in TMF$^{-/-}$ mice

Shai Bel[a], Yoav Elkis[a], Hila Elifantz[a], Omry Koren[b], Rotem Ben-Hamo[a], Tal Lerer-Goldshtein[a], Roni Rahimi[a], Shomron Ben Horin[c], Abraham Nyska[d], Sally Shpungin[a], and Uri Nir[a,e,1]

[a]The Mina and Everard Goodman Faculty of Life Sciences and [e]Quaresa Institute for Cancer Research, Bar-Ilan University, Ramat-Gan 5290002, Israel; [b]Faculty of Medicine, Bar-Ilan University, Safed 1311502, Israel; [c]Department of Gastroenterology, Sheba Medical Center, Tel-Hashomer 52621, Israel; and [d]Sackler School of Medicine, Tel-Aviv University, Tel Aviv 36576, Israel

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Tata Element Modulatory Factor (TMF/ARA160) is a multifunctional Golgi-associated protein, which accumulates in colonic enterocytes and goblet cells. Mice lacking TMF/ARA160 (TMF$^{-/-}$) produce thick and uniform colonic mucus that resists adherent bacterial colonization and diminishes susceptibility of these mice to induced acute colitis, through a mechanism that is not fully understood. Here, we show that mucus secretion by goblet cells is altered in the colon of TMF$^{-/-}$ mice, resulting in the formation of a highly oligomerized colonic gel-forming mucin, MUC2. Microbiome analysis revealed a shift in the microbiota of TMF$^{-/-}$ mice leading to predominance of the Firmicutes phylum and a significantly higher abundance of probiotic beneficial bacterial species. Notably, this trait was transmissible, and when cohoused with wild-type animals, TMF$^{-/-}$ mice influenced the microbiota and diminished the susceptibility of wild-type mice to chemically induced dextran sulfate sodium colitis. Thus, altered mucus secretion in TMF$^{-/-}$ mouse colons is accompanied by a reprogrammed intestinal microbiota, leading to a transmissible reduced sensitivity to induced colitis.

co-housing | inflammatory bowel disease | mucus granule

The mouse and human gut serve as a home for a multitude of microorganisms including fungi, bacteria, and viruses (1). Recent advances in sequencing technologies have provided substantial insight into the delicate balance between intestinal bacterial populations (2). An abundance of new data support the notion that many human conditions such as obesity, cancer, inflammatory bowel diseases (IBDs), and others are influenced and even triggered by a shift in balance between bacterial communities, or dysbiosis, in our body (3). Although the gut microbiota differ to some extent between mice and humans, specific families and species of bacteria can have similar pathogenic or beneficial effects in both species (4).

As part of its defense mechanisms, the gut epithelium secretes a viscous, gel-like mucus into the gut lumen. The main component of this mucus is the MUC2 mucin, an evolutionarily conserved, heavily glycosylated protein. The MUC2 protein, containing >200 cysteine residues that form intrachain and interchain disulfide bonds during folding and multimerization, is manufactured and secreted by intestinal goblet cells (5, 6). In these cells, the synthesis of the MUC2 protein is followed by translocation to the endoplasmic reticulum, where MUC2 folds and forms disulfide dimers via cysteine knots at its C-terminal end. Next, in the Golgi apparatus, the MUC2 dimers are heavily glycosylated before being densely packed in secretory vesicles, where the N-terminal ends of the dimers are believed to form oligomeric structures via disulfide bonds. Direction and retention of the MUC2 mucin in secretory vesicles is crucial for correct folding and multimerization of the colonic mucus because of the chemical environment inside these vesicles (7). Just before secretion from the goblet cells, the vesicle membranes fuse to each other, combining their mucus cargo (7). After secretion from the goblet cell theca, the MUC2 mucin forms a dense and viscous polymeric gel layer, largely due to interchain disulfide bonds, on the luminal surface of the epithelium (8). The outer face of this coating is degraded into a second, more diffuse layer by both bacterial and host factors (5). In healthy humans and mice, the inner mucus layer is impregnable to bacteria (5), whereas the outer layer serves as both a scaffold—preventing the bacteria from being cleared by peristalsis—and an energy source for various bacterial strains by bacterial consumption of mucus-released carbohydrates (9). Genetic ablation of the *muc2* gene in mice results in the development of spontaneous colitis, thus underscoring the protective role of the MUC2 gel-forming mucin by separating the host from the commensal bacteria (10). In ulcerative colitis patients and certain knockout (KO) mouse models, the inner mucus layer is penetrated by bacteria, which reach the colonic epithelia and thereafter trigger an inflammatory immune response (11). A recent study has further shown that in addition to its protective role, MUC2 plays a crucial role in delivering tolerogenic signals to the gut inflammatory cell population, thereby playing a dual role in intestinal homeostasis (12).

