Preview

Defining Dysbiosis in Inflammatory Bowel Disease

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In this issue of *Immunity*, Britton et al. (2019) demonstrate that the colonization of germ-free mice with microbiotas from inflammatory bowel disease patients induces an altered ratio of RORγt$^+$ regulatory T cells to T(h17) effector cells and recapitulates human disease severity in colitis-susceptible mice.

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Main Text

Besides genetic factors, the composition of the human intestinal microbiota has been long suspected to be a major risk factor for intestinal inflammatory bowel disease (IBD), but the identification of such "dysbiotic" microbiotas and underlying mechanisms in the host has been challenging due to the tremendous complexity and high intra-individual variability of intestinal compositions (Round and Mazmanian, 2009). The ability to categorize complex microbiotas into "symbiotic" or "dysbiotic" could have great potential to diagnose disrupted microbiota-host homeostasis and also for the improvement of current efforts to use fecal material transplantation (FMT) to treat IBD. In this issue of *Immunity*, Britton et al. (2019) addressed this question by transferring microbial collections from two independent cohorts of IBD patients or healthy subjects into gnotobiotic and colitis-susceptible mice and examining the impact on intestinal T cell populations. Their findings suggest that defining local tissue-resident T cell subsets after colonization of germ-free (GF) mice even with complex xeno-microbiotas may be the preferred method to identify IBD-suppressing and/or -curing microbiotas.

Britton et al. (2019) used human microbiotas derived from healthy donors or two independent cohorts of IBD patients. Samples were derived from patients with ulcerative colitis (UC) and Crohn's disease (CD) and were collected during active disease or in remission phase. 16S RNA amplicon sequencing of fecal samples and cultured microbiotas failed to discriminate healthy and IBD donors in their cohorts; the groups showed no significant differences in alpha diversity. The authors then colonized groups of adult GF mice with these microbial collections and assessed the mucosal immune response. Whereas each collection varied in its effect on the intestinal T helper (Th) cell compartment, microbiotas derived from IBD patients induced on average higher frequencies of RORγt$^+$ Th17 cells (and to a lesser degree Th2 cells) but did not cause signs of disease. Besides T effector cell differentiation, microbial colonization of the intestinal tract is known to expand a mutualistic Foxp3$^+$ regulatory T (Treg) cell population that secretes high amounts of interleukin (IL)-10 (Geuking et al., 2011). Surprisingly, microbiotas from healthy donors and IBD patients were both able to expand IL-10-secreting Foxp3$^+$ Treg cells in a similar manner, and there was no correlation to the frequencies of RORγt$^+$ T effector cells. Thus, total Foxp3$^+$ Treg cell numbers induced by microbial colonization cannot predict "healthy" or "dysbiotic" microbiotas.

Foxp3$^+$ Treg cells can be derived from thymic origin or differentiate locally from naive T cells in tissues. Distinguishing thymic-derived versus peripherally induced Treg cells has been challenging due to high similarity in phenotypic markers. Britton et al. (2019) used Foxp3 and RORγt co-expression and the absence of the Ikaros family member Helios (*Ikzf2*) to identify a subpopulation of Treg cells induced only after colonization of the intestinal tract with commensal bacteria (Ohnmacht et al., 2015; Sefik et al., 2015). Despite some variation, microbiotas from healthy donors—but not IBD patients—induced on average higher frequencies of RORγt$^+$ Treg cells in the intestinal lamina propria irrespective of whether the authors transferred fecal slurries or cultured bacteria. These findings imply that microbiota-induced Treg cells protect from colitis and further suggest that the intestinal Treg cell niche can partially compensate low RORγt$^+$ Treg cell numbers due to a dysbiotic microbiota with increased frequencies of non-RORγt$^+$ Treg cells.

Mechanistically, dendritic cells from mice reconstituted with the microbiotas from IBD donors that induced lower numbers of RORγt$^+$ Treg cells showed higher expression of costimulatory receptors, as shown in mouse models of disease (Ohnmacht et al., 2015). It remains unclear whether reduced RORγt$^+$ Treg cells fail to regulate dendritic cells or whether the microbiota directly alters dendritic cell function. Addressing this question will require detailed analyses as the microbiota may act on multiple cell types including innate,

stromal, and epithelial cells, which may ultimately determine the frequencies of RORγt$^+$ Treg cells.

Next, Britton et al. (2019) assessed whether the different human microbiotas were able to regulate disease severity in a gnotobiotic colitis model. The authors used a colitis model based on T cell transfer into Rag-deficient mice; disease in this model is dependent on both T cells and microbiota. Colonization of Rag-deficient mice with IBD microbiota prior to T cell transfer aggravated a variety of disease parameters including body weight loss, cellular infiltration into tissues, and fecal lipocalin irrespective of whether the microbiota was derived from patients with active disease or patients in remission. Disease severity was also independent of whether the microbiota was derived from CD or UC patients. The authors additionally investigated potential secondary effects of inflammation on microbial composition but did not find major alterations. Colonization with IBD microbiota induced more Th17 cells and a population of highly pro-inflammatory T cells co-expressing the cytokines IL-17 and interferon (IFN)-γ; few Foxp3$^+$ Treg cells were induced of which roughly half co-expressed RORγt. However, under these conditions the authors did not find differences between healthy and IBD donors. Thus, the T cell transfer colitis model in gnotobiotic mice is able to reveal dysbiotic human microbiotas but the frequency of RORγt$^+$ Treg cells cannot be used as a predictive marker in this setting. This is probably due to the highly acute nature of the disease and the relatively few Foxp3$^+$ Treg cells induced. Nevertheless, the co-transfer of Foxp3$^+$ T cells is able to suppress transfer colitis and RORγt$^+$ Treg cells are more efficient suppressors in this context, as compared to RORγt$^-$ Treg cells (Yang et al., 2016).

