
Abstract
The intestinal epithelium forms a protective barrier against luminal contents and the external environment, mediated via intercellular tight junctions (TJs). The TJ can be disrupted via cell signalling induced by either enteric pathogens or pro-inflammatory cytokines, thereby contributing to various intestinal disorders ranging from acute infectious diarrhoea to chronic inflammatory bowel diseases. Probiotics, such as Lactobacillus rhamnosus GG (LGG), are reported to confer beneficial effects on epithelial cells, including antagonizing infections and reducing overt pro-inflammatory responses, but the underlying mechanisms of these observed effects require further characterization. We hypothesized that probiotics preserve barrier function by interfering with pro-inflammatory cytokine signalling. Caco-2bbe cells were seeded into Transwells to attain polarized monolayers with intercellular TJs. Monolayers were inoculated apically with the probiotic LGG 3emsp14;h prior to the addition of IFN-γ (100emsp14;ng ml(-1)) to the basolateral medium overnight. The monolayers were then placed in fresh basal medium±TNF-α (10emsp14;ng ml(-1)) and transepithelial electrical resistance (TER) measurements were taken over the time-course of TNF-α stimulation. To complement the TER findings, cells were processed for zona occludens-1 (ZO-1) immunofluorescence staining. As a measure of TNF-α downstream signalling, cells were immunofluorescently stained for NF-κB p65 subunit and CXCL-8 mRNA was quantified by qRT-PCR. Basal cell culture medium was collected after overnight TNF-α stimulation to measure secreted chemokines, including CXCL-8 (interleukin-8) and CCL-11 (eotaxin). Following LGG inoculation, IFN-γ priming and 24emsp14;h TNF-α stimulation, epithelial cells maintained TER and ZO-1 distribution. LGG diminished the nuclear translocation of p65, demonstrated by both immunofluorescence and CXCL-8 mRNA expression. CXCL-8 and CCL-11 protein levels were decreased in LGG-inoculated, cytokine-challenged cells. These findings indicate that LGG alleviates the effects of pro-inflammatory cytokines on epithelial barrier integrity and inflammation, mediated, at least in part, through inhibition of NF-κB signalling.