Tata Element Modulatory Factor (TMF/ARA160; TMF) is a Golgi-associated protein that is apically expressed in colonic enterocytes and goblet cells and has been shown to be involved in vesicle tethering to target membranes (13, 14). We recently showed that KO mice lacking TMF/ARA160 (TMF$^{-/-}$) have a thicker, more robust colonic mucus layer, which significantly reduces adherent bacterial load and protects the animals from experimental acute colitis (13). To gain a deeper insight into the

Significance

Our data demonstrate that a knockout of a single gene (tmf1) leads to the beneficial reprogramming of the gut resident microbiota. This reprogramming results in a diminished susceptibility of the genetically modified animals to induced colitis. Notably, the reprogrammed bacterial profile is transmissible, thereby conferring altered microbiome and reduced susceptibility to induced colitis in wild-type mice, when cohoused. Our findings open previously unreported avenues for unraveling regulatory factors that affect the gut homeostasis and mammalian sensitivity to the onset of inflammatory bowel diseases.

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[1]To whom correspondence should be addressed. E-mail: uri.nir@biu.ac.il.

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effect of an altered mucosal architecture on the gut microbiota and on the susceptibility of TMF$^{-/-}$ mice to colitis, we followed the MUC2 secretion process and the gut microbiome in these mice.

In this study, we show that colonic goblet cells of TMF$^{-/-}$ mice exhibit altered granular secretion of MUC2. Goblet cells of TMF$^{-/-}$ mice secrete dense granules, with delayed fusion and subsequent discharge of a highly oligomerized MUC2 protein. This modified MUC2 is part of a mucus layer, which is largely undegraded, that causes reprogramming of the TMF$^{-/-}$ gut microbiota, thereby enabling beneficial bacterial strains of the Firmicutes phylum to prosper. As a result, TMF$^{-/-}$ mice are protected from the development of chronic colitis, a trait that is transmissible to wild-type (WT) mice when these strains are cohoused.

Results

Altered Secretion and Oligomerization Profile of MUC2 in TMF$^{-/-}$ Colons. We previously showed that the gut epithelium of TMF$^{-/-}$ mice is lined with a thicker and more robust mucus layer compared with WT mice (13). To further explore the molecular mechanisms that could contribute to this modified mucosal architecture, we used transmission electron microscopy (TEM) on colon sections to follow the production and secretion process of MUC2 in TMF$^{-/-}$ and WT goblet cells. In WT mice the mucus-packed granules underwent fusion to each other as the secretion process progressed (Fig. 1*A*), and just before secretion, the WT goblet cell theca was filled with mucus as the individual granules had fused to each other (Fig. 1 *B* and *C*). However, in TMF$^{-/-}$ goblet cells, mucus granules did not fuse during the secretion process, even as the individual granules protruded into the colonic lumen (Fig. 1 *D*–*F*). Instead, the mucus was released from these cells in a stepwise manner from each granule separately (Fig. 1*F*). To further substantiate the involvement of TMF in the secretion process of MUC2, we coimmunostained human colonic section to determine the subcellular localization of MUC2 and TMF. This analysis revealed the colocalization of the two proteins in the theca of goblet cells (Fig. 1 *G*–*J*).

One of the factors that could affect the spatial structure and organization of MUC2 in the colonic mucus is the formation of disulfide bonds, which mediate the oligomerization of the protein (7). We therefore analyzed the state of S–S bonds in MUC2 from TMF$^{-/-}$ and WT mice colons. Western blot analysis was performed on mucus scraped from colons of the two mice cohorts by using SDS/PAGE analysis separation under reducing and nonreducing conditions. Under nonreducing conditions, MUC2 from WT mice colons appears as a smear, indicating natural mucus degradation by bacterial and host proteases (15). MUC2 from TMF$^{-/-}$ mice colons, conversely, did not seem to be degraded and appeared as a high-molecular-weight specie, which, because of its size, did not migrate into the SDS/PAGE (Fig. 1*K*). Under reducing conditions, MUC2 from both cohorts appeared as bands of similar molecular weight, indicating altered disulfide bond formation in TMF$^{-/-}$ colonic mucus (Fig. 1*L*). Notably, a higher level of MUC2 was found in samples from TMF$^{-/-}$ colons (Fig. 1*L*), supporting the increased stability of MUC2 in these animals. This observation also coincides with our previous finding (13) that the transcription level of the *muc2* gene is elevated in colons of TMF$^{-/-}$ mice.

Other mucins are produced in the colon, with the majority being cell-surface mucins (8). To test whether loss of TMF affects other mucins, we performed Western blot analysis of the membrane-bound Mucin 1 (16). Mucin 1 profile from TMF$^{-/-}$ and WT mice did not show any significant difference (Fig. S1).

TMF$^{-/-}$ Mouse Colons Harbor Altered Microbiota That Is Transmissible to Cohoused WT Animals. The secreted colonic mucus functions as both a barrier, separating bacteria from the gut epithelia, and a scaffold and energy source for bacteria (5). Because the microbiota