Finally, Britton et al. (2019) used a logistic model to investigate the parameters of the T helper cell response in unchallenged gnotobiotic mice that correlated with colitis severity and with the health status of the human microbiota donor. The number of intestinal RORγt$^+$ T helper cells positively correlated with murine and human colitis severity. Neither Th2 nor total Foxp3$^+$ Treg cell numbers correlated with disease severity, but when the authors used the frequency of induced RORγt$^+$ Treg cells the ileum or colon by microbial colonization, they found a strong correlation between RORγt$^+$ Treg cells and colitis severity in mice and human donors. In fact, RORγt$^+$ Treg cell frequencies were as good as murine colitis scores to predict human disease status. Thus, Britton et al. (2019) reveal not only a cross-species transferable role of IBD microbiotas but also a method—namely assessing the frequency of induced RORγt$^+$ Treg cells—to assess the colitogenic potential of a complex human microbiota.

These results are in agreement with previous studies demonstrating that RORγt$^+$ Treg cells are superior in the suppression of T cell transfer colitis (Yang et al., 2016) and that the deletion of RORγt in Foxp3$^+$ Treg cells results in enhanced disease scores in different chemically induced colitis models (Ohnmacht et al., 2015; Sefik et al., 2015). Moreover, the colonization of mice with a potential pathobiont called *Helicobacter hepaticus* does not result in intestinal inflammation when the host is able to induce antigen-specific RORγt$^+$ Treg cells (Xu et al., 2018). The current study therefore strengthens the importance of RORγt$^+$ Treg cells in microbial tolerance and offers the possibility to screen potential human donor microbiotas for FMT (Figure 1). The identification of such ideal microbiota donors is currently one of the major bottlenecks in the implementation of FMT for IBD patients.

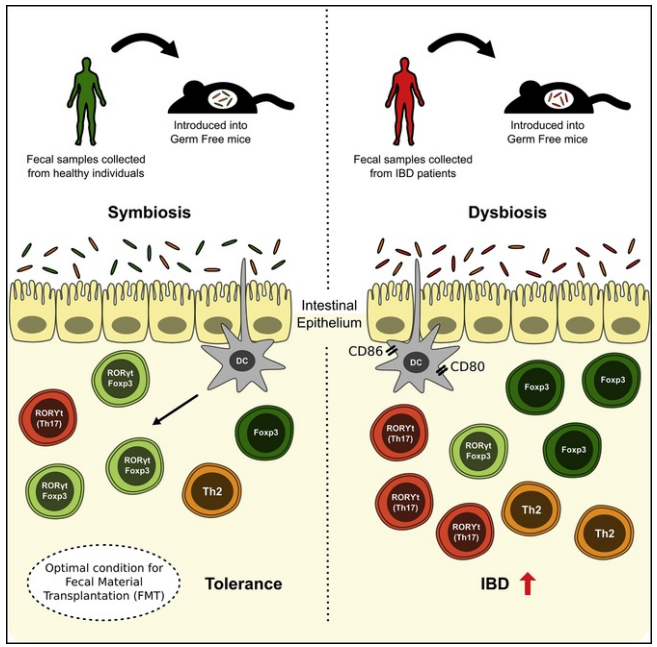

Figure 1 Frequency of RORγt$^+$ T Effector and T Regulatory Cells Reveal IBD-Prone or Healthy Human Microbiotas

Xeno-colonization of adult germ-free mice with human microbiotas from IBD donors result in enhanced RORγt$^+$ Th17 (and Gata3$^+$ Th2) effector cell frequencies in the lamina propria of ileum and colon and result in enhanced disease severity in colitis-susceptible mice. Microbiotas collected from healthy and IBD donors result in a similar increase in total Foxp3$^+$ Treg cells in the intestine of ex-germ-free mice. Microbiotas from healthy human donors induce on average higher frequencies of RORγt$^+$Foxp3$^+$ Treg cells in the lamina propria of ileum and colon and prevent disease

exacerbation. These observations are independent of whether fecal samples from ulcerative colitis or Crohn's disease were used and whether sampling occurred during remission or active disease. Frequencies of RORγt$^+$Foxp3$^+$ Treg and Th17 cells after microbial colonization were predictive for disease status of human microbial donors.

One caveat in the use of relative cell frequencies, as is done broadly in the field and in this study, lies in the difficulty of calculating total cell numbers from tissues because isolation procedures can vary not only for technical reasons but also due to differential microbial colonization and the degree of inflammation. In fact, such variations could potentially explain why previous studies came to divergent conclusion of the origins of Foxp3$^+$ Treg cells in the colon (Cebula et al., 2013; Lathrop et al., 2011). Moreover, monocolonization with individual bacterial strains or products from probiotic strains can also induce RORγt$^+$ Treg cells to a similar degree as in mice colonized with a specific-pathogen-free microbiota. Whether supplementation with one of those strains or products is able to induce tolerance toward a complex microbiota in a physiologic setting remains to be addressed (Sefik et al., 2015; Verma et al., 2018).

In the future it would be highly desirable to identify the underlying mechanisms both from the host and the intestinal microbiome that regulate the induction of RORγt$^+$ Treg cells not only in mice but also in humans. In addition, the identification of genetic risk loci with a potential impact on the generation or function of RORγt$^+$ Treg cells will be useful to the understanding of the pathophysiology of IBD. Furthermore, the study from Britton et al. (2019) implies that assessing the frequency and the characteristics of the human surrogate of RORγt$^+$ Treg cells, e.g., from biopsies, could help to diagnose disrupted microbiota-host homeostasis and may be relevant for potential applications regarding Treg cell-based therapies in IBD patients.

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