Review Article

Research Advance in Intestinal Mucosal Barrier and Pathogenesis of Crohn’s Disease

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To date, the etiology and pathogenesis of Crohn’s disease (CD) have not been fully elucidated. It is widely accepted that genetic, immune, and environment factors are closely related to the development of CD. As an important defensive line for human body against the environment, intestinal mucosa is able to protect the homeostasis of gut bacteria and alleviate the intestinal inflammatory and immune response. It is evident that the dysfunction of intestinal mucosa barriers plays a crucial role in CD initiation and development. Yet researches are insufficient on intestinal mucosal barrier’s action in the prevention of CD onset. This article summarizes the research advances about the correlations between the disorders of intestinal mucosal barriers and CD.

1. Introduction

CD and ulcerative colitis (UC) are inflammatory bowel disease (IBD). As a chronic, nonspecific, and granulomatous bowel disease, CD often occurs in the whole layer of intestinal wall, and, mostly, its lesions are segmentally and asymmetrically distributed. It may appear in any part of the gastrointestinal tract, especially in terminal ileum and adjacent colon [1]. CD has a long course as well as poor prognosis. Moreover, it occurs refractorily and repeatedly. According to the epidemiological investigation [2–7], the incidence of CD is higher in some developed countries in Europe and the United States and is increasing in Asia areas (especially in China). Nowadays, the etiology and pathogenesis of CD have not yet been fully recognized. Various genetic, immunologic, and environmental factors have been proved to be associated with the occurrence and development of CD, among which the immunologic factor is considered to be one of the most important factors [8–11]. The intestinal mucosal barrier dysfunction caused by immune abnormalities and infection is critical in the pathogenesis of CD. In this article, we mainly summarized the research advances about the correlations between the disorders of intestinal mucosal barriers and CD, including mechanical, chemical, immune, and biological barriers.  

2. The Structure and Function of Intestinal Mucosal Barrier

Intestinal mucosal barrier is composed of mechanical barrier, chemical barrier, immune barrier and biological barrier, constituting a defensive barrier between the human body and the surrounding environment. The mechanical barrier mainly consists of intestinal epithelial cells and epithelial tight junctions. Tight junction (TJ) is the main connection form between intestinal mucosal epithelial cells, and it also plays an important role in maintaining the integrity of structure and normal function of intestinal mucosal barrier. Chemical barrier is made up of many chemicals such as digestive acid secreted by gastrointestinal, digestive enzymes, lysozyme, mucopolysaccharides, glycoproteins, and glycolipids. Therefore, it is involved in the process of bacteriolysis to inhibit the invasion of pathogenic bacteria. Gut-associated lymphoid tissue (GALT) and secretory immunoglobulin A (SIgA) as well as some special cells (such as macrophages, natural killer cells, and intraepithelial lymphocytes) constitute the immune barrier, which is an important guarantee for the intestinal immunity homeostasis via identifying the autoantigens and exogenous antigens to regulate the immune response. Actually, biological barrier is a mutually dependent and interrelated microecosystem. It is mainly composed of
the resident intestinal flora, among which obligate anaerobe is the dominant bacterial community. Intestinal mucosal barrier is a barrier constituted between the organism and the surrounding environment. Those four barriers have distinguished structures and regulatory mechanism and each plays a different role in biological function. Intestinal mucosal barrier can effectively maintain the balance between pro- and anti-inflammatory factors and prevent pathogenic microorganism from entering into the tissues to keep the body healthy [12–15]. An important component of intestinal homeostasis and inflammation is the integrity of the intestinal barrier and the dysfunction of intestinal mucosal barrier is key to the occurrence of CD; therefore, maintaining the integrity of the intestinal mucosal barrier is of great significance in clinical CD prevention and treatment.

