Intestinal Alkaline Phosphatase Regulates Tight Junction Protein Levels

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BACKGROUND: Intestinal alkaline phosphatase (IAP) plays a pivotal role in maintaining gut health and well-being. Oral supplementation with IAP in mice improves gut barrier function and prevents luminal proinflammatory factors from gaining access to the circulation. In this study, we sought to explore the relationship between IAP and tight junction protein (TJP) expression and function.

STUDY DESIGN: The effect of IAP deletion on TJP levels was studied in mouse embryonic fibroblasts (MEFs) generated from IAP-knockout and wild type mice. Regulation of TJPs by IAP was assayed in the human colon cancer Caco-2 and T84 cells by overexpressing the human *IAP* gene. Tight junction protein levels and localization were measured by using RT q-PCR and antibodies targeting the specific TJPs. Finally, the effect of IAP on inflammation-induced intestinal permeability was measured by in vitro trans-well epithelial electrical resistance (TEER).

RESULTS: Intestinal alkaline phosphatase gene deletion in MEFs resulted in significantly lower levels of ZO-1, ZO-2, and Occludin compared with levels in wild-type control cells; IAP over-expression in Caco-2 and T84 cells resulted in approximate 2-fold increases in the mRNA levels of ZO-1 and ZO-2. The IAP treatment ameliorated lipopolysaccharide-induced increased permeability in the Caco-2 trans-well system. Furthermore, IAP treatment preserved the localization of the ZO-1 and Occludin proteins during inflammation and was also associated with improved epithelial barrier function.

CONCLUSIONS: Intestinal alkaline phosphatase is a major regulator of gut mucosal permeability and appears to work at least partly through improving TJP levels and localization. These data provide a strong foundation to develop IAP as a novel therapy to maintain gut barrier function. (J Am Coll Surg 2016;222:1009−1017. © 2016 Published by Elsevier Inc. on behalf of the American College of Surgeons.)

Gut barrier function plays a pivotal role in human health and disease. "Leaky gut," or the disruption of intestinal integrity, results in the permeation of luminal mediators into the circulation, leading to harmful immune responses and inflammation in various organs.[1] An impaired gut mucosal barrier has been implicated to play a causal role in intestinal disease such as Crohn's and ulcerative colitis, as well as in a variety of other diseases ranging from metabolic syndrome to osteoarthritis, Alzheimer's, and aging.[2-7] In addition, improving gut barrier function has been shown to be beneficial in the recovery of patients suffering from severe trauma, burns, and other conditions associated with critical illness.[8] Intestinal permeability to macromolecules is controlled by paracellular tight junction (TJ) formation. Tight junctions are composed of transmembrane and cytosolic proteins, such as claudins, Occludins, and zona occludens.[9] Downregulation of tight junction protein (TJP) levels has been implicated in many diseases.[1] Furthermore, paracellular permeability is dynamically regulated by altering the TJP localization and TJ integrity.[10]

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Abbreviations and Acronyms

IAP = intestinal alkaline phosphatase
DMEM = Dulbecco's modified minimum essential medium
IL = interleukin
LPS = lipopolysaccharide
MEF = mouse embryonic fibroblast
RT-PCR = real-time polymerase chain reaction
TEER = transepithelial electrical resistance
TJ = tight junction
TJP = tight junction protein
TNF = tumor necrosis factor

Activation of inflammatory pathways such as NF-κB and the resultant increase in cytokine production results in disrupted TJP levels and localization, in turn increasing the passage of intestinal contents to the systemic circulation.[11,12] Guo and colleagues[13] showed the adverse effects of the gram-negative bacterial proinflammatory factor lipopolysaccharide (LPS), a potent activator of the NF-κB pathway, on the formation of TJs and gut barrier function.