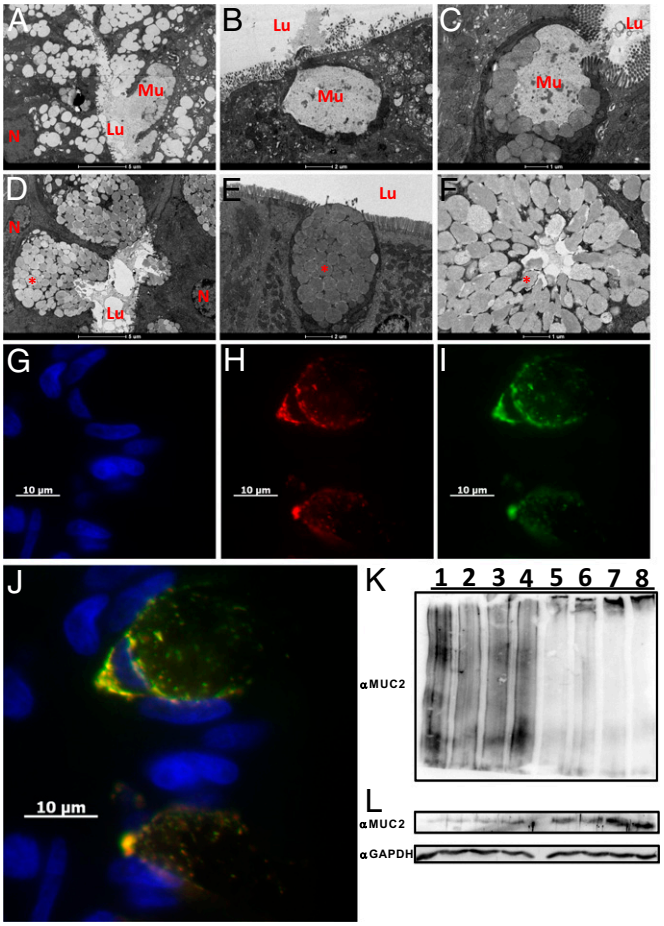

Fig. 1. Distinct goblet cell morphology and mucus secretion profile in TMF$^{-/-}$ mouse colons. (*A*–*F*) TEM of colon sections from WT (*A*–*C*) and TMF$^{-/-}$ (KO) (*D*–*F*) mice. Scale bars are as indicated. Lu, lumen; Mu, fused mucus granules; N, nucleus; *, mucus granules that do not fuse. (*G*–*J*) Coimmunofluorescence of TMF and MUC2 in goblet cells from human biopsies. Nuclei are visualized with Hoechst in blue (*G*), anti-TMF antibody in red (*F*), and anti-MUC2 in green (*I*). (*J*) All channels combined. Scale bars are as indicated. The images shown are representative of five biopsies. (*K* and *L*) Western blot analysis of colonic mucus from WT (lanes 1–4) and KO (lanes 5–8) mice colons without (*K*) or with (*L*) DTT as a reducing agent. Samples were stained with anti-MUC2 antibody, as indicated, and with anti-GAPDH to ensure equal loading. Each lane in the blot represents a sample from a different mouse.

composition in the colon is affected by changes in host-liberated glycans, which are released from degraded mucus (9), we next compared the commensal bacterial microbiota in colons of TMF$^{-/-}$ and WT mice.

Fecal samples from separately housed TMF$^{-/-}$ and WT mice (mice were housed in multiple cages to rule out stochastic "cage effect") were used for bacterial DNA extraction and bar-coded sequencing of the 16S rRNA gene V1–V3 regions. Principal coordinates analysis (PCoA) plots of weighted UniFrac distances (17) showed that TMF$^{-/-}$ mice host a significantly distinct bacterial population from that profiled in WT mice (Fig. 2*A*). Shannon–Wiener Diversity Index was calculated for each mouse in a given group to identify microbiota diversity of each mouse within each group. This analysis revealed that TMF$^{-/-}$ mice had a higher diversity index than WT mice housed alone, indicating a richer and more diverse bacterial microbiota in the TMF$^{-/-}$ mice (Fig. 2*B*).

The distinct microbiota of TMF$^{-/-}$ mice prompted us to examine whether this profile is transmissible to WT mice. This transmissibility would enable us to test whether there is a link

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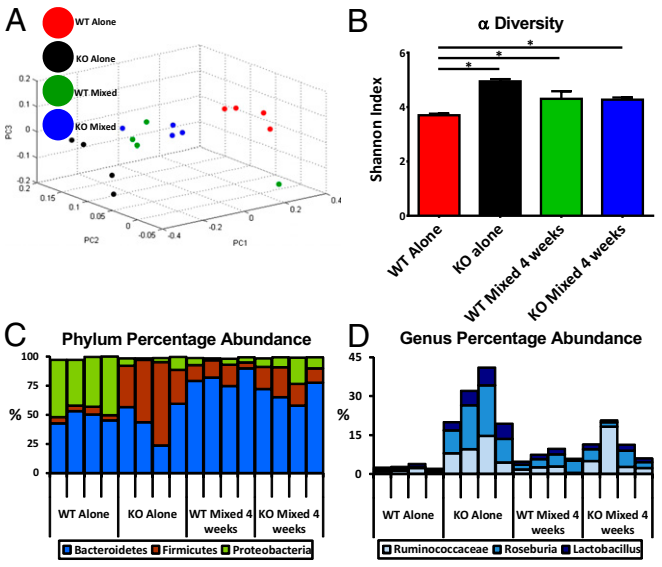

Fig. 2. Distinct microbiota profile in fecal samples of TMF$^{-/-}$ mice and transmission to WT mice. KO and WT mice were either housed alone or cohoused for 4 wk. Fecal samples were then collected, and microbiota composition was analyzed with 16S rRNA analysis. (*A*) PCoA plots of bacterial populations based on the first three principal components. Each dot represents one mouse. Colors represent the groups as shown near the colored circles. (*B*) Difference in Shannon–Wiener Diversity among the different groups. *$P < 0.05$ (ANOVA). (*C*) Taxon-based analysis at phylum level among the different groups. Data are shown as percent relative abundance. Phyla with >1% abundance are shown. Each bar represents one mouse. Each color represents one phylum as indicated at the bottom of the histograms. (*D*) Relative abundance of an uncultured member of the Ruminococcaceae, *Roseburia*, and *Lactobacillus* genus among the different groups. Each bar represents one mouse. Color index is shown at the bottom.

between the altered microbiota and the reduced sensitivity of TMF$^{-/-}$ mice to colitis. We therefore analyzed fecal samples from TMF$^{-/-}$ and WT mice cohoused for 4 wk. The microbiota profile of the TMF$^{-/-}$ and WT cohoused mice were indistinguishable from each other, but quite distinct from the two cohorts housed alone (Fig. 2*A*). Notably, the microbiome diversity index of the WT cohoused mice was higher than the WT mice housed alone, whereas the diversity index of the TMF$^{-/-}$ cohoused mice was lower than the TMF$^{-/-}$ mice housed alone (Fig. 2*B*).

Taxon-based analysis revealed that TMF$^{-/-}$ mice housed alone had a much higher ratio of Firmicutes to Bacteroidetes and Proteobacteria phylum than separately housed WT mice (Fig. 2*C*). When cohoused, TMF$^{-/-}$ mice conferred this ratio to WT mice, so that the two mice cohorts shared a similar ratio of Firmicutes to Bacteroidetes and Proteobacteria (Fig. 2*C*). At the family level, TMF$^{-/-}$ mice housed alone had a much higher abundance of the Lachnospiraceae, Ruminococcaceae, Lactobacillaceae, and Rikenellaceae families, compared with WT mice housed alone (Fig. S2). Again, this phenotype was transmissible to WT mice when cohoused with TMF$^{-/-}$ mice (Fig. S2). At the genus level, we found that TMF$^{-/-}$ mice had significantly higher relative abundance of an uncultured member of the Ruminococcaceae, *Roseburia*, and *Lactobacillus* groups compared with WT mice housed alone (Fig. 2*D*). These groups of bacteria are known to be beneficial and are associated with decreased susceptibility to IBDs (18–20). The transmissibility of these abundant probiotic bacteria to WT mice was significant but limited. Reciprocally, the level of these bacterial groups was reduced in the cohoused TMF$^{-/-}$ mice (Fig. 2*D*).

To examine whether mixing mice from different cages had an effect on colonic microbiota, we cohoused WT mice from different cages in one cage and TMF$^{-/-}$ mice from different cages in another cage for a period of 4 wk before collecting fecal samples for analysis. Bar-coded sequencing of the 16S rRNA gene V1–V3 regions from these samples did not show any significant effect of mixing mice from the same genotype (Fig. S3).

Resistance of TMF$^{-/-}$ Mice to Chronic Colitis Is Transmissible to Cohoused WT Animals. To examine the effects of the beneficial microbiota established in TMF$^{-/-}$ mice on mice's susceptibility to induced colitis, we carried out a sensitivity transmissibility test. We used an established model of acute and chronic colitis in which mice are given 3% (wt/vol) dextran sulfate sodium (DSS) in their drinking water for 5 d to induce acute colitis. This treatment is followed by 4 wk of plain drinking water, during which the chronic phase develops (21). WT and TMF$^{-/-}$ mice were cohoused for 4 wk and then treated with DSS. The cohoused groups were administered DSS in their drinking water, in parallel to WT and TMF$^{-/-}$ cohorts that were housed separately. The progression of colonic disease in each group was followed for 33 d by monitoring weight change and fecal scores.

During the first 10 d, encompassing the acute phase of the experimental model, TMF$^{-/-}$ mice housed alone exhibited an attenuated response to DSS, compared with the WT mice. This response was reflected in both the weight loss and fecal score parameters (Fig. 3 *A* and *B*), as described (13). However, in the cohoused group, clinical symptoms were reduced in the WT mice, whereas they were exacerbated in the cohoused TMF$^{-/-}$ mice (Fig. 3 *A* and *B*).

Development of chronic colitis was assessed in all groups at day 33 after mice were killed, by evaluating several inflammatory parameters. These included histopathological examination and determination of proinflammatory cytokine levels. Although colonic sections from TMF$^{-/-}$ mice housed alone did not reveal the development of chronic colitis (Fig. 3*D*), WT mice grown alone developed mild chronic colitis characterized by loss of crypt architecture and considerable infiltration (Fig. 3*C*). WT mice cohoused with TMF$^{-/-}$ mice showed only minimal signs of chronic colitis (Fig. 3*E*), and, notably, cohoused TMF$^{-/-}$ mice developed severe chronic colitis with massive infiltration and ulceration of the epithelial barrier (Fig. 3*F*). This histopathological analysis is summarized in Fig. 3*G*.

