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Glycans for good

Katherine A. Kaiser, Nicholas Arpaia*

Department of Microbiology & Immunology, Vagelos College of Physicians and Surgeons,
Columbia University Irving Medical Center, New York, NY, USA.

Abstract

The gut commensal bacterium *Bifidobacterium bifidum* promotes immune tolerance by facilitating the induction of colonic regulatory T cells.

Colonization of mucosal barriers by commensal microbes is important for the development and conditioning of the host immune system. In the mammalian gastrointestinal tract, resident bacterial communities can also support host metabolism through the digestion of complex carbohydrates, synthesis of essential vitamins and nutrients, and the biotransformation of bile acids. More recently, it has been appreciated that some bacterial components and metabolites can strongly influence the inflammatory potential of immune cells and profoundly affect in vivo phenotypes (1). Although considerable efforts have been made to isolate particular mucosa-associated microbes that contribute to host protection or disease susceptibility, the effects of individual bacterial species remain largely unknown (2). In this issue of *Science Immunology*, Verma *et al.* explore the mechanism by which the gut commensal bacterium *Bifidobacterium bifidum* induces peripheral regulatory T (T_{reg}) cells within the mouse gastrointestinal tract (Fig. 1) (3). Using an ex vivo screening approach to test for the ability of individual probiotic strains to promote the differentiation of T_{reg} cells from naïve CD4⁺ T cell precursors, the authors identify *B. bifidum* as a highly immunomodulatory microbe that potently induces T_{reg} cells both in vitro and in vivo. In subsequent experiments that used a number of germ-free and gnotobiotic animal models, the authors report the molecular cross-talk between *B. bifidum* and host immune cells that dampens inflammation and prevents colitis.

Over the past 15 years, many studies have demonstrated that the administration of specific strains of probiotic bacteria can promote the induction of intestinal T_{reg} cells and suppress inflammation (4, 5). One of the earliest publications in this area described a role for *Bacteroides fragilis* polysaccharide A (PSA) in promoting the differentiation of mouse intestinal T_{reg} cells (5). Verma *et al.* build on this growing body of literature to show that molecules isolated from *B. bifidum* can also influence mucosal immunity. Interestingly, in comparison with T_{reg} cells generated during long-term monocolonization with *B. fragilis*, the authors observed a robust expansion of colonic T_{reg} cells within just 3 weeks after monocolonization of germ-free mice with *B. bifidum*. In addition to expressing high levels of the immunosuppressive cytokine interleukin-10 (IL-10), a substantial proportion of these

*Corresponding author. na2697@cumc.columbia.edu.

newly expanded Foxp3⁺ T_{reg} cells express RAR-related orphan receptor- γ (ROR γ t), which is indicative of a subset of T_{reg} cells whose development is dependent on the detection of discrete microbial signals. T_{reg} cells induced in the context of *B. bifidum* colonization also exhibit low expression of Helios and NRP1, two markers believed to be specifically expressed by thymically derived, as opposed to peripherally induced, T_{reg} cells. Together, these data support the notion that the presence of *B. bifidum* within the intestine regulates peripheral T_{reg} cell development.

Higher frequencies of colonic T_{reg} cells may arise from their increased differentiation from naïve CD4⁺ T cells or by the expansion of preexisting intestinal T_{reg} cell populations. To tease apart these possibilities, the authors used an OT-II T cell receptor (TCR)–transgenic T cell transfer model in which T cells that recognize the food antigen ovalbumin (OVA) contribute to oral tolerance. Delivery of naïve, congenic, OT-II T cells into recipient mice who were fed OVA and monocolonized with *B. bifidum* resulted in increased numbers of differentiated T_{reg} cells, as compared with that of mice colonized with a control bacterium, *Lactobacillus paracasei*. This biased differentiation of naïve OT-II T cells into T_{reg} cells indicated that the presence of *B. bifidum* within the colonic microbiota can support de novo induction of peripheral T_{reg} cells.

T_{reg} cells generated in the context of *B. bifidum* colonization have a highly diverse TCR repertoire and recognize a wide range of antigenic targets besides *B. bifidum* itself. The broad tolerogenic capacity of *B. bifidum*-induced T_{reg} cells was further demonstrated by using a CBir TCR transgenic model of bacteria-induced colitis, in which transgenic T cells are activated in response to bacterial flagellin. Delivery of naïve CBir transgenic T cells into Rag1^{−/−} recipients that lack adaptive T and B cells resulted in unrestricted inflammatory responses against the commensal microbiota. Notably, recipients gavaged with *B. bifidum* were protected from colitis, highlighting the beneficial role of *B. bifidum* in promoting tolerance to a variety of different antigens in vivo. Inappropriate immune responses to gut commensals are a feature of intestinal inflammatory disorders. Identifying targetable pathways that dampen these responses through the induction of T_{reg} cells may bring about long-term improvement in patients who suffer from inflammatory bowel disease.

