COMMENTARY

Microbial metabolites control gut inflammatory responses

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An estimated 100 trillion bacteria populate the human gut and are separated by a single layer of intestinal epithelial cells (IECs) from the innate and adaptive immune cells of the lamina propria. Considering that both commensal and pathogenic microbes share many molecular features and that these features are detected by pattern recognition receptors (PRRs) displayed by immune cells, it is necessary to understand how appropriate inflammatory responses against pathogens are coordinated in the presence of such high numbers of innocuous bacteria. Current thinking holds that the mucosal immune system tolerates commensal microbes while maintaining the ability to mount a robust protective response against pathogens. This process must operate within the context of a dynamic equilibrium of microbial diversity that rapidly fluctuates in response to an environment of ingested dietary materials (1). Lack of response to pathogenic onslaught leaves the host vulnerable to barrier penetration and systemic infection, whereas aberrant inflammatory responses against the commensal community result in tissue damage and dysbiosis that perturbs the microbial community and facilitates inflammatory bowel disease (IBD). It has long been appreciated that commensal bacteria are necessary for digestion of plant-derived starches, bile acid production, and vitamin acquisition. Observations that byproducts of these processes are reduced in IBD patients have led many groups to test whether these microbial metabolites shape intestinal immunity. In PNAS, Chang et al. (2) show that a short-chain fatty acid (SCFA), n-butyrate, produced as an end product of bacterial anaerobic fermentation of starches, has potent anti-inflammatory properties and inhibits proinflammatory responses by intestinal macrophages. These data indicate, along with an additional emerging body of evidence, that certain bacterial species communicate with the immune system through their metabolites to influence both the magnitude and the quality of the immune response (Fig. 1).

Specific bacterial species have previously been associated with the presence of certain T-cell subsets within the intestines. For example, colonization of mice with a single commensal microbe, segmented filamentous bacteria (SFB), is sufficient for induction of the Th17 subset of $CD4^+$ T cells (3) (Fig. 1*A*). These cells produce the cytokines IL-17 and IL-22, promote the production of antimicrobial peptides and tissue repair (4), and enhance resistance to *Citrobacter* infection (3). Generation of Th17 cells can also be promoted via sensing of bacteria-derived ATP by dendritic cells (DCs), promoting the expression of Th17-inducing cytokines IL-6 and IL-23 (5). SFB-colonized mice do not have increased levels of intestinal ATP, however, indicating that multiple pathways that sense colonization by specific microbes can converge on the induction of the same immune phenotype. This observation is also true for induction of colonic regulatory T (T_{reg}) cells.

Colonization of mice with *Clostridia* species from clusters IV, XIVa, and XVIII isolated from human feces enhances T_{reg} cell abundance and also increases the production of potent anti-inflammatory molecules such as the cytokine IL-10 (6, 7) (Fig. 1*B*). Addition of cecal extracts from these mice to human and mouse IEC cell lines led to their production of TGF-β1, a major cytokine involved in the differentiation of T_{reg} cells in the intestines. Cecal extracts contained high concentrations of SCFA and addition of a combination of purified acetate, propionate, and butyrate to IECs also induced TGF-β1 in vitro. These observations suggest that production of SCFA by *Clostridia* is likely responsible for the increase in the T_{reg} cell numbers. Before this observation, the reduction of inflammatory responses was thought to be mediated by microbe-derived acetate signaling through the short-chain free fatty acid receptor, GPR43, present on neutrophils and eosinophils (8). GPR43 signaling was proposed to dampen inflammatory responses and ameliorate disease in animal models of colitis, inflammatory arthritis, and allergic airway disease. Most recently, however, GPR43 expression on colonic T_{reg} cells has been suggested to promote their expansion in response to orally administered SCFA and to confer protection in an experimental model of colitis induced on adoptive T-cell transfer into lymphopenic recipients (9).

Aside from recognition of SCFA by G protein–coupled receptors (GPCRs), GPCR-independent mechanisms have been shown to account for the anti-inflammatory effects

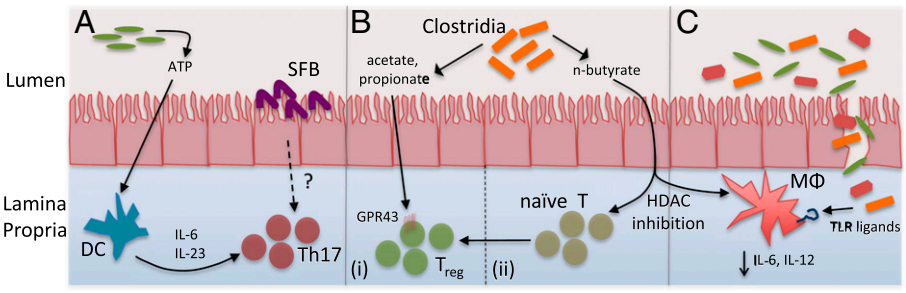

Fig. 1. Multiple bacterial species and microbial metabolites shape immunity in the intestines. (*A*) Colonization by SFB or delivery of bacterial-derived ATP promotes Th17 induction by unknown and indirect mechanisms involving DCs. (*B*) *Clostridia* species, via production of SCFA, promote the proliferation (*i*) and de novo induction (*ii*) of T_{reg} cells in the colonic lamina propria via distinct mechanisms. (*C*) HDAC inhibitory activity of n-butyrate suppresses proinflammatory cytokine production by intestinal macrophages (MΦ).

