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INSIGHTS

The microbial one-hit wonder

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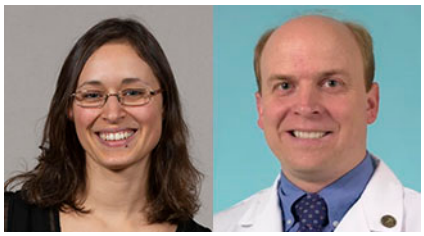
Intestinal inflammation, in the absence of infection, occurs from contributions by genetics and environment. Chen et al. (2021. *J. Exp. Med.* https://doi.org/10.1084/jem.20210324) challenge this concept by demonstrating that a dominant transmissible dysbiotic microbial community predisposes to intestinal inflammation in absence of genetic alterations.

Decades of studies have led to the understanding that alterations, or dysbiosis, of the gut microbiota can be a significant contributor to intestinal inflammation. Yet our understanding of how we arrive at these dysbiotic states, how they are maintained, and why it is so difficult to reverse them have remained cryptic. In this issue, Chen et al. (2021) identify that a genetic alteration in goblet cells results in a mucus secretory defect that induces a dysbiotic state potentiating intestinal inflammation, and once established, this dysbiosis becomes dominant, transmissible over the healthy microbiota and genetics, and drives an inflammatory phenotype, suggesting that in some situations "one hit" from the dysbiotic gut microbiota could be sufficient to potentiate intestinal inflammatory disease (see figure).

Forkhead box protein O1 (Foxo1) belongs to the family of proteins that are mainly studied for their role as transcription factors. Prior work demonstrated that loss of Foxo1 in immune cells results in the development of intestinal inflammation (Wu et al., 2018). Furthermore, Foxo1 has been implicated in controlling cell proliferation and apoptosis in multiple organisms and cell types (Eijkelenboom and Burgering, 2013). In this manuscript, Chen et al. use an intestinal epithelial cell (IEC)–specific Cre recombinase system (*Vil1*^{Cre}; see panel A of figure), as well as epithelial cell subset–specific Cre recombinases to investigate the role of Foxo1 in the control of intestinal barrier integrity and subsequent susceptibility to an intestinal inflammation (Chen et al., 2021). The authors narrow down the phenotype to reduced secretion of mucus by goblet cells resulting in a thinning of the mucus layer. In epithelial cells Foxo1 is located cytosolically and interacts with Atg5 to regulate autophagy and subsequent mucin granule release (see panel A of figure). The importance of the cytosolic location was confirmed with *Foxo1*^{AAA} animals that express a Foxo1 variant that is restricted to the nucleus, which fails to correct the loss of Foxo1 in IECs. The lack of transcriptional activity of Foxo1 in goblet cells is further highlighted by the nearly identical mRNA profiles of *Vil1*^{Cre} *Foxo1*$^{fl/fl}$ and *Foxo1*$^{fl/fl}$ mice. Not even genes involved in mucus production were altered in absence of Foxo1. However, loss of Foxo1 by IECs resulted in a reduction in the mucus layer, increase in mucinase-producing bacteria, decrease in short-chain fatty acid (SCFA)–producing bacteria, and increased susceptibility to colitis (see panel A of figure). This phenotype was transmissible and dominant in cohoused WT mice and could be rescued by SCFA or continuous supplementation with SCFA-producing bacteria. SCFA have been shown to promote mucus production and secretion, suggesting that loss of SCFA-producing bacteria in this self-sustaining dominant microbial community underlies the transmissibility of this colitogenic phenotype to WT mice (see panel A of figure). This is an excellent example of how a single genetic alteration can result in additive effects and a potentially transmissible phenotype in the absence of genetic alterations in the recipient.



Insights from Brigida A. Rusconi and Rodney D. Newberry.

The effects of a dysbiotic community are often framed as a loss of protective commensals or the gain of detrimental bacteria. Chen et al. show in this issue that changes in the host's mucus layer promote the presence of bacterial taxa with strong mucin-degrading activities that displace beneficial commensals, such as *Akkermansia muciniphila*. Oral supplementation with *A. muciniphila* reduced dextran sulfate sodium (DSS) colitis severity and improved intestinal barrier function in *Vil1*^{Cre} *Foxo1*$^{fl/fl}$ mice (see panel A of figure). These results point to the requirement of continuous supplementation of beneficial commensals in hosts that are not able to maintain the niche due to genetic alterations. Similar shifts in the intestinal microbial community have been observed in other animal models with impaired mucus. The mucus provides a unique niche for

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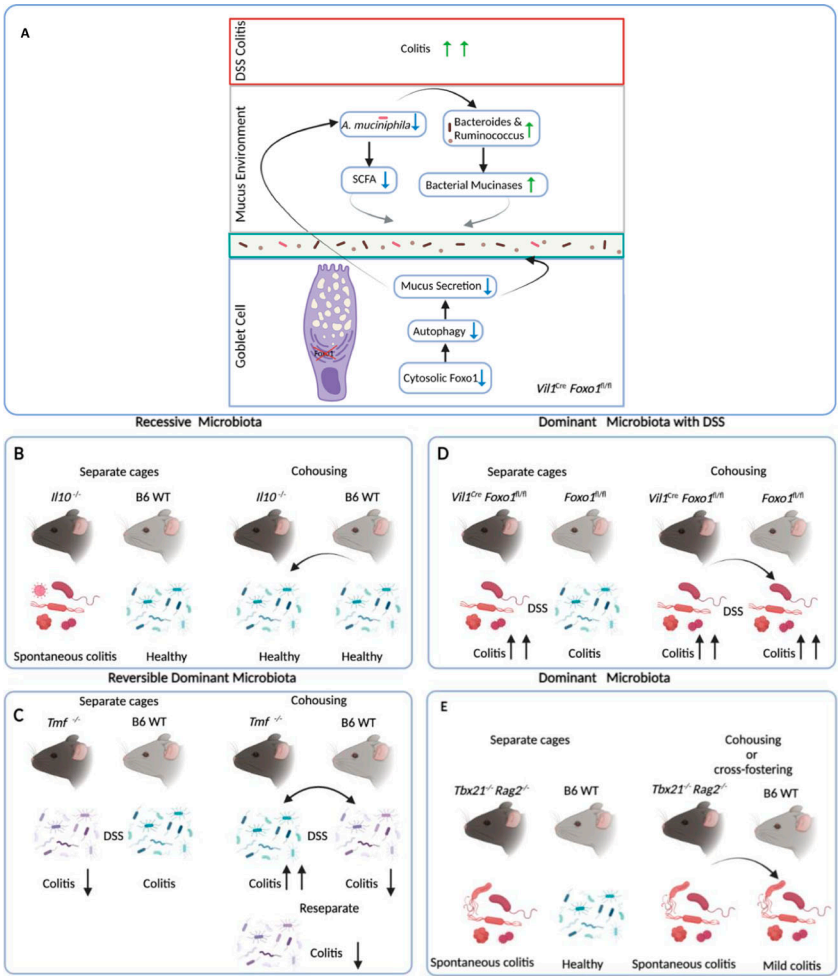

Defects in mucus secretion due to the loss of Foxo1 in goblet cells cause thinning of the mucus layer impacting the microbial community and increasing susceptibility to DSS colitis (Chen et al., 2021). **(A)** Schematic of the findings described by Chen et al. *Vil1*^{Cre} *Foxo1*$^{fl/fl}$ mice have a thinning of the mucus layer due to autophagy defect, causing a loss of *A. muciniphila* and SCFA with an increase in mucinase-rich members of the *Bacteroides* and *Ruminococcus*. This dysbiotic state is dominant and self-sustaining compared with other IBD mouse models. **(B)** *Il10* KO model microbiota is recessive when cohoused with WT (Shanmugam et al., 2014). **(C)** *Tmf* $^{-/-}$ and WT microbiotas are both dominant when cohoused (Bel et al., 2014). **(D)** IEC-specific *Foxo1* Cre model microbiota is dominant in cohousing over Cre negative (Chen et al., 2021). **(E)** TRUC microbiota is dominant in cohousing or cross-fostering over WT (Garrett et al., 2007).

commensals as both an anchor and nutrient source. At the same time, the mucus barrier prevents invasion of pathogenic bacteria and controls colonization by sequestering anti-microbial peptides. It is therefore not surprising that *Vil1*^{Cre} *Foxo1*$^{fl/fl}$ mice lack bacteria that produce SCFAs, which reside in the mucosal layer. SCFAs have been shown to control intestinal barrier function by affecting tight junctions (TJs; Waldecker et al., 2008). Indeed, dietary supplementation with individual SCFAs improved cellular localization of TJs in *Vil1*^{Cre} *Foxo1*$^{fl/fl}$ mice. As a result of improved TJs, dietary supplementation with SCFA reversed luminal permeability and reduced susceptibility to DSS colitis. Considering the regulatory function of SCFA on the intestinal immune compartment (Olszak et al., 2014), the reduced inflammation observed in animals receiving SCFAs could be partially mediated by effects on immune cells and not only the epithelial compartment. *Vil1*^{Cre} *Foxo1*$^{fl/fl}$ mice do not have any defects in the immune compartment at steady-state but could still have an abnormal inflammatory response upon injury due to intrinsic changes to the immune compartment.