3. CD and Mechanical Barrier

The intestinal epithelial tight junction (TJ) is an important part of the intestinal mechanical barrier, and it is indeed the most essential structure to maintain the function of mechanical barrier. TJ is mainly composed of occludin, claudin, junction adhesion molecules (JAMs), and ZOs [16–18], among which claudin is the main frame protein, as the transmembrane protein in the claudin protein family, claudin-1, always plays a significant role in maintaining the integrity of intestinal epithelial TJ and the normal function of intestinal mechanical barrier [19, 20]. TJ possesses many protein complexes which are able to regulate the paracellular permeability. The intestine infection may be followed by TJ impairment, leading to intestinal epithelial permeability increase and intestinal mucosal barrier damage. This has been recognized as the key process to initiate the intestinal inflammation as well as the immune reaction. IFN-gamma can affect the expression of claudin-2 and occludin proteins through different mechanisms, like inducing the apoptosis of intestinal epithelial cells and destroying the integrity of intestinal epithelial TJ, eventually leading to IBD [21–23]. The aberrant increase of TNF-alpha level in the colonic mucosa of CD significantly reduced expression of occluding, claudin-1, and ZO-1 protein and mRNA and finally resulted in the structure impairment and TJ dysfunction. A new study [24] has also suggested that the inhibition of p38MAPK/p53 signaling pathway can increase the expression of TJ proteins (ZO1, protein-1, and occludin) and alleviate injury to the intestinal mucosal barrier.

4. CD and Chemical Barrier

The mucus secreted by gastrointestinal tract together with various other substances forms the intestinal mucosal chemical barrier, which is the key component of the body’s natural immune system. Among all these substances, mucus is the most effective one in protecting the surface of intestinal mucosa. Intestinal mucous layer consisting of goblet cells and mucin (MUC) secreted by intestinal epithelial cells is the first defensive line to resist against extraneous pathogen through protecting and lubricating intestine. The intestinal mucous layer can be divided into external mucous layer which provided a suitable symbiotic environment for the gut microbiota and the internal mucous layer which protected the integrity of intestinal mucosal barrier by preventing microorganism from invading intestinal epithelium. Normally, only when the body is in a disease state caused by some abnormal factors could the bacteria penetrate the internal mucous layer and destroy the intestinal epithelium subsequently. MUC is not only the main component of the intestinal mucous layer but also the most important functional unit in mucus [25]. The mucin in the colorectum can be mainly divided into MUC1, MUC2, MUC3A, MUC3B, MUC4, MUC13, and MUC17, among which MUC2 is the most important one [26, 27]. It has been proven that the allelic polymorphism of MUC1 and MUC2 is closely associated with CD. Moreover, a large number of inflammatory cytokines (such as IL-4, IL-6, IL-13, TNF-alpha, and IFN-gamma) can promote the secretion of MUC in epithelial cells cultured in vitro [28, 29]. Studies [30, 31] have shown that MUC2 has direct antibacterial effect by forming the antiprotease substrates to defend the bacterial invasion. In Th1 and Th2 colitis rats model, MUC1 could regulate Th17 immune response and inhibit inflammatory response as Th17 cytokines stimulated MUC1 generation whose negative feedback regulated Th17 generation, so as to downregulate T17 mediated immune response, finally inhibiting the inflammatory reaction [32].

