Breaking Down the Gut Barrier: Cytokine-Mediated Epithelial Dysfunction as a Hallmark of Inflammatory Bowel Disease Progression

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The intestine plays a vital role not only as the main organ for absorbing nutrients and water but also as a crucial barrier protecting the body from harmful and toxic substances, often introduced through daily dietary intake [1]. With a total surface area of about 400 m^2, the gastrointestinal (GI) tract is home to an estimated 10^{14} microorganisms. This microbial community outnumbers human cells by a factor of 10 and contains over 100 times the genetic material found in the human genome. Under normal circumstances, this microbiota offers significant benefits, including protecting against harmful pathogens and helping to regulate the immune system [2]. However, changes in the composition of gut bacteria, known as dysbiosis, can disrupt the balance of intestinal homeostasis. This disruption occurs through altered signaling pathways, ultimately affecting how the bacteria interact with the host [3]. Gut bacteria are crucial for breaking down indigestible carbohydrates to produce short-chain fatty acids (SCFAs), synthesizing essential vitamins like B12, K, and folate, and regulating lipid metabolism. These functions are vital for maintaining the integrity of the intestinal barrier. In addition to supporting metabolism, gut bacteria protect the intestinal lining by preventing the colonization of harmful pathogens and producing antimicrobial compounds, ensuring a stable gut environment [4]. Moreover, the gut microbiota actively supports the development and maturation of the immune system, which is essential for defending against infections and maintaining tolerance to harmless substances in the gut [5]. While the gut microbiota is generally beneficial, not all bacteria are harmless. For instance, Bacteroides fragilis, typically a well-behaved commensal, can invade intestinal tissues and cause significant problems in individuals with weakened immune systems.

Imbalances in the relationship between gut bacteria and the host can trigger excessive inflammation, potentially leading to conditions like inflammatory bowel disease (IBD), irritable bowel syndrome, and other gastrointestinal disorder [6]. IBD, which includes ulcerative colitis (UC) and Crohn's disease (CD), is characterized by chronic inflammation in parts of the GI tract. Evidence points to intestinal barrier dysfunction as a key factor in the development of IBD. For instance, some patients with IBD exhibit reduced mucus production or antimicrobial peptides, and mice with genetic deficiencies in mucosal barrier components show increased susceptibility to intestinal inflammation. Although the exact causes of IBD remain unclear, proposed mechanisms include mucosal barrier defects, dysbiosis, persistent infections, and immune dysregulation. The intestinal epithelium, a selectively permeable barrier, plays a dual role: protecting the body from pathogens while selectively allowing nutrient absorption. During inflammation, as seen in IBD, this barrier becomes compromised, further highlighting its importance in IBD pathogenesis [6]. Recent studies show that disruptions in the epithelial cell layer and the molecular circuits governing its renewal and repair can trigger IBD. Far from being a simple physical barrier, the intestinal epithelium is a dynamic tissue that responds to signals from both the gut microbiota and the immune system.

These responses are critical for maintaining the balance of the microbiota, regulating immune responses, and preserving gut health [7]. Ultimately, the intestinal epithelium acts as a bridge between the microbiota and the immune system. When signaling between epithelial cells and nearby immune cells goes awry, it can lead to immune dysregulation, which may contribute to the onset of IBD [7].

