Tailoring gut immune responses with lipoteichoic acid-deficient Lactobacillus acidophilus

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INTRODUCTION

The gastrointestinal tract possesses a highly specialized immunologic system comprised of both innate and adaptive immune components. These defense systems act in concert to maintain a state of alertness or physiological inflammation in the gut that enables the recognition and clearance of invading pathogens while remaining tolerant to the commensal microbiome (Sansonnetti, 2004). By virtue of their antigen processing and presenting abilities, dendritic cells (DCs) are at the forefront of intestinal immune responses. Previously, we have demonstrated that treatment with genetically modified Lactobacillus acidophilus is sufficient to tilt the immune balance from proinflammatory to regulatory in experimental models of colitis and colon cancer. Given the significant role of DCs in efficiently orchestrating intestinal immune responses, characterization of the signals induced within these cells by the surface layer molecules, such as lipoteichoic acid (LTA), and proteins of L. acidophilus is critical for future treatment and prevention of gastrointestinal diseases. Here, we discuss the potential regulatory pathways involved in the downregulation of pathogenic inflammation in the gut, and explore questions regarding the immune responses to LTA-deficient L. acidophilus that require future studies.

Keywords: Lactobacillus acidophilus, lipoteichoic acid, S-layer proteins, gut inflammation, dendritic cells, immune regulation

As highlighted by the development of intestinal autoinflammatory disorders when tolerance is lost, homeostatic interactions between gut microbiota, resident immune cells, and the gut epithelium are key in the maintenance of gastrointestinal health. Gut immune responses, whether stimulatory or regulatory, are dictated by the activated dendritic cells (DCs) that first interact with microorganisms and their gene products to then elicit T and B cell responses. Previously, we have demonstrated that treatment with genetically modified Lactobacillus acidophilus is sufficient to tilt the immune balance from proinflammatory to regulatory in experimental models of colitis and colon cancer. Given the significant role of DCs in efficiently orchestrating intestinal immune responses, characterization of the signals induced within these cells by the surface layer molecules, such as lipoteichoic acid (LTA), and proteins of L. acidophilus is critical for future treatment and prevention of gastrointestinal diseases. Here, we discuss the potential regulatory pathways involved in the downregulation of pathogenic inflammation in the gut, and explore questions regarding the immune responses to LTA-deficient L. acidophilus that require future studies.
Oral consumption of probiotics has been associated with multiple health benefits, including induction of mucus-secreting cells, maintenance of intestinal permeability, production of antimicrobial factors, colonization resistance, and immune cell activation or suppression of pathogenic intestinal autoinflammation.

**Lactobacillus acidophilus** AND ITS SURFACE LAYER COMPONENTS

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**Lactobacillus acidophilus** and its surface layer contribute to bacterial survival and host–microbial cell interactions, which is believed to facilitate adhesion, colonization, and invasion of host cells (Reichmann and Grundling, 2011). In contrast, LTA is regarded as the Gram-positive counter-part of the potent and proinflammatory Gram-negative stimulus, lipopolysaccharide (LPS; Sriskandan and Cohen, 1999; Su et al., 2006). LTA is a zwitterionic glycolipid found in the cell wall of many Gram-positive bacterial strains, including *L. acidophilus*, which is believed to facilitate adhesion, colonization, and invasion of host cells (Reichmann and Grundling, 2011). In addition, the likely role of LTA in Lactobacillus adhesion to mucosal surfaces, this molecule promotes immune cellular activation via TLR2 signaling, which then activates downstream proinflammatory cytokine signaling cascades (Schwandner et al., 1999; Chiu et al., 2009; Chang et al., 2010; Saber et al., 2011). Notwithstanding, conflicting reports suggested that LTA from certain Lactobacillus species induces anti-inflammatory cytokine production (IL-10), and only results in the generation of proinflammatory mediators in preexisting inflammatory conditions (i.e., co-culture with interferon-gamma (IFN-γ), Kaj et al., 2010; Kang et al., 2011). Taken together, these data contend that the functions of LTA might differ between bacterial species (beneﬁcial lactobacilli versus pathogenic) as well as depending on the status of the local cytokine milieu (steady state versus proinﬂammatory). However, a caveat of these studies is that the work was performed in vitro, which prompts the following question: what is the physiological role of lactococcilli-derived LTA?

**IMMUNE REGULATION INDUCED BY LTA-DEFICIENT L. acidophilus**

To clarify the in vivo effects of *L. acidophilus*-LTA, we recently developed a *L. acidophilus* strain lacking the gene encoding LTA.
Figure 1: Immune regulation established by lipoteichoic acid (LTA)-deficient Lactobacillus acidophilus. (A) In steady state conditions, molecules expressed on the cell surface of L. acidophilus activate dendritic cells (DCs) to promote effector Th1 and Th17 responses that are held in check by the accompanying generation of induced regulatory T cells (iTregs). However, in preexisting inflammation or susceptible individuals, immune activation by L. acidophilus-LTA exacerbates inflammatory responses and fails to promote immune regulation. Oral intake of mutant strains lacking LTA expression (LTA−−L. acidophilus) predominantly results in suppression of exacerbated immune responses via the induction of regulatory IL-10-secreting DCs (B), which then promote the conversion of naive T cells into iTregs. (B) Confocal microscopy analysis of DCs (CD11c+, green; CD11b+, red) that produce IL-10 (white) in the colons of healthy control mice after treatment with LTA-deficient L. acidophilus.
Although the exact signaling pathways whereby LTA-deficient L. acidophilus not only prevented chemical and pathogenic T cell-induced colitis, but also quickly resolved established colitis, as measured by diminished percent weight loss, lower diarrhea and fecal occult blood scores, and reduced disease activity index (Mohamadzadeh et al., 2011). By the same token, LTA-deficient L. acidophilus dramatically reversed colonic preneoplasia in genetically predisposed animals (Khazaie et al., 2012). While protection from colitis in our studies correlated with an increase in IL-10-producing DCs and the number of iTregs (Mohamadzadeh et al., 2011; Khan et al., 2012), polyposis reversal coincided with an overall dampening of local and systemic immunity that was linked with restoration of Treg function and stability (Khazaie et al., 2012). Importantly, proinflammatory Tregs have also been identified in colorectal cancer (CRC) patients (Blatter et al., 2012), further supporting the clinical applicability of LTA-deficient L. acidophilus for the treatment of intestinal maladies given its potential ability to prevent the formation of proinflammatory FoxP3+ TCRγδ Tregs.