5. CD and Immune Barrier

The immunological factor has been considered to be the key factor in the occurrence and development of CD. Intestinal mucosal immune barrier is essential for maintaining intestinal immune homeostasis. GALT is made up of lymphoid nodule, free lymphoid tissue, plasma cells, and the intestine-related tissue composed of lymphocyte in the epithelium. GALT is an important immune organ to maintain the integrity of intestinal mucosal barrier. SlgA secreted immune globulin with diverse functions and is a main antibody that plays an important role in effects of anti-infection and immunomodulation in defense system of mucosa. A related study [33] found that the level of SlgA expression in patients with CD decreased obviously compared to the normal controls, and its level was negatively correlated with the severity of CD. It can be concluded that the intestinal mucosal immune system will lose the immune tolerance ability when the pathogenic bacteria and its antigen intrude into body; then the pathogen invades the intestinal epithelium and destroys the intestinal mucosal barrier. Paneth cells (PC), which are the typical cells of small intestine, are vitally important components of intestinal mucosal barrier and the main effector cells of small intestinal mucosal barrier. PC contain a variety of antibacterial material such as defensins, lysozyme, and SlgA [34–36], in which both defensins and lysozyme have the spectrum antimicrobial activity and can promote the innate immune response by killing the bacteria and keeping the steady state of intestinal flora [37]. Antibacterial peptide is alkaline peptide and maintains the balance of intestinal flora and the integrity of intestinal mucosal...
barrier via interacting with the bacteria in mucosal surface to keep endothelial cells away from being invaded [38–40]. Lysozyme can hydrolyze the peptidoglycan in pathogenic bacteria and change the osmotic pressure between intra-cellular and extracellular states. Recent researches [41, 42] indicate the therapeutic potential of lysozyme on various systemic inflammatory diseases. The functional lysozyme can also be used as a tracking reagent for microbial population in antibacterial tests. Besides, the nucleotide-binding oligomerization domain 2 (NOD2) expressed in PC could identify the bacterial peptidoglycan and kill the pathogens through the generation of antimicrobial peptide and induction of bacteria autophagy in the cell as well as the modulation of immunity [43, 44]. Researches [45, 46] have shown that the NOD2 gene mutation in CD may increase the susceptibility of the disease through influencing the interaction between ileal microbes and intestinal mucosal immunity. T cell immunoglobulin and mucin domain-3 (TIM-3), the newly discovered T cell immunoglobulin and mucin domain, is expressed specifically and merely on surface of the mature and active T cells. TIM-3 may be involved in the process of regulating T cells proliferation and activation and inhibiting the immune response mediated by Th1 cells [47–49]. TIM-3 plays an important role in chronic inflammatory and autoimmune diseases in humans [50, 51] and is a possible candidate for the treatment of disease in clinic. Simultaneously, TIM-3 also plays a critical role in regulating the activities of macrophages, dendritic cells, monocytes, natural killer cells, mast cells, and endothelial cells. The level of TIM-3 expression in Th1 cells of the intestinal mucosa in CD patients increased more obviously than in healthy persons, as decreasing the expression level of TIM-3 in Th1 cells may provide a new cure for a number of chronic inflammatory diseases in clinical practice [49]. Furthermore, regulating the levels of Th17 and Treg cells in intestinal mucosa could alleviate the intestinal inflammatory response and improve the integrity of intestinal epithelium mucosal barrier via increasing the expression of T1 proteins and mRNA and inhibiting the apoptosis of intestinal epithelial cells [52–55]. The severity of colitis is closely related to the level of IL-18 in intestinal epithelial cells, and, as a microbial modulator, the NOD-like receptor protein 6 (NLRP6) inflammasome can drive the microbial community stability [56–58]. Both IL-18 and NLRP6 inflammasome have key roles in maintaining homeostasis and intestinal barrier function.

6. CD and Biological Barrier

The biological barrier is constituted by normal flora and deposited in intestinal mucosa to maintain the integrity of the intestinal mucosal barrier. Normally, the microecological environment in intestine maintains homeostasis through the interdependence and mutual restrictions between probiotics and pathogenic bacteria. IBD is accompanied with alteration of intestinal flora, which could induce intestinal infection when body is affected by abnormal factors [59–61]. Both the *Bifidobacterium* and *Lactobacillus* are the probiotics. On one hand, they could restrict the pathogenic bacteria; on the other hand, they could repair the damaged mucosal barrier by adjusting the level of inflammatory cytokines. A study had demonstrated that lactic acid bacteria could decrease the levels of IL-6, TNF-alpha, toll-like receptor 4 (TLR4), and NF- kappaB mRNA and increase the level of IL-10 mRNA observably at the same time [62]. In the feces of patients with CD, the amount of *bacteroid, Bacillus*, and *Streptococcus* were increased, while the amount of *Bifidobacterium* was decreased [63]. Prebiotics would protect the integrity of intestinal epithelium barrier by promoting the expression of ZO-1 and occludin protein [64, 65]. Studies [66–69] have shown that the normal gut microbiota could prevent bacteria from contacting with the intestinal epithelium, and probiotics could balance the intestinal flora in experimental colitis model of rats through regulating the intestinal mucosal barrier and the levels of related immune cells. Therefore, probiotics may repair the damaged mucosa and maintain the integrity of intestinal mucosal barrier.

7. Conclusion

The mechanical, chemical, immune, and biological barriers play important role in protecting the gut against bacteria homeostasis, regulating the intestinal immune response and reducing the inflammatory response. Yet the comprehensive and systematic researches are insufficient on intestinal mucosal barrier's action in the prevention of CD onset. Therefore, it is of great significance to conduct more thorough studies and randomized controlled trials with largescale, multicentre, and high-quality. In addition, interventions by which to maintain the structural integrity and proper function of intestinal mucosal barrier are expected to be a rational and reliable approach in the prevention of CD in the future.

Competing Interests

There is no potential conflict of interests.

Authors’ Contributions

Kuan Wang, Lu-yi Wu, Chuan-zi Dou, and Xin Guan contributed equally to this manuscript with the literature retrieval, sorting, and analysis; Kuan Wang wrote the manuscript; Huan-gan Wu and Hui-rong Liu conducted and revised the manuscript.

