transgenic CD8 and CD4 mouse T cells specific for OT1 and OT2, respectively. We also demonstrate that OT2 expression by L. reuteri can be recognized in vivo and generate antigen-specific Tregs. METHODS: We generated expression of OT1 and OT2 peptides in L. reuteri and L. plantarum in three subcellular locations: secreted, cell membrane-associated, and cell wall-associated. For in vitro assays, mouse dendritic cells (DCs) were pulsed with heat-killed cell pellets from each of these constructs or culture supernatant from secreted constructs, then incubated with either OT1 CD8+ or OT2 CD4+ T cells pre-stained with CellTrace Violet (CTV) for 3.5 days. For in vivo analyses, mice were gavaged with L. reuteri transformed with either empty vector or wall-associated OT2, or given ovalbumin in drinking water, followed by adoptive transfer of CTV-stained OT2 cells. OT2 cells were recovered from mesenteric lymph nodes (mLN) 7 days after adoptive transfer and stained for Foxp3.

RESULTS: All OT2 constructs induced significant in vitro proliferation (decreased CTV staining) of OT2 cells compared to strains expressing empty vector, while secreted and membrane-associated OT1 constructs induced significant proliferation of OT1 cells. In vivo, L. reuteri expressing wall-associated OT2 induced significant OT2 proliferation in mLN compared to L. reuteri transformed with empty vector. A significantly higher proportion of these proliferated cells were Tregs (Foxp3+) compared to proliferated cells in mice treated with L. reuteri transformed with empty vector. CONCLUSION: These findings suggest that Lactobacillus species can generate antigen-specific proliferation of T cells in vitro, and that L. reuteri may be capable of inducing antigen-specific Tregs in vivo. Expression of antigens by this species may represent a method of generating tolerance in the intestine, which could lead to novel therapies for controlling inflammation in IBD.

LACTOCoccus LACTis DELIVERY OF SURFACE LAYER PROTEIN A PROTECTS MICE FROM COLITIS BY RE-SETTING HOST IMMUNE REPERTOIRE
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Inflammatory bowel disease (IBD) is characterized by gastrointestinal inflammation comprised of Crohn’s disease and ulcerative colitis. Centers for Disease Control and Prevention report that 1.3% of the population of the United States (approximately 3 million people) were affected by the disease in 2015, and the number keeps increasing over time. IBD has a multifactorial etiology, from genetic to environmental factors. Most of the IBD treatments revolve around disease management, by reducing the inflammatory signals. We previously identified the surface layer protein A (SlpA) of Lactobacillus acidophilus that possesses anti-inflammatory properties to mitigate murine colitis. Herein, we expressed SlpA in a clinically relevant, food-grade Lactococcus lactis to further investigate and characterize the protective mechanisms of the actions of SlpA[1]. Oral administration of SlpA-expressing L. lactis (R110) mitigated the symptoms of murine colitis. Oral delivery of R110 resulted in a higher expression of IL-27 by myeloid cells, with a synchronous increase in IL-10 and cMAF in T cells. Consistent with murine studies, human dendritic cells exposed to R110 showed exquisite differential gene regulation, including IL-27 transcription, suggesting a shared mechanism between the two species, hence positioning R110 as potentially effective at treating colitis in humans.