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Interferon-γ and tumor necrosis factor-α disrupt epithelial barrier function by altering lipid composition in membrane microdomains of tight junction

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Abstract Tight junctions (TJs) are specialized membrane microdomains of plasma membrane and play an important role in barrier function. IFN-γ and TNF-α have been implicated in intestinal barrier dysfunction. In the present study, we analyzed the effect of IFN-γ and TNF-α on epithelial barrier function and determined the contribution of apoptosis to this process using T84 cells, a model intestinal epithelial cell line. We found that TNF-α and IFN-γ synergistically affected the epithelial barrier and disrupted the structure of TJs. We demonstrated for the first time that treatment with TNF-α and IFN-γ changed lipid composition and fatty acyl substitutions of phospholipids in membrane microdomains of TJs. Alterations of lipid environment affected TJs barrier function and partly removed flotillin-1 and displaced occludin from membrane microdomains of TJs to detergent-soluble fractions. The distribution of claudin isoforms was unaffected by TNF-α and IFN-γ treatment. These findings indicated the interaction between inflammatory cytokines and alterations of lipid composition in membrane microdomains of TJs in the inflammatory processes. The apoptosis inhibitor did not prevent cytokine-induced decrease in TER and increase in permeability to FITC-dextran. Our results suggest that the cytokines directly influence TJ function and modulate both the membrane microdomain localization of TJ proteins and lipid composition of TJs. The effects of proinflammatory cytokines on TJ structure and function provide a mechanism in the pathophysiology of Crohn's disease (CD). Understanding the intracellular mechanisms involved could be important in devising future therapeutic strategies to induce retightening of the leaky TJ barrier.

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Introduction

Epithelial paracellular permeability is regulated by the apical junction complex including the tight junction, adherens junction and desmosome. Tight junctions (TJs) play an important regulatory role in barrier function. TJs are situated at the membrane between apical and lateral regions of polarized epithelial cells and selectively regulate the passage of molecules and ions via the paracellular pathway [1]. Inflammatory diseases of intestinal mucosa are characterized by immune cells activation and compromised barrier function. Abnormal intestinal permeability is observed in patients with Crohn's disease (CD) and the intestinal permeability is related to alterations of tight junction [2–4]. Proteins of the tight junction are composed of occludin, the claudin family, junctional adhesion molecule-JAM and ZO-1, ZO-2 and ZO-3 [5,6].

Interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) trigger the proinflammatory cascade and disrupt tight junction barrier. A link between increased gut permeability and inflammatory cytokines in inflammatory bowel disease has been noted. It was shown that the levels of TNF-α and IFN-γ were increased in Crohn's disease [7,8]. TNF-α and IFN-γ are critical to the barrier disruption. Disruption of epithelial barrier by TNF-α and IFN-γ represents a major pathophysiological consequence of intestinal inflammation. The intracellular mechanisms involved in TNF-α and IFN-γ modulation of intestinal TJ barrier remain unclear. Understanding the intracellular mechanisms involved could be important in devising future therapeutic strategies to block disruption of the leaky TJ barrier.

A previous study suggested that TJs were lipid raft-like microdomains of the plasma membrane [9]. Lipid rafts have been identified as distinct membrane subdomains, which are insoluble in non-ionic detergents and can be isolated as detergent-insoluble membrane microdomains. Evidence shows that TJ serves as a platform for trafficking and signaling protein complexes [10]. It was suggested that TJ membrane microdomains play an important role in spatial organization of TJs and in regulation of paracellular permeability in epithelial cells [9]. Cytokine-induced change in epithelial permeability was associated with redistribution of TJ proteins [11].

