Title:
An assay for inducing APRIL production by intestinal epithelial cells to probe breastmilk's immuno-modulatory effects

Background:
Breastmilk is the infant's natural primary source of immuno-modulatory factors, such as immunoglobulin A (IgA), during the post-natal period. The advent of increasingly common pediatric food allergies suggests that early immune system development and early environmental exposure determine susceptibility to allergies. IgA prevents allergies by inhibiting the excessive uptake of sensitizing food antigens through the infant's intestinal epithelium. However, the infant's gut does not produce IgA due to inhibited plasma B-cell development until approximately one month after birth. Normally, mucosal lamina propria B-cells convert from producing IgM to producing IgA via class switch recombination (CSR). Two pathways for activating the production of IgA, T-cell dependent (TD) and T-cell independent (TI) pathways, utilize end plasma B-cell CSR but involve different upstream factors. APRIL is a critical factor in the TI pathway for IgA production and is directly synthesized by epithelial cells upon intestinal stimulation, such as stimulation by breastmilk. APRIL binds to B-cells and activates CSR to IgA. Because TD pathway factors dominate in the early gut and do not correlate with increased IgA-producing plasma B-cell densities during the one-month critical time window, the slower-forming TI pathway may be responsible for the low allergy-protecting IgA densities during early life.

Objective:
Here, an assay for culturing and stimulating intestinal epithelial cells was developed to measure APRIL production in response to stimulants. This assay will be used to probe the immuno-modulatory effects of breastmilk stimulation on the infant's gut epithelium for TI pathway factors as defense against pediatric food allergies in early life.

Results:
- 10% Fetal Bovine Serum - Minimum Essential Medium (FBS-MEM) is preferred for longer incubation periods, such as three to four days. The high concentration of growth factors in 20% FBS-MEM may negatively feedback to inhibit cell activation.
- Cell stimulation with media containing lipopolysaccharide (LPS) elicited enhanced APRIL expression compared to stimulation with media containing flagellin (FLG), regardless of whether the cells were grown in 10% or 20% FBS-MEM.
- Cells that were cultured for three or more days, followed by incubation with LPS-media for three or more days, elicited the highest fold change in APRIL expression.
- For the experimental trials performed thus far, qPCR is the best quantification method for detecting APRIL mRNA. ELISA (enzyme-linked immunosorbent assay) detection of APRIL protein in media supernatant is weaker but should not be discounted in future assay development.
Conclusion:
-LPS is the optimal stimulant for inducing APRIL expression.
-Further assays for culturing and stimulating intestinal epithelial cells may grow and stimulate cells in 10% FBS-MEM for longer periods beyond three days to potentially induce higher APRIL production.
-Although qPCR best detected fold changes in APRIL expression between control and stimulated cells, continuing to compare results between qPCR and ELISA detection methods will strengthen the assay’s reliability.
-The developed assay should be adapted to grow cells on a transwell with a permeable membrane as a model system for the gut’s epithelium.

References: