Modulation of gut microbes: Interplay between peptidoglycan recognition proteins and lysozyme transgenic goat milk.

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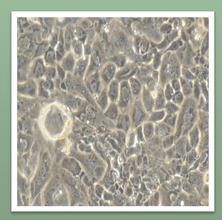
Introduction

Many of the species in the gut are symbiotic aiding in digestion, producing host beneficial byproducts and occupying niches that would otherwise house pathogens. Imbalance of these functional groups, dysbiosis, has been linked to diseases including inflammatory bowel diseases and allergic conditions. Formation of the microbiome after birth is primarily driven by diet, thus antimicrobials present in milk are a major contributor to shaping the microbiome. In fact, there is evidence that cessation of breast feeding is the primary determinate of abrupt changes in abundance of microbial communities during childhood, known as bacterial succession. The first strains to colonialize infants are mainly aerobic organisms some of which are opportunistic pathogens, such as Enterobacteria, *Streptococcus and Staphylococcus* making the antimicrobials in milk critical to host protection.

To interrogate the effect of milks' antibacterial peptides on the microbiome, our lab has genetically engineered goats to produce human lysozyme (hLZ) in their milk at 67% of the level of human milk. Lysozyme is an antimicrobial enzyme that is naturally found in tears, saliva and milk, however, it is found at comparatively low levels in goat milk. To study the effects of hLZ on microbial communities and intestinal health, milk with disease and increase the relative abundance of from hLZ transgenic goats (hLZ milk) was fed to pigs resulting in a reduction in Firmicutes associated Bacteroidetes families associated with better gut health. Thus, hLZ milk could substantially improve the health of populations in developing countries where malnutrition and diarrheal disease impacts children beyond the age of breastfeeding. However, a full understanding of the mechanism by which hLZ asserts its effects is unknown and is critical prior to human consumption. Using RNAseq on porcine jejunal IPEC-J2 cells treated with hLZ milk, we previously found that hLZ upregulated peptidoglycan recognition protein 3 (PGLYRP-3). Peptidoglycan recognition proteins (PGLYRPs) are innate immune proteins implicated in early establishment and maintenance of equilibrium of commensal populations. In humans and pigs, there are four PGLYRPs and, due to their function and higher levels of secretion in the GI tract, we investigated the effect of hLZ on PGLYRP-3 and 4 as a mechanism by which hLZ asserts its effects. Here we examine gene expression and secretion of PGLYRPs in IPEC-J2 cells in the presence of hLZ milk with and without different bacterial species to determine the how the interplay between lysozyme in hLZ milk and PGLYRPs modulates the microbiome.

Experimental Design



The Ability of hLZ Goat Milk to Regulate PGLYRP-3 and 4 Expression is Time Dependent

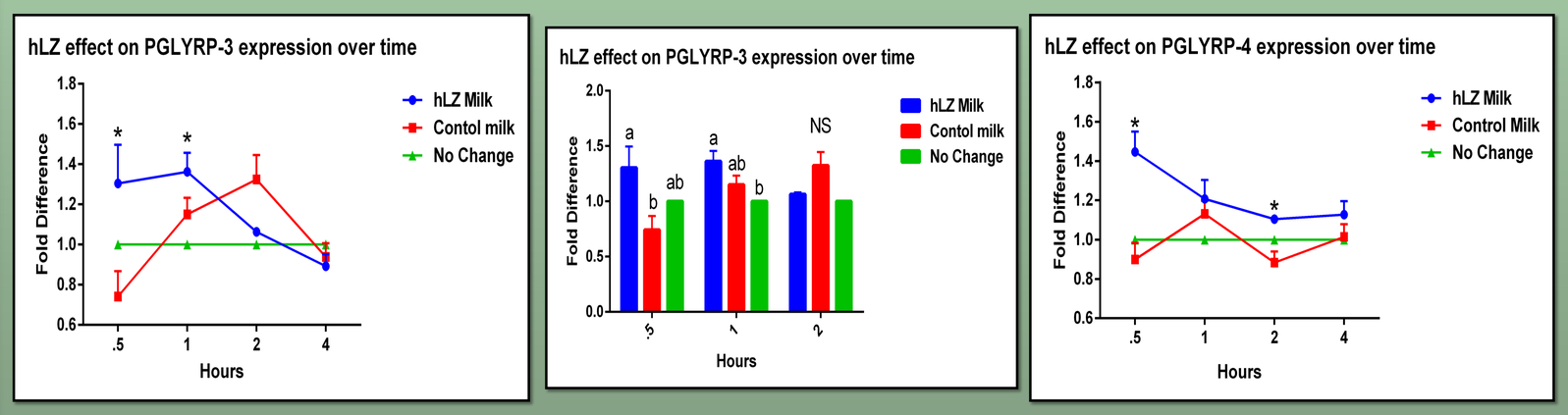


Figure 2: IPEC-J2 cells were treated with either hLZ or control milk for different periods of time. After treatment cells were harvested and qRT-PCR performed. Fold-changes in gene expression were analyzed by ANOVA and differences between groups determined by the Tukey test with $p \leq 0.05$ considered significant (*). One hour time point represents two separate experiments with n=9, half hour and two hour time points n=5. Data is presented as mean ± SEM.