The differences in lipid composition are largely dependent on the level of differences in the ratio of lipid species and in the distribution of fatty acid species within a membrane. Lipid interactions are very important for the formation of lipid rafts [12]. Inflammatory cytokines may alter lipid composition in membrane microdomains of tight junction, hence, in the present study, the cytokine-dependent alterations in epithelial barrier function were investigated by using T84 cells as an in vitro model. We found that treatment with TNF-α and IFN-γ led to changes of lipid composition and fatty acyl substitutions in phospholipids in membrane microdomains of TJs in T84 cells. We observed that IFN-γ and TNF-α displaced flotillin-1 and occludin from membrane microdomains of TJs to TritonX-100-soluble fractions and localization of claudin-1 and claudin-4 was unaffected. Changes in epithelial permeability were associated with the disruption in TJ ultrastructure. Finally, inhibition of apoptosis did not alter the cytokine-induced increase in permeability. The findings provide new useful insight into the mechanism by which proinflammatory cytokine including TNF-α and IFN-γ compromises barrier function in inflammatory bowel disease.

Materials and methods

Materials

The mixture of Dulbecco's modified Eagle's medium (DMEM) and F-12 nutrient mixture (Ham) and fetal bovine serum were purchased from GibcoBRL (GibcoBRL, Grand Island, New York, USA). Complete protease inhibitor tablets were purchased from Boehringer Mannheim (Indianapolis, IN). ECL Western blotting analysis system was purchased from Amersham (Piscataway, NJ). Rabbit polyclonal antibody against occludin, claudin-1 and mouse polyclonal antibodies against claudin-4 was from Zymed Laboratories Inc. (San Francisco, CA). Mouse anti-flotillin-1 monoclonal antibody was from BD Transduction Laboratories (Lexington, KY). Alex-Fluor-635 and Alex-Fluor-488 secondary antibodies, fluorescein isothiocyanate-labeled dextran (molecular weight 3000D, FD-3) and 1,6-diphenyl-1,3,5-hexatriene (DPH) were purchased from Molecular Probes (Eugene, OR). PVDF membrane was purchased from Bio-Rad Laboratories. IFN-γ and TNF-α were purchased from R&D Systems (Minneapolis, MN). Annexin V-FITC apoptosis detection kit and caspase-3/CPP32 inhibitor Z-DEVD-FMK were obtained from Biovision (Mountain View, USA).

Epithelial cell culture and TNF-α and IFN-γ incubation

The epithelial T84 cells, obtained from ATCC (Rockville, MD) were grown in a 1:1 mixture of DMEM and F-12 medium supplemented with 5% heat-inactivated fetal bovine serum, 14 mM $NaHCO_3$, 15 mM HEPES buffer, at a pH of 7.3 and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin). They were cultured on 75 cm^2 flasks (Corning Costar, Cambridge, MA) at 37 °C in a humidified atmosphere of 5% CO_2. For subcultures, the cell suspension was obtained from confluent monolayers by exposing to 0.25% trypsin and 1 mM EDTA in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline. The experiments were performed with cells subcultured for three passages and cultured to confluence. The cell monolayers were incubated in the continuous presence of IFN-γ (100 U/mL) and TNF-α (10 ng/mL) or preincubated with IFN-γ (100 U/mL) for 19 h then TNF-α (10 ng/mL) was added.

Isolation of membrane microdomains of TJs

T84 cells were grown to confluence on 75 cm^2 flasks. After being washed three times with ice-cold PBS, the cells were scraped into extraction buffer (50 mM Tris, 25 mM KCl, 5 mM $MgCl_2 \cdot 6H_2O$, 2 mM EDTA, 40 mM NaF, 4 mM Na_3VO_4, pH 7.4) containing 1% TritonX-100 and protease inhibitors mixture solution. The sucrose concentration of the cell lysate was adjusted to 40%, placed at the bottom of an ultracentrifuge tube and overlaid with a sucrose step gradient of 30, 25, 20 and 5% in extraction buffer. The samples were centrifuged at 250,000×*g* for 18 h at 4 °C with a Ti90 rotor in an Optima L-80XP ultracentrifuge (Beckman Coulter Inc. Fullerton, CA).