Moreover, in vitro co-culture of DCs with LTA-deficient L. acidophilus led to a regulatory DC phenotype, as demonstrated by enhanced IL-10 secretion, low expression of costimulatory molecules, and concomitant decreases in IL-12 and TNF-α production. Alternatively, no beneficial effects could be induced in IL-10−/− mice in vivo, highlighting the important role of this anti-inflammatory cytokine in the control of pathogenic intestinal inflammation in our system, similar to previous findings by others (Asseman et al., 1999; Grangette et al., 2005; Rubtsov et al., 2008). Activation of mitogen-activated protein kinases (MAPK) signaling pathways differentially controls features of both innate and adaptive immune responses (Dong et al., 2002). Favorably IL-10 production by regulatory DCs has previously been found to be dependent on extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) activation, while suppressed IL-12 secretion resulted from impaired p38 activation (Qian et al., 2006). Indeed, significant and sustained ERK1/2 activation was measured in the colonic tissues of mice orally treated with LTA-deficient L. acidophilus, whereas the wild-type strain promoted p38 phosphorylation (Saber et al., 2011). Furthermore, DC stimulation with LTA-deficient L. acidophilus resulted in only weak TLR2-dependent cytokine production and did not enhance the expression of this PRR; these data indicate that LTA is in fact the proinflammatory molecule most strongly associated with TLR2 activation by L. acidophilus in DCs, and that the in vivo regulatory response noted after LTA-deficient L. acidophilus treatment is a direct consequence of its absence. Collectively, the favorable effects of LTA-deficient L. acidophilus may be due to weak TLR2 activation and downstream signaling, together with the predominant activation of alternative DC-related PRRs, such as CLR (Konstantinov et al., 2008), by different surface-associated molecules, including SlpA (summarized in Figure 1A).

**CONCLUDING REMARKS AND FUTURE DIRECTIONS**

Although the exact signaling pathways whereby LTA-deficient L. acidophilus promotes the generation of regulatory DCs and, consequently, iTregs, are currently under intensive investigation, data obtained thus far clearly demonstrate that IL-10-dependent pathways (Figure 1) underlie the protective effects of LTA-deficient L. acidophilus. In addition, work by others point to SlpA as a potential regulatory molecule in L. acidophilus (Konstantinov et al., 2008). Notably, as seen in the wild-type L. acidophilus strain, the presence of this S-layer protein alone is not sufficient to counterbalance the proinflammatory actions of LTA. Additional studies performed in our laboratories demonstrated that a mutant strain expressing LTA and SlpA, but not SlpX and SlpB, was unable to afford any protection against colitis (Zadeh et al., 2012). In fact, oral treatment with this LTA−/−SlpA+ strain led to a higher number of TNF-α-producing colonic DCs, in addition to sustained IL-12 production by DCs in the colon, when compared to the LTA-deficient parental strain (Zadeh et al., 2012). These findings may be interpreted to imply that the other S-layer proteins expressed by L. acidophilus NCFM also contribute to the regulation of LTA-induced inflammation; however, attempted deletion of SlpA in this strain resulted in slightly lower expression levels of the protein when compared to the parental strain, which then suggests that even small perturbations in the amount of SlpA expressed can exacerbate LTA-mediated inflammation. Consequently, ongoing studies aim to investigate the specific contribution of the S-layer components (i.e., SlpA) to conserve and support gut homeostasis by creating restricted mutant strains of L. acidophilus using molecular techniques previously described (Goh et al., 2009) and purifying our protein of interest, SlpA. Thus, the therapeutic value of both SlpA+SlpB− and SlpX−/− strains of L. acidophilus and purified SlpA will be determined in vivo.

In other respects, it is likely that LTA-deficient L. acidophilus confers additional benefits to the host through mechanisms independent of the immunomodulatory effects mentioned above. For instance, intestinal epithelial cells not only create a protective barrier against invading pathogens, but also sense and interact with microbes through PRRs to influence subsequent innate immune responses (Wells et al., 2011). Accordingly, the status of the mucosal epithelium is central to gastrointestinal health and accumulating evidence indicates that aberrant epigenetic modification of colonic tissue contributes to CRC development (Luo and Grady, 2011). As these changes can arise in the presence or absence of pathogenic intestinal inflammation, we recently tested the effects of LTA-deficient L. acidophilus treatment on the epigenetic landscape of the intestinal mucosa and found that this bacterium induced the expression of CRC-associated, epigenetically controlled genes that are often downregulated in cancer-promoting pathogenic conditions (Lightfoot et al., 2012). These important results create a strong position to precisely define the bacterial gene products that may dampen detrimental gut inflammation and protect against inflammatory conditions, including inflammatory bowel disease and colon cancer, not only through immune cell modulation, but also via direct interactions with the gut epithelium.

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