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References

[1] Chinese Society of Gastroenterology IBD Working Group, "Consensus on diagnosis and management of inflammatory bowel disease," Chinese Journal of Digestion, vol. 32, no. 12, pp. 796–813, 2012.

[2] K. T. Ihia, E. V. Loftus, W. J. Sandborn, and S.-K. Yang, "An update on the epidemiology of inflammatory bowel disease in Asia," American Journal of Gastroenterology, vol. 103, no. 12, pp. 3671–3828, 2008.

[3] J. J. Zheng, X. S. Zhu, Z. Huangfu, Z. X. Gao, Z. R. Guo, and Z. Wang, "Crohn's disease in mainland China: a systematic analysis of 50 years of research," Chinese Journal of Digestive Diseases, vol. 6, no. 4, pp. 175–181, 2005.

[4] H. Zhang and Q. Ouyang, "Retrospective analysis of 515 cases of Crohn's disease hospitalization in China: nationwide study from 1990 to 2003," Journal of Gastroenterology and Hepatology, vol. 21, no. 6, pp. 1099–1015, 2006.

[5] J. J. Zheng, X. S. Zhu, Z. Huangfu, X. H. Shi, and Z. R. Guo, "Prevalence and incidence rates of Crohn's disease in mainland China: a meta-analysis of 55 years of research," Journal of Digestive Diseases, vol. 11, no. 3, pp. 161–166, 2010.

[6] Y. F. Wang, Q. Ouyang, and R. W. Hu, "Progression of inflammatory bowel disease in China," Journal of Digestive Diseases, vol. 11, no. 2, pp. 76–82, 2010.

[7] S.-K. Yang, S. Yun, J.-H. Kim et al., "Epidemiology of inflammatory bowel disease in the Songpa-Kangdong district, Seoul, Korea, 1986–2005: a KASID study," Inflammatory Bowel Diseases, vol. 14, no. 4, pp. 542–549, 2008.

[8] R. J. Chiodini, S. E. Dowd, S. Galandiuk, B. Davis, and A. Glassing, "The predominant site of bacterial translocation across the intestinal mucus barrier occurs at the advancing disease margin in Crohn's disease," Microbiology, 2016.

[9] R. J. Xavier and D. K. Podolsky, "Unraveling the pathogenesis of inflammatory bowel disease," Nature, vol. 448, no. 7152, pp. 427–434, 2007.

[10] G. Bamias and F. Cominelli, "Immunopathogenesis of inflammatory bowel disease: current concepts," Current Opinion in Gastroenterology, vol. 23, no. 4, pp. 365–369, 2007.

[11] E. Betelli, Y. Carrier, W. Gao et al., "Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells," Nature, vol. 441, no. 7090, pp. 235–238, 2006.

[12] A. c. Luissitt, C. A. Parkos, and A. Nusrat, "Inflammation and the intestinal barrier: leukocyte-epithelial cell interactions, cell junction remodeling, and mucus repair," Gastroenterology, 2016.

[13] M. C. Arrieta, L. Bistritz, and J. B. Meddings, "Alterations in intestinal permeability," Gut, vol. 55, no. 10, pp. 1512–1520, 2006.

[14] A. Farhadi, A. Banan, J. Fields, and A. Keshavarzian, "Intestinal barrier: an interface between health and disease," Journal of Gastroenterology and Hepatology, vol. 18, no. 5, pp. 479–497, 2003.

[15] L. Antoni, S. Nuding, J. Wehkamp, and E. F. Stange, "Intestinal barrier in inflammatory bowel disease," World Journal of Gastroenterology, vol. 20, no. 5, pp. 1165–1179, 2014.

[16] M. G. Lauketter, P. Nava, and A. Nusrat, "Role of the intestinal barrier in inflammatory bowel disease," World Journal of Gastroenterology, vol. 14, no. 3, pp. 401–407, 2008.

[17] M. Utech, M. Bruwer, and A. Nusrat, " Tight junctions and cell-cell interactions," Methods in Molecular Biology, vol. 341, pp. 185–195, 2006.

[18] A. Nusrat, C. A. Parkos, P. Verkade et al., "Tight junctions are membrane microdomains," Journal of Cell Science, vol. 113, no. 10, pp. 1771–1781, 2000.

[19] X. Guo, J. N. Rao, L. Liu et al., "Polyamines are necessary for synthesis and stability of occludin protein in intestinal epithelial cells," American Journal of Physiology—Gastrointestinal and Liver Physiology, vol. 288, no. 6, pp. G1159–G1169, 2005.