Effect of Commensal Bacteria in the Presence of hLZ Milk on Expression of PGLYRP-3

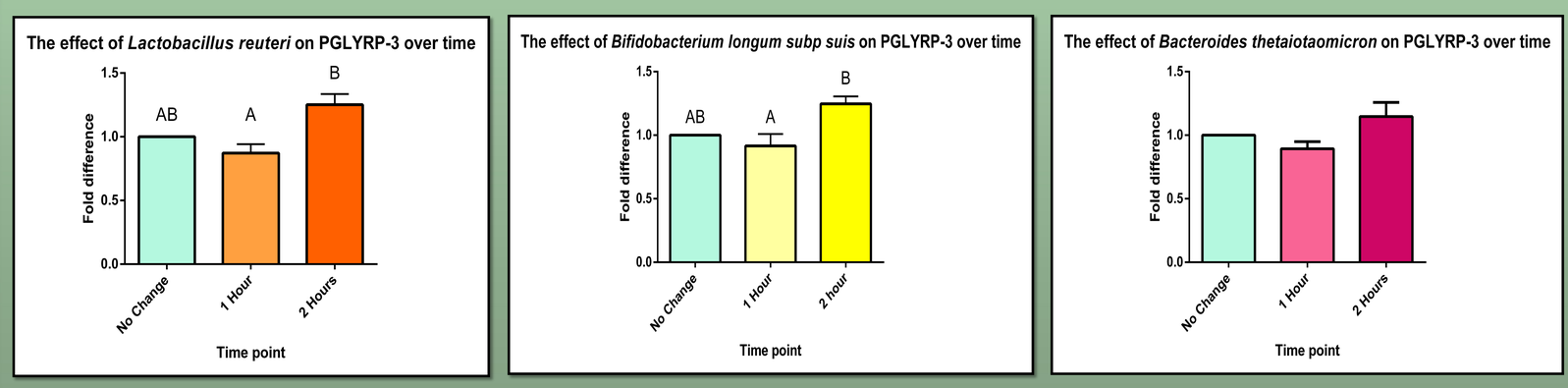
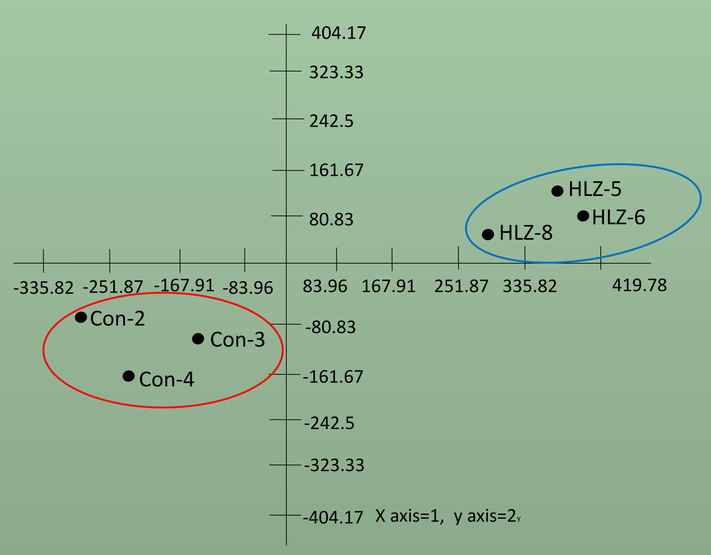


Figure 3: IPEC-J2 cells were co-cultured with a mixture of hLZ milk and either *Lactobacillus reuteri* or *Bifidobacterium longuum subp suis or Bacteroides thetaiotaomicron* for various time periods. After treatment cells were harvested and fold-changes in gene expression were determined via qRT-PCR. Data represents n=6 and is presented as mean ± SEM. Fold-changes in gene expression were analyzed by ANOVA and differences between groups determined by the Tukey test with $p \leq 0.05$ considered significant.

hLZ Goat Milk Shifts Microbiota of Pigs



Bacteroidetes — hLZ enriched biomarkers of gut health → Bifidobacteriaceae, Lactobacillaceae, Bacteroidetes

Firmicutes — hLZ reduced disease associated bacteria → Streptococcaceae, Clostridiaceae

Figure 1: Six week old pigs were fed either control or hLZ milk twice a day (n=3). At the end of two weeks feces was analyzed by 16S rRNA gene sequencing to determine bacterial populations present. Principle component analysis demonstrated significant differences between groups ($p \leq 0.05$).

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Secretion of PGLYRP-3/4

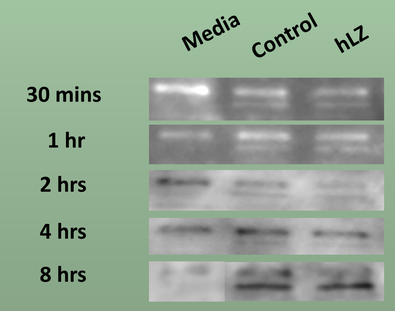

Figure 4: IPEC-J2 cells were treated with control or hLZ milk. After respective time, media was collected and western blot was preformed with an antibody that binds both PGLYRP-3 and 4.

Conclusions

- hLZ goat milk increases PGLYRP-3 and 4 expression in a time dependent manner.
- PGLYRP-3 and 4 are secreted into the media of cells treated with hLZ or control milk in very low concentrations.
- Some commensal bacterial species can induce expression of PGLYRP-3 in the presence of hLZ milk in a time dependent manner.

Future Directions

- Elucidate the mechanism by which commensal species effect expression of PGLYRP-3.
- Determine how a pathogenic bacteria, *E. Coli*, influences PGLYRP-3/4 expression over time compared to commensal species.
- Distinguish between sequestration of peptidoglycan or direct bactericidal activity by as primary anti-inflammatory role of PGLYRP-3/4.
- Determine if there is a synergistic anti-inflammatory effect of lysozyme and PGLYRPs in response to pathogenic bacterial challenge.