Research shows that intestinal permeability is influenced by multiple factors, including external stimuli, epithelial cell apoptosis, cytokines, and the immune system. Dysfunction of the intestinal barrier caused by immune responses plays a significant role in the onset and worsening of various autoimmune and inflammatory conditions, such as inflammatory bowel disease (IBD), food allergies, celiac disease, and diabetes [8]. Cytokines like interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) are key drivers of inflammation in IBD and have been shown to disrupt the intestinal epithelial barrier. Experiments using intestinal epithelial cells (Caco2 and T84) treated with these cytokines revealed changes in the organization of tight junction proteins, such as ZO-1, JAM-A, occludin, claudin-1, and claudin-4, which weakened the barrier function. This disruption is primarily driven by the activation of myosin light chain kinase (MLCK), which leads to the phosphorylation of myosin light chain (MLC). This process destabilizes the tight junctions (TJ), weakening the barrier. Encouragingly, inhibiting the phosphorylation of MLC caused by TNF-α and IFN-γ has been shown to restore barrier integrity. Additionally, these cytokines disrupt the balance of claudin and occludin proteins, further increasing intestinal permeability. Cytokines from the Th2 immune response, such as interleukin-4 (IL-4) and interleukin-13 (IL-13), also play a role in modulating the barrier. Studies on colonic epithelial cells (T84 and HT-29/B6) have shown that IL-4 and IL-13 can increase intestinal permeability. This effect is associated with epithelial cell apoptosis and the expression of claudin-2, a tight junction protein that forms permeability-enhancing pores. Further investigations revealed that the effects of IL-4 and IL-13 on the intestinal barrier are mediated by phosphoinositide 3-kinase (PI3K). Blocking PI3K activity has been shown to prevent IL-4/IL-13-induced barrier dysfunction, even without activating STAT-6, a downstream signaling molecule. These changes not only compromise the barrier but also interfere with glucose absorption. The role of zonulin, a protein involved in tight junction regulation, has also been highlighted in animal studies. Blocking the zonulin receptor in IL-10-deficient mice reduced intestinal permeability, lowered TNF-α secretion, and prevented further inflammation, emphasizing the importance of controlling intestinal permeability in managing inflammatory conditions [9]. The zonulin/receptor pathway is crucial for regulating tight junction formation through actin reorganization mediated by protein kinase C (PKC). In IL-10-deficient mice, defects in this pathway lead to increased expression of inflammatory cytokines like IFN-γ and TNF-α, further compromising the intestinal barrier.

The intestinal barrier plays a crucial role in maintaining gut homeostasis, and any damage or dysfunction to this barrier is closely associated with both local and systemic inflammation. This is primarily due to the direct contact of bacteria or their products with the epithelial cells, which leads to their translocation into the bloodstream [10]. When bacteria or bacterial products come into contact with the intestinal epithelial cells, they activate immune cells through signaling pathways that depend on TLR4/MyD88 in the lamina propria. This activation, which involves the interaction of lipopolysaccharide (LPS) from gut bacteria,

triggers the secretion of pro-inflammatory mediators that prolong local inflammation. Interestingly, research has shown that the administration of an LPS-TLR4 signaling inhibitor like TAK-242 can attenuate this inflammatory signaling pathway. Local intestinal inflammation is known to contribute to the development of various gastrointestinal diseases, including inflammatory bowel disease (IBD), Crohn's disease, and ulcerative colitis. The major consequence of a compromised intestinal barrier is the increased transport of LPS through the paracellular route into the systemic circulation. Once in the bloodstream, LPS binds to lipopolysaccharide-binding protein (LBP) or lipoproteins and interacts with receptors like TLR4 on immune cells, which in turn triggers the inflammatory response. However, TLR4 alone cannot bind LPS without the help of CD14, which acts as a cofactor. CD14 facilitates the transfer of LPS to TLR4 and MD2, allowing for proper LPS recognition [11]. The binding of these molecules leads to a cascade of signals that result in the homodimerization of TLR4 and activation of MyD88-mediated intracellular pathways. This signaling cascade activates NF-κB, which promotes the transcription of pro-inflammatory cytokines, such as IL-1, TNF-α, and IL-6. Activated macrophages, or macrophages in peripheral tissues triggered by circulating LPS, contribute to tissue inflammation [12]. The further infiltration of immune cells such as neutrophils and monocytes into the affected tissues extends the inflammation, which disrupts the normal tissue homeostasis. For example, in the liver, increased inflammation results in insulin resistance and lipogenesis, which can lead to fatty liver disease. Similarly, an increase in adipose tissue inflammation or skeletal muscle insulin resistance plays a key role in the development of diabetes.

In conclusion, the intestinal barrier plays a crucial role in maintaining the balance and health of the gut. When this barrier is damaged or disrupted, it can trigger both local and systemic inflammation, often due to the direct contact between bacteria or bacterial products and the epithelial cells. This process eventually activates NF-κB, leading to the increased production of pro-inflammatory cytokines like TNF-α, IL-6, and IL-1. The interaction between the intestinal epithelium, microbiota, and the immune system plays a vital role in regulating gut permeability. Understanding these complex interactions is key to unlocking insights into the development of inflammatory bowel disease (IBD) and could pave the way for new therapeutic strategies.

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