The brush border enzyme intestinal alkaline phosphatase (IAP) is expressed and secreted exclusively in the small-intestinal epithelium.[14] The IAP functions as an anti-inflammatory factor, detoxifying a variety of proinflammatory mediators that exist within the gut lumen, including adenosine triphosphate and the toll-like receptor (TLR) ligands: LPS, flagellin, and CpG DNA.[15,16] We previously showed that IAP knockout mice have an impaired ability to detoxify luminal LPS and appear to be more susceptible to gut-derived inflammatory conditions.[17,18] Interestingly, IAP is down-regulated in settings in which gut barrier dysfunction plays a critical role in the development of diseases such as colitis.[19] Furthermore, IAP treatment has been shown to be beneficial in colitis in both humans and mice.[17,20] More recently, we showed that IAP levels are decreased in critically ill patients and that IAP supplementation improved the gut barrier function in a relevant mouse model.[21] Given the beneficial effects of IAP in regard to intestinal permeability, we speculated that this enzyme might represent a key regulator of TJP levels and TJ formation.

In this study, we show that the IAP deletion lowers intestinal junction protein levels in vitro, similar to our previous findings shown in mice. We demonstrate that IAP upregulates the expression of TJPs in various human colon cell lines. Finally, we show that IAP supplementation improves the barrier function in a Caco-2 transwell system, likely by preserving TJ formation and integrity.

METHODS

Reagents

Intestinal alkaline phosphatase, LPS (*E coli* serotype 055:B5), and Ripa buffer were purchased from Sigma-Aldrich, and TRIzol was purchased from Invitrogen. The iScriptReverse Transcription Supermix for RT-qPCR and iQ SYBR Green Supermix Kit were obtained from BIO-RAD, and the Coomassie (Bradford) Blue Protein Assay Kit was from Fisher Scientific. Goat anti-human ZO-1 and rabbit anti-human Occludin were purchased from Santa Cruz Biotechnology, and Alexa Flour secondary antibodies were obtained from Lifetechnology.

Cell culture

Primary mouse embryonic fibroblasts (MEFs) were isolated from day 13.5 wild-type C57BL/6 or Akp3 knock-out embryos, passaged at a density of 1×10^6 cells per 10-cm plate every 3 days (reference). The MEFs were maintained in Dulbecco's modified minimum essential medium (DMEM) supplemented with 55 μM β-mercaptoethanol. Human colon cancer Caco-2 and T84 cells were purchased from American Type Culture Collection and maintained in DMEM or in a 1:1 mixture of Ham's F12 medium and Dulbecco's modified Eagle's medium culture media, respectively. All culture media were supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, and 1% antibiotic-antimycotic solution obtained from GIBCO.

Plasmid constructs and transfection

Complementary DNA synthesized from RNA extracts from the human colon cancer Caco-2 cell line was used to amplify the human IAP gene by PCR using Q5 High-Fidelity DNA Polymerase (New England Biolabs). The following primers were used: forward, 5′-TTAAGCTT ATGCAGGGGCCCTGGGTGCTGCTG-3′; reverse, 5′-TTGGTACCGGGAGCAGCG GACGCCCCCAGCA -3′. The amplified product was digested with HindIII and KpnI, and then inserted into HindIII/KpnI-digested-pDsRed1-N1 vector (Clontech) upstream of the red fluorescent gene and named pDsRed1-IAP. The insert was confirmed using sequencing. The empty pDsRed1-N1 vector was used as a control. Plasmids were transfected into Caco-2 and T84 cells on a 12-well plate using 1 μg/well of pDsRed1-IAP or pDsRed1-Control using Lipofectamine LTX (Invitrogen). Stable transfectant clones expressing pDsRed1-IAP or pDsRed1-Control were selected with 1 mg/mL G418 (InvivoGen). Transfection efficiency was evaluated by real-time polymerase chain reaction (RT-PCR) analysis using the following primers: forward, 5′-GTTCCTGGTGTCCCCACTTC-3′; reverse, 5′GGCACCCCCAACCCATC-3′.