[20] A. M. Hopkins, S. V. Walsh, P. Verkade, P. Boquet, and A. Nusrat, "Constitutive activation of Rho proteins by CNF-1 influences tight junction structure and epithelial barrier function," Journal of Cell Science, vol. 116, part 4, pp. 725–742, 2003.

[21] C. Xu, X. Li, B. Qin, and B. Liu, "Effect of tight junction protein of intestinal epithelium and permeability of colonic mucosa in pathogenesis of injured colonic barrier during chronic recovery stage of rats with inflammatory bowel disease," Asian Pacific Journal of Tropical Medicine, vol. 9, no. 2, pp. 148–152, 2016.

[22] L. E. M. Willemsen, J. P. Hoetjes, S. J. H. van Deventer, and E. A. F. van Tol, "Abrogation of IFN-γ mediated epithelial barrier disruption by serine protease inhibition," Clinical and Experimental Immunology, vol. 142, no. 2, pp. 275–284, 2005.

[23] D. Schuhmann, P. Godoy, C. Weiss et al., "Interfering with interferon-γ signalling in intestinal epithelial cells: selective inhibition of apoptosis-maintained secretion of anti-inflammatory interleukin-18 binding protein," Clinical and Experimental Immunology, vol. 163, no. 1, pp. 65–76, 2011.

[24] J. Ouyang, Z. H. Zhang, Y. X. Zhou et al., "Up-regulation of tight-junction proteins by p38 mitogen-activated protein kinase/p53 inhibition leads to a reduction of injury to the intestinal mucosal barrier in severe acute pancreatitis," Pancreas, vol. 45, no. 8, pp. 1136–1144, 2016.

[25] J. P. Pearson and I. A. Brownlee, "The interaction of large bowel microflora with the colonic mucus barrier," International Journal of Inflammation, vol. 2010, Article ID 321426, 9 pages, 2010.

[26] M.-H. Zhao, C. Gong, J.-M. Lou, and B.-S. Feng, "Relationship between mucins and inflammatory bowel disease," Shi jie Huaren Xiaohua Zazhi, vol. 22, no. 27, pp. 4100–4106, 2014.

[27] A. Swidsinski, B. C. Sydora, Y. Doerfler et al., "Viscosity gradient within the mucus layer determines the mucosal barrier function and the spatial organization of the intestinal microbiota," Inflammatory Bowel Diseases, vol. 13, no. 8, pp. 963–970, 2007.

[28] M. G. Smirnova, L. Guo, J. P. Birchall, and J. P. Pearson, "LPS up-regulates mucin and cytokine mRNA expression and stimulates mucin and cytokine secretion in goblet cells," Cellular Immunology, vol. 221, no. 1, pp. 42–49, 2003.

[29] A. Sabbah, T. H. Chang, R. Harnack et al., "Activation of innate immune antiviral responses by Nod2," Nature Immunology, vol. 10, no. 10, pp. 1073–1080, 2009.

[30] M. E. Lidell, D. M. Moncada, K. Chadee, and G. C. Hansson, "Entamoeba histolytica cysteine protease cleave the MUC2 mucin in its C-terminal domain and dissolve the protective colonic mucus gel," Proceedings of the National Academy of Sciences of the United States of America, vol. 103, no. 24, pp. 9298–9303, 2006.

[31] C. Caballero-Franco, K. Keller, C. De Simone, and K. Chadee, "The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells," 9298–9303, 2006.
A. Negroni, L. Stronati, M. Pierdomenico et al., “Signature biomarkers in Crohn’s disease: toward a molecular classification,” *Mucosal Immunology*, vol. 1, no. 5, pp. 399–411, 2008.

Z. Zhang and Z. Liu, “Paneth cells: the hub for sensing and regulating intestinal flora,” *Science China Life Sciences*, vol. 59, no. 5, pp. 463–467, 2016.

D. A. Elphick and Y. R. Mahida, “Paneth cells: their role in innate immunity and inflammatory disease,” *Gut*, vol. 54, no. 12, pp. 1802–1809, 2005.

Q.-J. Tang, L.-M. Wang, K.-Z. Tao et al., “Expression of polymeric immunoglobulin receptor mRNA and protein in human paneth cells: paneth cells participate in acquired immunity,” *American Journal of Gastroenterology*, vol. 101, no. 7, pp. 1625–1632, 2006.

