Infant Commensal Complexed with Milk Secretory Immunoglobulin A Prevents Enteric Infection

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Enteric infections plague millions worldwide each year, with the highest rates of morbidity and mortality in infants under 1 year of age. Breast milk provides marked protection against enteric pathogens through several mechanisms including antimicrobial proteins like lactoferrin and lysozyme, oligosaccharides to promote colonization of commensals that competitively exclude pathogens, and secretory immunoglobulin A (SIgA) known to supply passive immunity to the neonate. Recently, milk SIgA has gained new appreciation for its role in shaping the commensal biota through antigen-independent interactions, but to date there have been no evaluations of the impact of this relationship on enteric pathogens. This study aimed at investigating the functional outcome of a commensal:SIgA complex on the prevention of enteropathogenic invasion in human colonocyte cells in vitro, and in dampening the host inflammatory response. Invasion assays were completed by pre-treating a co-culture of Caco-2 colonocytes and HT29-MTX cells (90:10 ratio) with a commensal:SIgA complex confirmed through flow cytometry (1000 ug SIgA/1e7cfu ratio) for 1 h followed by inoculation with enterohemorrhagic *Escherichia coli* (EHEC), *Salmonella enterica* serovar Typhimurium (*Salmonella*), or *Campylobacter jejuni* (*C. jejuni*). A gentamycin protection assay eliminated extracellular bacteria for *Salmonella* and *C. jejuni* assays, and the colony forming units were counted from colonic cell lysates to determine invaded pathogen. Commensal pretreatment alone reduced *Salmonella* invasion by 50%, but had no effect on EHEC adhesion and increased *C. jejuni* invasion. However, commensal:SIgA complex reduced *Salmonella* invasion by almost 90% ($p<.001$), EHEC adhesion by 45% ($p<.051$) and *C. jejuni* invasion by 100%. The commensal:SIgA complex reduced mammalian cell gene expression of the pro-inflammatory cytokine IL-8 by 6-fold and altered tight junction binding protein expression. In addition, the commensal:SIgA complex significantly reduced the trans-epithelial electrical resistance (TEER), a measure of intestinal permeability, when co-cultures were pre-treated prior to *Salmonella* challenge. Mechanisms behind these effects warrants investigation. This may be through direct host-microbial cross-talk that strengthens intestinal barrier function, reduction of pathogen load, or alteration of metabolite secretion of the commensal and/or pathogen with SIgA. This study provides a better understanding of the multifarious roles of milk SIgA in shaping the infant gut microbial community and may offer a novel mechanism to prevent enteric infectious disease in the newborn.