While widely accepted, the contribution of the gut microbiome and dysbiosis to inflammatory bowel disease (IBD) may be variable and complex, from potentially being a less prominent component in very-early-onset IBD, which can be a monogenetic disorder, to potentially a more dominant component, which may be corrected with fecal microbiota transplant (FMT). Mouse models reflect the variability of dysbiosis on susceptibility to intestinal inflammation, with the dysbiotic microbiotas often viewed as recessive or dominant (Kiesler et al., 2015). Cohousing WT mice with *Il10*$^{-/-}$ mice prevents spontaneous colitis in the latter, indicating that this recessive dysbiotic microbial community can be corrected by exposure to a dominant WT intestinal community (see panel B of figure; Shanmugam et al., 2014). In addition, other genetic models can be rescued by cohousing or fecal transfer from WT mice (Kiesler et al., 2015). Conversely, in some situations the protective microbial community is not entirely dominant or recessive. Deletion of tata element modulatory factor (*Tmf*$^{-/-}$) results in decreased susceptibility to colitis due to a more diverse microbial community (Bel et al., 2014). When these mice are cohoused with WT mice, WT become more protected from colitis, while the *Tmf*$^{-/-}$ become more susceptible (see panel C of figure). In this case, the microbiotas cause a reversal of outcomes, likely due to exchange of key taxa. However, when *Tmf*$^{-/-}$ mice are separated following cohousing, the microbial community drifts back to a protective state (see panel C of figure). The concept of a dominant colitogenic dysbiotic community is illustrated by the *Tbx21*$^{-/-}$ *Rag2*$^{-/-}$ (TRUC) mice (Garrett et al., 2007). Cohousing WT mice with TRUC mice or cross-fostering WT mice with TRUC dams induced colitis in WT mice (see panel E of figure). This demonstrates that not only can the colitogenic dysbiotic microbiota dominate, it can be maternally transmitted and potentiate colitis independent of the genetics of the offspring (Garrett et al., 2010). In this issue, Chen et al. describe a microbial dysbiosis occurring as a result of a genetic defect in goblet cells, which is not only necessary, but sufficient to induce increased susceptibility to intestinal inflammatory insults in genetically normal mice (Chen et al., 2021). Germ-free *Foxo1*$^{fl/fl}$ mice reconstituted with fecal content from *Vil1*^{Cre} *Foxo1*$^{fl/fl}$ mice have reduced mucus thickness and worsened DSS colitis. Since germ-free mice have an abnormal

intestinal immune compartment, the authors used cohousing of *Vil1*^{Cre} *Foxo1*$^{fl/fl}$ with *Foxo1*$^{fl/fl}$ littermates to confirm the transmission of a dysbiotic state to *Foxo1*$^{fl/fl}$ and increased sensitivity to DSS colitis (see panel D of figure; Chen et al., 2021). The use of littermates is a critical factor for studies that have a microbial component, since preweaning events can define the adult microbial community. For example, an initial publication described constitutive inflammasome KO mice to have a dysbiotic state that could be transmitted to WT mice (Elinav et al., 2011). However, subsequent studies using littermates showed that the dysbiotic community does not develop if the animals are raised together even in the F2 generation (Lemire et al., 2017). The data from Chen et al. allow for an expansion of the concept of microbially driven diseases as it demonstrates that there can be a transmissible disease state in absence of underlying genetic alterations. A recent publication by Petersen et al. describes a similar dominant microbial community in a genetic model of obesity (Petersen et al., 2019). Cohousing of T-*Myd88*$^{-/-}$ with WT littermates led to an increase of weight gain in the latter despite being on a regular diet.

The implications of the roles of the microbiota in these models has profound impact on our interpretations of the drivers and origins of IBD and how it might be treated. It has been appreciated that despite extensive genome-wide associations studies, the observed genetic polymorphisms do not account for all of the predicted heritability of IBD (Gordon et al., 2015), and that this "missing heritability" may in part be explained by the gut microbiota, which is largely maternally acquired. Overlaying these observations might suggest that an individual lacking genetic risk to develop dysbiosis might obtain a dominant and persistent dysbiotic microbiota from a mother with this genetic risk, thus contributing to the missing heritability phenomenon. Further, it might suggest that these individuals, or individuals acquiring this dysbiotic microbiota in other ways, could be refractory to FMT in the absence of apparent genetic risk for dysbiosis. Thus, the observations presented here further raise the question of whether dysbiotic gut microbial communities could provide one hit to potentiate intestinal inflammation.

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