Examination of proinflammatory cytokines [TNF-α, IL-1β, and keratinocyte-derived chemokines (KCs)] at day 33 revealed a similar trend. TMF$^{-/-}$ mice housed alone showed baseline levels of cytokines, whereas WT mice housed alone showed elevated levels of these proinflammatory agents (Fig. 3 *H–J*). In the cohoused group, WT mice showed low levels of cytokines, whereas TMF$^{-/-}$ mice had the highest levels of the proinflammatory cytokines measured (Fig. 3 *H–J*).

Reseparated TMF$^{-/-}$ Mice Regain Their Beneficial Microbiota Composition and Restore Their Diminished Susceptibility to Colitis. Because the TMF$^{-/-}$ mice cohoused with WT mice had a reduction in some beneficial bacterial species of the Firmicutes phylum and exerted an exacerbated response to the induced colitis, we next examined whether this effect was reversible. We tested whether the increased sensitivity to induced colitis and the altered microbiota of cohoused TMF$^{-/-}$ mice could revert to their original profile after reseparation from the WT cohort. Fecal samples from TMF$^{-/-}$ mice cohoused for 8 wk with WT mice were collected, after which the TMF$^{-/-}$ mice were separated for an additional 4 wk, and fecal samples were again collected. Although an additional 4 wk of cohousing with WT mice had no significant effect on the microbiota composition of the TMF$^{-/-}$ mice, reseparation of the two groups for 4 wk had a marked effect, and the TMF$^{-/-}$ mice exhibited a complete restoration of their original microbiome profile. Specifically, the Firmicutes to Bacteroidetes and Proteobacteria ratio increased, and levels of the beneficial

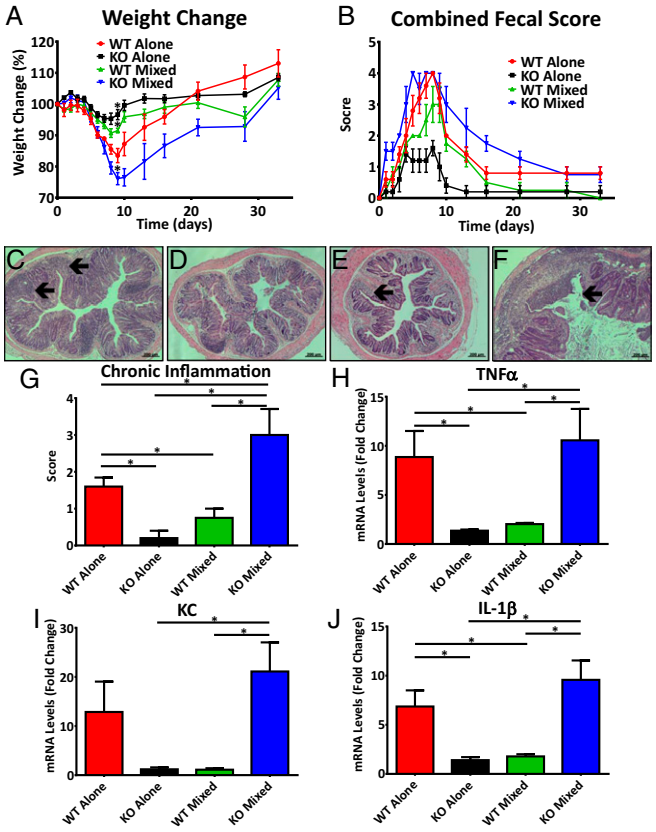

Fig. 3. Diminished sensitivity of TMF$^{-/-}$ mice to induced chronic colitis is transmissible to WT mice. KO and WT mice were either housed alone or cohoused for 4 wk before 3% DSS treatment for a 5-d period, followed by plain water for an additional 27 d. (*A*) Weight loss is presented as mean percent of initial weight ± SE. *$P < 0.05$ (ANOVA; $n = 5$). (*B*) Fecal score was determined as described in *Materials and Methods* (±SE; $n = 5$). (*C–F*) Colon histology of WT (*C*) and KO (*D*) mice either housed alone (*E*) or cohoused (*F*) for 4 wk before DSS treatment. Arrows indicate tissue damage (either ulceration or infiltration). Scale bars are as indicated. Images are representative examples from the different groups ($n = 5$). (*G*) Chronic inflammation. Histopathological semiquantitative scores are presented as the mean score (determined as described in *Materials and Methods*) ± SE. *$P < 0.05$ (ANOVA; $n = 5$). (*H–J*) Real-time quantitative RT-PCR analysis of proinflammatory cytokine mRNAs: TNF-α (*H*), KCs (*I*), and IL-1β (*J*) represented as mean expression levels relative to 18S RNA ± SE. *$P < 0.05$ (ANOVA; $n = 5$).

bacterial groups Ruminococcaceae, *Roseburia*, and *Lactobacillus* returned to the pre-cohousing state (Fig. 4 *A* and *B*). Furthermore, unweighted pair group method with arithmetic mean-based phylogenetic tree revealed that these reseparated mice possessed microbiota that were most similar to the TMF$^{-/-}$ mice housed alone (Fig. 4*C*). We then challenged these reseparated TMF$^{-/-}$ mice with DSS-induced acute colitis (13) alongside WT mice bred and housed separately. The reseparated TMF$^{-/-}$ mice exhibited recovery from their increased sensitivity and showed an attenuated response to the DSS treatment compared with the WT mice. This attenuated response was reflected in reduced weight loss, fecal score, and histopathological score (Fig. 4 *D–F*).

