STRONG CHILDREN'S RESEARCH CENTER

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ABSTRACT

Title: Measuring the Impact of Breast Milk on the Expression of Immunomodulatory Factors in the Intestinal Epithelial Cells Utilizing a Caco-2 Cell Model

Background: Breast milk is an intricate biological fluid composed of a combination of immune cells, immunoglobulin A (SIgA), probiotics, cytokines, and other immunomodulatory factors.[1] Research has shown that exposure to such molecules could affect the development and maintenance of both the infant's gut microbiome and immune system, making breast milk a vital component supporting the largely undeveloped infant immune system in the beginning of life.[2] One of the key immune factors in breast milk is secretory immunoglobulin A (SIgA), an antibody thought to play a role in humoral immunity by halting the interaction of antigens with the gut's epithelium, thereby maintaining structural barrier integrity and preventing an allergic reaction.[3] The biological mechanisms by which human milk can impact the infant gut mucosal immune responses are largely unknown, [2] including the impact of breast milk on the gut epithelial cells, the first line of exposure.

Objective: To develop a cell culture model of the infant gut epithelium could be used to assess changes in the RNA expression levels of particular immunomodulatory factors that play a role in the development and upkeep of the infant's immune system.

Design/Methods: The Human-Isolated Colon Carcinoma (Caco-2) Cell Line was used to model the gut epithelium. Caco-2 cells were grown to confluency on a transwell system that allowed a polarized monolayer, including tight junctions, to form. Caco-2 cells were passaged a minimum of seven times when thawed from liquid nitrogen before plating on the transwell inserts to assure single cell suspension and appropriate growth.

To confirm the system's accuracy for measuring changes in the RNA expression levels of immunomodulatory factors, positive controls were developed. Known stimulants (lipopolysaccharide or LPS and flagellin) were used to induce a change in the expression levels of particular cytokines (IL-8 and NF-kB). The expression of IL-8 has been found to be up-regulated post-stimulation with flagellin in a dose-dependent manner.[4] Similarly, the production of NF-kB has been shown to increase after stimulation with LPS, a ligand for the Toll-Like Receptor (TLR) 4 pathway in the gut epithelium that aids in the production of pro-inflammatory cytokines (such as IL-8).[5]

Cells were stimulated with these control stimulants or breast milk from either the basolateral or apical compartment of the transwell system. Resulting RNA was reverse transcribed and measured for fold change in expression levels relative to a known housekeeping gene (B-Actin) utilizing qPCR.

Results/Conclusions: Caco-2 cells reached confluency and formed a polarized monolayer on the transwell inserts after 168 hours (seven days) in culture. Transepithelial Electrical Resistance (TEER) was measured to assess confluency and all cells with a minimum TEER of 350 Ohms*cm^2 were assessed as being confluent. Immunofluorescent staining positive for the epithelial barrier protein Claudin-1 supported the use of Caco-2 Cells at this time point.

Stimulation of Caco-2 Cells with potential positive control treatments (LPS or Flagellin) was also assessed. Results showed that both apical and basolateral stimulation with flagellin

yielded an up-regulation of IL-8, as previously shown.[4] LPS treatments were not as strongly conclusive, but there was evidence to suggest a down-regulation of NF-kB by both apical and basolateral stimulation. Based on these results, apical stimulation with flagellin was used as the positive control to assert the functionality of the Caco-2 Cell model.

Data collected suggests a notable effect by breast milk on the Caco-2 cell monolayer. Both apical and basolateral stimulation by breast milk was found to up-regulate the expression level of IL-8. Stimulation from either chamber by breast milk yielded conflicting results for the expression levels of NF-kB. As well, IL-1B was shown to be up-regulated by apical stimulation and down-regulated by basolateral stimulation. As a next step, this system will be used to assess epithelial cell RNA by RNAseq to give a complete understanding of the potential changes that occur in the expression levels of immunomodulatory factors. This could potentially give new insights to the effects of breast milk on the developing infant gut microbiome and immune system.

Citations:

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