

## TRANSLATIONAL MEDICINE: BENCH TO BEDSIDE

# Butyrate and Mucosal Inflammation: New Scientific Evidence Supports Clinical Observation

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Short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, are bacterial metabolites generated via the fermentation of dietary fibers. Luminal SCFAs are recognized as a preferred energy substrate for the colonic epithelium. As early as the 1980s, SCFAs were observed to offer therapeutic benefit in some forms of colitis.<sup>1</sup> Since then, clinical trials have produced variable results in confirming the efficacy of SCFAs for diversion colitis or for inflammatory bowel disease (IBD)-associated colitis. Putative mechanistic explanations for these clinical effects have been put forth along the way, particularly regarding butyrate.<sup>2</sup> Now, more than two decades after the initial clinical description, research is illuminating the basic mechanisms by which SCFAs influence gut immune responses to promote homeostasis. These findings have stimulated a resurgence of interest in the topic. This *Translational Medicine: From Bench to Bedside* mini-review focuses on recently published papers evaluating the basic immunologic effects of butyrate on mucosal inflammation and integrity.

Butyrate limits intestinal inflammation by promoting the formation of the regulatory T cells (Tregs), a population of adaptive immune cells that suppress inflammatory responses.<sup>3,4</sup> Furusawa *et al.* sought to identify the mechanism by which commensal microbiota induce Tregs.<sup>3</sup> They found that “germ-free” mice have lower numbers of Tregs than conventionally raised mice, which have an intact gut microbiome. They also observed that a high-fiber diet led to greater Treg numbers than a low-fiber diet. Metabolomic analysis identified an increase in SCFA production in mice fed the high-fiber diet. Using dietary supplementation to increase cecal concentrations of acetate, propionate, and butyrate, the authors found the most significant increase in Tregs occurred in animals receiving butyrate. In a related study, Arpaia *et al.* illustrate the importance of resident microbe metabolites (SCFAs) in extrathymic Treg generation.<sup>4</sup> Tregs were induced by fecal extracts from conventionally raised mice, but not by fecal extracts from germ-free mice or mice treated with antibiotics. This group further showed that supplementation with butyrate in drinking water, was sufficient to induce Tregs in mice. In both studies, the increase in Tregs was attributed to the inhibition of histone H3 deacetylases (HDACs, a class of regulatory proteins that function as inhibitors of gene expression). Treatment with butyrate relieved HDAC inhibition of FoxP3, a protein important for formation of Tregs.

Butyrate also modulates the function of innate immune cells. Chang *et al.* observed a reduction in pro-inflammatory cytokines in macrophages treated with butyrate *in vitro* and in macrophages isolated from mice given butyrate in their drinking water.<sup>5</sup> The implicated mechanism was again related to butyrate's ability to inhibit HDACs, and thus the inflammatory cascade. This finding is interesting as it demonstrates that microbes produce metabolites that suppress “first-line” innate immune cells from mounting an inflammatory response against these microbes.

Singh *et al.* proposed another mechanism of action for butyrate in the innate immune system. They found that this SCFA activates a receptor for niacin in the colon called Gpr109a.<sup>6</sup> Genetic ablation of this receptor resulted in an increased susceptibility to colitis. Dendritic cells and macrophages isolated from Gpr109a knockout mice showed reduced capacity to promote T-cell differentiation into Tregs even in the presence of butyrate. Furthermore, the authors linked their findings to colon carcinogenesis by showing Gpr109a-deficient mice had increased susceptibility to both colitis-associated and genetically driven (Apc) colon cancers. Finally, the authors also demonstrated that butyrate signaling through Gpr109a on epithelial cells promoted expression of the pro-homeostatic cytokine IL-18. Taken together, the authors concluded butyrate is important in promoting an immune tolerant colon mucosa, which is resistant to neoplasia.

The effect of butyrate is not limited to immune cells. Kelly *et al.* demonstrated that butyrate increases colonic epithelial cell oxygen consumption, leading to a phenomenon known as “physiological hypoxia”.<sup>7</sup> Physiologic hypoxia is a good thing as it supports normal intestinal barrier function through the activity of hypoxia-inducible factor (HIF). Disruption of the gut microbiota with antibiotics reduces luminal SCFAs and epithelial aerobic metabolism. These changes lead to HIF destabilization and reduced barrier function. Thus, butyrate also has a role in maintaining healthy colon barrier function, which prevents the flux of potentially pathogenic microbes across the epithelium.

Together these studies paint a remarkably positive picture for SCFAs, and butyrate in particular, in promoting and maintaining mucosal homeostasis. However, a few caveats should be considered before we move to offering SCFA or butyrate enemas to all

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our colitis patients. As previously noted, butyrate's efficacy in human colitis trials conducted to date has been inconsistent. This discrepancy was also present in the mouse models of colitis. Where butyrate reduced inflammation in a T-cell-driven mouse model of colitis,<sup>3</sup> it did not lessen colitis severity in a model initiated by disruption of the epithelial barrier.<sup>5</sup> The pathophysiology of human IBD is complex, but generally involves both immune cell activation and epithelial barrier dysfunction. Perhaps a missing link in understanding the inconsistent clinical trial results lies in how butyrate or other SCFAs affect the growth and differentiation state of normal epithelial cells. These functions are critical to wound healing that is required to achieve mucosal healing in IBD. To date, *in vitro* studies evaluating butyrate's effects on the colon epithelium have been done exclusively in colon cancer cell lines that do not accurately reflect the normal epithelium. Fortunately, new methodologies now exist that allow the culture of normal (non-cancer) human epithelial cells<sup>9</sup> and can be tailored to address these critical unanswered questions.

In summary, these recent studies provide new mechanistic insight as to how bacterial derived metabolites (SCFAs) impact the mucosal immune system and suppress inflammatory signaling. Butyrate appears to have the most powerful effects on innate immune cells, adaptive immune cells, and epithelial barrier function. These exciting findings strongly indicate that butyrate or other microbial metabolites deserve rigorous evaluation as therapeutic targets for IBD and colon cancer. Additional mechanistic studies will hopefully guide us in how these can be most effectively applied and overcome the limitations of efficacy observed in clinical trials over the last two decades.

### CONFLICT OF INTEREST

**Guarantor of the article:** Matthew A. Ciorba, MD.

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