Diving deeper into the molecular interactions between *B. bifidum* and intestinal leukocytes, the authors identified specific *B. bifidum*-derived ligands that recapitulate the immunological changes that were observed after treatment with intact bacterial preparations. Subfractionation and nuclear magnetic resonance analysis of cell surface polysaccharides revealed a mixture of neutral glycans capable of inducing T_{reg} cells in vitro, with evidence for β -1-6-glucan being primarily responsible. Administration of *B. bifidum* neutral cell surface β -glucan/galactan polysaccharides (CSGG) alone ameliorated progression of inflammatory colitis, indicating CSGG to be a functional component of *B. bifidum* in promoting immune tolerance.

Several reports have identified that particular bacterial-derived polysaccharides and short-chain fatty acids generated by bacterial metabolism of complex carbohydrates can promote T_{reg} cell differentiation (6, 7). Gut-resident dendritic cells (DCs) are key antigen-presenting cells that shape the intestinal T cell compartment through activation of the TCR and

production of polarizing cytokines (8). Toll-like receptor (TLR) ligands, including evolutionarily conserved pathogen-associated molecular patterns, promote DC activation and elicit inflammatory programs that influence T cell lineage commitment. In the setting of *B. fragilis* colonization, TLR2 expression on both DC and T cells has been shown to contribute to PSA-driven T_{reg} induction (7, 9). Dasgupta *et al.* identified that recognition of PSA by plasmacytoid DCs (pDCs) is a critical step that defines a pro-tolerance phenotype, and differentiation of T_{reg} cells occurred via reciprocal inducible costimulator (ICOS)–ICOS ligand (ICOS-L) interactions between regulatory pDCs and cognate T cells (9). In a similar vein, previous studies in the Im laboratory identified a mixture of probiotic bacteria that supports the differentiation of intestinal DCs that exhibit a “regulatory phenotype,” marked by high expression of IL-10, transforming growth factor- β (TGF- β), Cox-2, and indoleamine 2,3-dioxygenase (10).

In this context, the generation of T_{reg} cells with diverse specificities after colonization with *B. bifidum* prompted Verma *et al.* to examine whether bacterial products directly influenced T_{reg} cell differentiation or whether T_{reg} cell induction occurred indirectly by activating regulatory DCs (rDCs). In support of their earlier work and that of others in the field, the authors demonstrate that surface glycans of *B. bifidum* act to modify DC gene expression through a partially TLR2-dependent mechanism. Although incubation of naïve T cells with bacterial products was unable to promote T_{reg} cell differentiation, delivery of the neutral fraction of surface glycans (CSGGs) to DCs enhanced rDC-associated gene expression, including up-regulation of *Pdcd1*, *Tgfb1*, *Csf2*, *Ptgs2*, *Il10*, and *Il27*. Increased production of TGF- β by conventional DCs (cDCs) as a result of stimulation with CSGGs was primarily responsible for the generation of T_{reg} cells because antibody-mediated neutralization of TGF- β almost entirely prevented their induction. CSGG treatment of cDCs isolated from TLR2^{-/-} mice or animals deficient for the downstream signaling adaptor MyD88 exhibited a partial but important reduction in their ability to support T_{reg} cell induction. Signaling through TLR3, TLR6, TLR7, and TLR9 as well as through the C-type lectin receptors Dectin1 and Dectin2 had no observable role in CSGG-mediated T_{reg} cell induction. These results describe a molecular mechanism by which DC-mediated detection of specific *B. bifidum*-derived glycans induces transcriptional changes that, in turn, alter T cell differentiation. This is in contrast to *B. fragilis* PSA, which, in addition to promoting regulatory pDCs, has also been reported to bias T_{reg} cell differentiation by directly activating TLR2 expressed by T cells.

The identification of *B. bifidum*-derived molecules capable of drastically altering DC inflammatory gene signatures provides a method for the manipulation of adaptive immune responses within the gastrointestinal tract. Co-opting pathways that are influenced by gut microbes to modulate immune cells may provide powerful opportunities to bolster protective immunity or limit systemic inflammation. The present study circumvents the need for administering nonspecific probiotics by providing a small subset of polysaccharides capable of achieving T_{reg} differentiation in vivo while highlighting β -1-6-glucan as a potentially key effector within the mixture. Future studies may probe the use of these immunomodulatory polysaccharides in the treatment of inflammatory bowel disorders as well as investigate the role of *B. bifidum* in altering response rates to existing therapeutic interventions.

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Fig. 1. Induction of intestinal T_{reg} cells by colonization with *B. bifidum*.

Neutral CSGG of *B. bifidum* are recognized by TLR2 on CD103⁺ intestinal DCs. TLR2 promotes an rDC phenotype, marked by up-regulation of tolerogenic cytokines. Among these, TGF- β production by DCs induces Foxp3⁺ROR γ t⁺ T cells with broad antigenic specificity.