Collectively, these results suggest that TMF$^{-/-}$ mice housed alone exhibit diminished susceptibility to the development of both acute and chronic colitis and that this characteristic is transmissible to WT mice. In a reciprocal manner, TMF$^{-/-}$ mice acquire an increased sensitivity to acute and chronic colitis when cohoused with the WT cohort, a trait that is reversible upon reseparation of the two groups.

Discussion

The intestinal tract serves as a habitat for a vast number of commensal bacteria. Because most bacterial species cannot be cultured, the recent advances in high-throughput analytical methods have provided much information regarding how the composition of these bacteria affect their host, from modulating gene expression and immune system functions to obesity and cancer progression (3). The mucus layer, mainly comprising the MUC2 mucin, is responsible for keeping these bacteria separated from the epithelium (5). Recent studies have shown that in patients suffering from IBD and in certain mouse KO models that spontaneously develop colitis, the inner mucus layer is penetrated by bacteria, leading to barrier breakdown (11).

We previously described the diminished susceptibility of TMF$^{-/-}$ mice to experimentally induced acute colitis, due to a thicker and more robust colonic mucus layer, which reduces adherent bacterial load (13). In the present study, we analyzed the mucus secretion

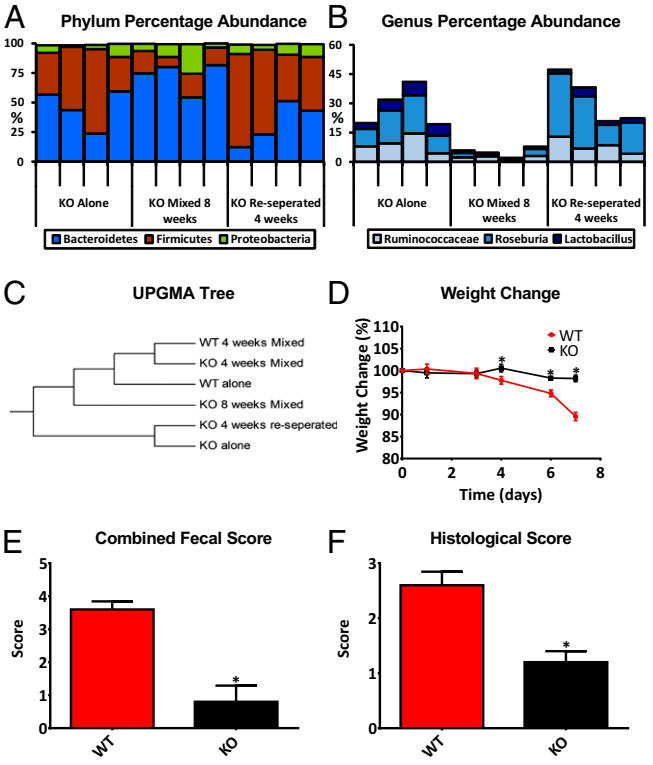

Fig. 4. Reprogramming of the gut microbiota and the colitis susceptibility in reseparated TMF$^{-/-}$ mice. KO mice were either housed alone or cohoused for 8 wk with WT mice, after which they were reseparated for an additional 4 wk. (*A* and *B*) Fecal samples were collected from each mouse in the different groups, and microbiota composition was analyzed via 16S rRNA analysis. (*A*) Taxon-based analysis at phylum level among the three groups. Data are shown as percent relative abundance. Phyla and families with >1% abundance are shown. Each bar represents one mouse. (*B*) Relative abundance of an uncultured member of the Ruminococcaceae, *Roseburia,* and *Lactobacillus* genera among the different groups. Each bar represents one mouse. (*C*) Tree clustering based on unweighted pair group method with arithmetic mean of the different mice groups in all experiments. (*D–F*) WT mice housed alone and KO mice cohoused with WT mice for 8 wk and then reseparated for another 4 wk were treated with 3% DSS in drinking water for 7 d. (*D*) Weight loss is presented as mean percent of initial weight ± SE. *$P < 0.05$ (Student *t* test; $n = 5$). (*E*) Fecal score was determined as described in *Materials and Methods* (± SE). *$P < 0.05$ (Student *t* test; $n = 5$). (*F*) Histopathological semiquantitative scores are presented as the mean score (determined as described in *Materials and Methods*) ± SE. *$P < 0.05$ (Student *t* test; $n = 5$).

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process in colonic TMF$^{-/-}$ goblet cells and investigated the cause of the reduced sensitivity of TMF$^{-/-}$ mice to DSS-induced colitis.

TMF was previously shown to be involved in vesicle tethering by bringing vesicle and target membranes into close apposition before fusion (14). Using TEM analysis on colon sections from WT and TMF$^{-/-}$ mice, we found that mucus-packed granules in goblet cells of TMF$^{-/-}$ mice do not fuse before mucus secretion, as is seen in WT mice, but, rather, are discharged of their cargo late in the secretion stage. This finding implies that TMF is required for mucus granule fusion, after granule translocation from the *trans*-Golgi compartment. These findings are corroborated by previous, independent proteomic studies, which indicated that TMF is part of the mucus granule complex and even associates with the granule membrane (22, 23). To confirm this association, we used florescence microscopy to show colocalization of TMF with the MUC2 mucin in human colonic goblet cells.

