Milk fermented by *Lacticaseibacillus casei* improve barrier function and alter sterol metabolism in intestinal epithelial cells

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Introduction: How fermented dairy products can modify immunity and metabolism to benefit human health is largely unknown. In this study, we investigated the capacity of milk and milk fermented by *Lacticaseibacillus casei* BL23 (BL23-milk) and ATCC334 (A334-milk) to improve transcellular barrier integrity of intestinal epithelial cells (IEC).

Methods: Cell-free preparations of UHT milk fermented by *L. casei* BL23 or ATCC334 were applied onto differentiated Caco-2 cell monolayers in transwell inserts. IFNg was then applied and transepithelial electrical resistance (TER) across cell the monolayers was quantified 24 h later. To determine IEC responses to milk and *L. casei*, transcriptome analysis was performed using RNA-seq. An average of 2 million reads were obtained per sample. Follow up studies were performed using EGFR inhibitor, AG1478.

Results: BL23-milk and A334-milk, but not milk alone, significantly increased TER in an IFNg-dependent manner. IFNg application increased expression of genes in known IFNg modulatory pathways. Several of these genes were downregulated in IECs exposed to milk, BL23-milk, or A334-milk ($p < 0.05$). Only BL23-milk reduced expression of CLDN2 ($p = 0.02$). Only BL23- and A334-milk conferred increased expression of genes required for sterol metabolism ($p < 0.05$). AG1478 prevented *L. casei*-induced increase in TER.

Discussion: Secreted compounds resulting from *L. casei* growth in milk improved barrier function of Caco-2 cells exposed to IFNg. These results were not strain specific and were dependent on EGFR activation. Transcriptomic analysis strongly indicates a role for sterol metabolism in *L. casei*-dependent regulation of intestinal cell responses. These results support ongoing efforts to understand how the dairy matrix influences the capacity of probiotics to promote gastrointestinal health.