Author contributions: N.A. and A.Y.R. wrote the paper.

The authors declare no conflict of interest.

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of SCFA. Two reports recently demonstrated that the histone deacetylase (HDAC) inhibitory activity of SCFA leads to the de novo generation of T_{reg} cells from naïve $CD4^+$ T-cell precursors (10, 11) (Fig. 1*B*). This is in contrast to GPR43-dependent expansion of the preexisting colonic T_{reg} cell pool (9). The capacity for each SCFA to induce T_{reg} cells positively correlated with the level of HDAC inhibition, with n-butyrate being most potent, and led to increased histone H3 acetylation within genetic loci required for T_{reg} cell induction. Furthermore, DC pretreated with butyrate exhibited a markedly reduced proinflammatory signature, further aiding in the conversion of naïve $CD4^+$ T cells to T_{reg} cells on coculture in the presence of TGF-β1 (11).

The anti-inflammatory effect of n-butyrate on intestinal macrophages reported by Chang et al. (2) was similarly dependent on HDAC inhibition and was associated with the accumulation of histone 3 lysine 9 acetylation (H3K9Ac) (Fig. 1*C*). Intestinal macrophages are the most abundant immune cell type in the lamina propria and, because they are unable to proliferate, are repopulated by a pool of newly recruited circulating monocytes (12). However, unlike circulating monocytes or macrophages from other tissues, intestinal macrophages are hyporesponsive to toll-like receptor (TLR) stimulation (13). These observations suggested that an active process must restrain inflammatory responses of myeloid cells on their arrival in the lamina propria and induce TLR-ligand hyporesponsiveness to microbial signals present within this niche. The data presented by Chang et al. (2) provide a link between these two observations. First, treatment of bone marrow–derived macrophages (BMDMs) with n-butyrate was sufficient to induce hyporesponsiveness to lipopolysaccharide (LPS) stimulation. Furthermore, depletion of microbiota-derived SCFA on treatment of mice with broad-spectrum antibiotics followed by supplementation with n-butyrate or vehicle alone showed that hyporesponsiveness of intestinal macrophages was dependent on SCFA provision and that in their absence intestinal macrophages regained the ability to respond to LPS.

As mentioned above, this effect was due to the HDAC inhibitory activity of n-butyrate, as similar observations were made when BMDM were treated with a well-characterized HDAC inhibitor, trichostatin A (TSA) (2). Interestingly, hyporesponsiveness of macrophages treated with n-butyrate was not due to a global effect on gene expression. Only induction of LPS secondary response genes (SRGs), which require de novo protein synthesis, was blunted. In particular, *Tnfa* and *Ccl2*, the genes encoding the TNF-α and monocyte chemotactic protein-1 (MCP-1), respectively, were unaffected by n-butyrate. Conversely, genes encoding the proinflammatory cytokines IL-6 and IL-12p40 were strongly repressed on n-butyrate exposure. It has been proposed that regulation of SRG expression may be cell lineage specific (14).

The work by Chang et al. provides a previously unidentified mechanistic explanation of why intestinal macrophages are hyporesponsive to stimulation by luminal bacteria.

n-butyrate may orchestrate chromatin modifications that elevate the threshold for nucleosome remodeling and allow for selective SRG repression (15). In support of this, Chang et al. (2) show that n-butyrate treatment results in deposition of H3K9Ac at *Il6* and *Il12b* loci. Although typically associated with transcriptional activation, these data are consistent with reports that H3K9Ac modifications can recruit Mi-2β (16), a member of the Mi-2/NuRD repressor complex, and that Mi-2β expression is upregulated on HDAC inhibition (17). Most importantly, Mi-2β recruitment to SRG loci is refractory to LPS-mediated induction of gene expression (18).

The work by Chang et al. (2) provides a previously unidentified mechanistic explanation of why intestinal macrophages are hyporesponsive to stimulation by luminal bacteria and lends support to a growing body of evidence that microbial metabolites shape tolerance mechanisms in the intestines. The most intriguing part of these observations, however, is that hyporesponsiveness is selective. Intestinal macrophages are still capable of generating normal levels of TNF-α and MCP-1 and maintain potent phagocytic and bactericidal activities (2, 13). One can speculate as to why these genes have been evolutionarily selected for exclusion from n-butyrate–mediated repression. In this regard, an attractive hypothesis is that TNF-α and MCP-1 may facilitate the influx of monocytes and neutrophils that are not yet conditioned by SCFA and are poised to generate proinflammatory cytokines in response to microbes if a significant breach of the intestinal barrier has occurred. The resulting inflammatory environment may overcome suppression by other innate and adaptive cell types, foremost T_{reg} cells, and allow for the tolerant state to give way to inflammatory responses that are needed to clear infection and initiate tissue repair.

The study by Chang et al. (2) and several other recent reports (6, 9–11) underscore the pleiotropic effects of microbial metabolites that engage multiple pathways in different cell types and likely moderate innate and adaptive immune cell responses against the commensal microbiota. These studies have begun to unravel the chemical vernacular of host–commensal interactions. Deciphering how specific microbial metabolites affect different host cell types offers an exciting field of research for investigators who seek to crack the commensalism communication code.

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