When the newly synthesized MUC2 protein reaches the Golgi apparatus, it is packed into secretory vesicles, where it forms disulfide bonds and oligomerizes (7). Formation of the disulfide bonds is necessary for giving the mucus its viscous nature (8). The exact form of the disulfide bonds and final structure of the MUC2 oligomer are not yet fully understood; however, it is well perceived that transport and retention in the secretory vesicles are crucial for MUC2 oligomerization, mainly due to distinct chemical environment in the vesicles (7, 24, 25). Based on our findings described here, we suggest that in TMF$^{-/-}$ mouse goblet cells, the prolonged retention of MUC2 in secretory granules results in more extensive disulfide bond formation and oligomerization of MUC2, thereby leading to the formation of increased mucus viscosity. This observation, of extensive interchain bonds, is supported by the fact that the mucus from TMF$^{-/-}$ does not undergo natural degradation by host and bacterial proteases as does mucus from WT mice, which appears as a smear in a Western blot analysis.

The colonic mucus acts to protect the gut epithelium and as a scaffold and energy source for the commensal gut microbiota (9). Furthermore, the gut microbiota composition is largely dependent on host-released glycans (9). Recently published work has shown that genetic alteration of a single fucosyltransferase isoform, which adds fructose to the colonic mucin, dramatically alters gut bacterial composition in mice, and, notably, this mutation drives gut infection in humans (26). Extension of the above-described study showed that host mucosal glycans liberated by certain bacterial groups can facilitate expansion of enteric pathogens after antibiotic treatment (27). The altered characteristics of the colonic mucus layer in TMF$^{-/-}$ mice, especially its reduced susceptibility to degradation, could result in altered glycans release. This finding could account for the distinct bacterial populations, represented by the altered microbiota, which we found in fecal samples from TMF$^{-/-}$ mice. This distinct population is of significantly greater diversity than that of WT mice, a trait that has been shown to be important in prevention of dysbiosis (28). TMF$^{-/-}$ mice had a higher ratio of Firmicutes to Bacteroidetes and Proteobacteria phylum than WT mice. Notably, a similarly skewed ratio is found in healthy individuals compared with patients suffering from IBD (28). Specifically, TMF$^{-/-}$ mice harbor a larger abundance of bacterial species from the Firmicutes phylum, such as an uncultured member of the Ruminococcaceae, *Roseburia*, and *Lactobacillus,* which are known to be probiotic and even possess therapeutic value against IBD (18–20). In addition to these changes in microbiota, we analyzed our sequencing data at the species level and looked for bacterial species that are known to degrade colonic mucus. Of the known mucus-degrading species (29), we found only *Prevotella* spp. in high quantity in the colons of WT mice. This specie was virtually absent from colons of TMF$^{-/-}$ mice, which implies the undegradable nature of the colonic mucus in these mice (Fig. S4).

When TMF$^{-/-}$ and WT mice were cohoused, the increase in beneficial bacterial species was transmissible, and the microbiota of the WT mice was altered within 4 wk. The WT mice also exerted a reciprocal effect on the TMF$^{-/-}$ mice, reducing their bacterial diversity and the abundance of beneficial strains colonizing these mice.

To evaluate the relevance of the altered microbiota of TMF$^{-/-}$ mice to diminished susceptibility to induced colitis, we examined the sensitivity of cohoused cohorts to acute and chronic colitis. We found a protective transmissible effect on WT mice when cohoused with TMF$^{-/-}$ mice, showing that not only the altered colonic mucus but also the subsequently altered microbiota in TMF$^{-/-}$ mice has a protective effect.

To our surprise, the TMF$^{-/-}$ mice cohoused with the WT mice were actually more susceptible to experimental colitis, exhibiting more severe disease than even the WT mice housed alone. Because TMF$^{-/-}$ mice are born with a reprogrammed microbiota and DSS is known to erode the mucus barrier, we suggest that when TMF$^{-/-}$ mice are cohoused with WT mice, they are exposed to novel pathogenic species against which they did not develop immunity. The erosion of the mucus barrier by the DSS in these cohoused TMF$^{-/-}$ mice likely triggers a severe immune response to these newly introduced pathogenic species. Interestingly, this effect was reversible, and upon reseparation, the commensal composition of TMF$^{-/-}$ gut recovered to its pre-cohousing state; consequently, these TMF$^{-/-}$ mice regained their diminished susceptibility to induced colitis. This reversible effect highlights the role of the unique colonic mucus of TMF$^{-/-}$ mice in shaping and reprogramming the gut microbiota.

Collectively, we describe here a tight correlation between the transmissibility of the probiotic bacterial population from TMF$^{-/-}$ to WT mice and the reduced sensitivity to induced colitis, acquired by the recipient mice. This finding suggests that the main cause for the diminished susceptibility of TMF$^{-/-}$ mice to colitis stems from the altered and reprogrammed microbiota that they harbor, in addition to their altered mucus layer. Thus, manipulation of the intestinal TMF system might allow deliberate reprogramming of the gut bacterial microbiota, thereby rendering it beneficial, with reduced sensitivity to IBD.

Materials and Methods

Mice. TMF/ARA160 KO mice from ICR background (30) were crossed with C57BL/6J mice (Harlan) for 10 generations. WT refers to C57BL/6J mice (Harlan). Each group of mice examined in the colitis models and subjected to microbiome analysis were housed in at least two different cages, to rule out stochastic caging effect. Cages containing the groups WT alone, TMF$^{-/-}$ alone, and TMF$^{-/-}$ reseparated held two mice per cage, whereas cages containing the groups WT mixed and TMF$^{-/-}$ mixed held four mice per cage (two from each strain). Mice were maintained under specific pathogen-free conditions with controlled temperature (25 °C) and photoperiod (12/12-h light/dark cycle) and allowed unrestricted access to standard mouse chow and water. All of the protocols were approved by the Institutional Animal Care and Use Committee at Bar-Ilan University. All mice used in the experiments were born and bred at the same facility.

Chronic Colitis Model. Chronic colitis was induced by using an established model (21). Briefly, mice were administered 3.0% DSS (molecular mass of 36–50 kDa; MP Biomedicals) in water ad libitum over a 5-d period, followed by plain water for 27 additional days. Weight change was monitored, as indicated, and fecal score was determined as described (13) in a blinded fashion.

Histological, Immunohistochemical, and Immunofluorescence Analysis of Human Colonic Sections. Intestinal biopsies of patients undergoing colonoscopy for reasons unrelated to IBD (e.g., screening colonoscopy) were obtained during the procedure and fresh frozen in −80 °C. All patients gave a written informed consent, and the study was approved by the Institutional Ethics Committee of the Sheba Medical Center. Tissue fixation, staining procedures, and antibodies used were described (13).

Histological Assessment of Colitis. Histopathological analysis and semiquantitative scoring was performed by a board-certified toxicological pathologist according to the scoring system described by Cooper et al. (31), taking into consideration the grades of extension (laterally, along the mucosa and deep into the mucosa, submucosa, and/or muscular layers) of the inflammation and ulceration. Analysis was performed in a blinded fashion.

Quantitative Real-Time PCR Analysis. RNA isolation and reverse transcription were performed as described (13). Quantitative real-time PCR was performed by using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad) with Fast SYBER Green Master Mix (Applied Biosystems) and analyzed with CFX Manager Software (Bio-Rad). Expression levels were normalized to 18S and are presented as fold change. Primers used were described (13).

TEM. Mice colons were incubated overnight in Karnovsky fixation buffer (2.5% glutaraldehyde, 1% paraformaldehyde in cacodylate buffer, pH 7.4). Post-fixation, staining, sectioning, and imaging were performed as described (30).

Western Blot Analysis. Mice colons were removed and cut open along the longitude axis. Mucus from the distal 5 cm of the colon was scraped into PBS supplemented with 2x Complete protease inhibitor mix (Roche) and immediately frozen in liquid nitrogen. Thawed samples were incubated for 5 min in 100 °C with sample buffer either with or without DTT as a reducing agent, before being loaded onto 4% SDS/PAGE and transferred to a poly(vinylidene fluoride) membrane under 400-mA current for 4 h in ice. Anti-Mucin2 antibody (1:1,000) (sc-15334, H-300; Santa Cruz Biotechnology) was used to evaluate MUC2 expression. Anti-GAPDH antibody (1:1,000) (sc-25778, FL-335; Santa Cruz Biotechnology) was used to ensure equal loading. For MUC1 analysis, whole colons were lysed with radioimmunoprecipitation assay (RIPA) buffer as described, and lysates were subjected to a Western blot analysis (13). Anti-Mucin1 antibody was a gift of D. Wreschner (32).

Stool Microbiota Analysis. Fecal DNA was isolated by using QIAamp DNA stool Mini Kit (QIAGEN) according to the manufacturer's instructions.

Microbiota Analysis. Fecal DNA was subjected to a single-step 30-cycle PCR by using HotStarTaq Plus Master Mix Kit (Qiagen) under the following conditions: 94 °C for 3 min, followed by 28 cycles of 94 °C for 30 s; 53 °C for 40 s; and 72 °C for 1 min, after which a final elongation step at 72 °C for 5 min was performed. Primers used were the 16S rRNA universal primers 27fmod-519R which targets the 16S rRNA genes regions V1–V3.

Sequencing of 16S rRNA genes of the gut microbial community was performed at MR DNA on an Ion Torrent PGM following the manufacturer's guidelines. Taxonomy was determined by Molecular Research DNA Laboratories. Statistical analysis of sequence data were performed with Mothur (33). Sequences were trimmed and checked for chimeras. Chimeras were removed, and Shannon indexes as well as PCoA were performed to determine diversity and similarities between communities, respectively.

Statistical Analysis. ANOVA was performed to identify significant differences between the means of four independent groups (WT, KO, cohoused WT, and cohoused KO) in the experiments. Unpaired two-tailed *t* test was performed when comparing two groups of mice. $P < 0.05$ was considered significant.

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