Y.-R. Yang, Z.-J. Liu, and H.-D. Liang, “Role of defensins in the pathogenesis of inflammatory bowel disease,” *World Chinese Journal of Digestology*, vol. 18, no. 29, pp. 3010–3016, 2010.

J.-M. Yuk, D.-M. Shin, H.-M. Lee et al., “Vitamin D3 induces autophagy in human monocytes/macrophages via calcitriol,” *Cell Host & Microbe*, vol. 6, no. 3, pp. 231–243, 2009.

A. Dupont, Y. Kaconis, I. Yang et al., “Intestinal mucus affinity and biological activity of an orally administered antibacterial and anti-inflammatory peptide,” *Gut*, vol. 64, no. 2, pp. 222–232, 2015.

H. W. Koon, D. Q. Shih, J. Chen et al., “Cathelicidin signaling via the toll-like receptor protects against colitis in mice,” *Gastroenterology*, vol. 141, no. 5, pp. 1852–1863, 2011.

L. Zheng, Y. Wan, L. Yu, and D. Zhang, “Lysozyme as a recognition element for monitoring of bacterial population,” *Talanta*, vol. 146, pp. 299–302, 2016.

J. Chung, S.-K. Ku, S. Lee, and J.-S. Bae, “Suppressive effects of lysozyme on polyphosphate-mediated vascular inflammatory responses,” *Biochemical and Biophysical Research Communications*, vol. 474, no. 4, pp. 715–721, 2016.

C. R. Homer, A. L. Richmond, N. A. Rebert, J. p. Achkar, and C. McDonald, “ATG16L1 and NOD2 interact in an autophagy-dependent antibacterial pathway implicated in Crohn’s disease pathogenesis,” *Gastroenterology*, vol. 139, no. 5, pp. 1630.e2–1641.e2, 2010.

A. Negroni, L. Stronati, M. Pierdomenico et al., “Activation of NOD2-mediated intestinal pathway in a pediatric population with Crohn’s disease,” *Inflammatory Bowel Diseases*, vol. 15, no. 8, pp. 1145–1154, 2009.

A. Biswas, T. Petnicki-Ocwieja, and K. S. Kobayashi, “Nod2: a key regulator linking microbiota to intestinal mucosal immunity,” *Journal of Molecular Medicine*, vol. 90, no. 1, pp. 15–24, 2012.

T. Petnicki-Ocwieja, T. Hrcir, Y.-J. Liu et al., “Nod2 is required for the regulation of commensal microbiota in the intestine,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 37, pp. 15813–15818, 2009.

C. Zhu, A. C. Anderson, A. Schubart et al., “The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity,” *Nature Immunology*, vol. 6, no. 12, pp. 1245–1252, 2005.

L. Monney, C. A. Sabatos, J. L. Gaglia et al., “Thi-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease,” *Nature*, vol. 415, no. 6871, pp. 536–541, 2002.

K. Morimoto, S. Hosomi, H. Yamagami et al., “Dysregulated up-regulation of T-cell immunoglobulin and mucin domain-3 on mucosal T helper 1 cells in patients with Crohn’s disease,” *Scandinavian Journal of Gastroenterology*, vol. 46, no. 6, pp. 701–709, 2011.

S. Han, G. Chen, B. Shen, and Y. Li, “Tim-3: an activation marker and activation limiter of innate immune cells,” *Frontiers in Immunology*, vol. 4, article 449, 2013.

C. Zhu, A. C. Anderson, and V. K. Kuchroo, “TIM-3 and its regulatory role in immune responses,” *Current Topics in Microbiology and Immunology*, vol. 350, pp. 1–15, 2011.

C. Zhao, C. Bao, J. Li et al., “Moxibustion and acupuncture ameliorate Crohn’s disease by regulating the balance between Th1 and Treg cells in the intestinal mucosa,” *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 938054, 11 pages, 2015.

H.-X. Shang, A.-Q. Wang, C.-H. Bao et al., “Moxibustion combined with acupuncture increases tight junction protein expression in Crohn’s disease patients,” *World Journal of Gastroenterology*, vol. 21, no. 16, pp. 4986–4996, 2015.

K. Wei, D. Zhang, C. Z. Dou et al., “Study on the regulating effect of moxibustion on NF-κB p65, TNF-α, and IL-1β in Colon of CD Rats,” *World Chinese Medicine*, 8, pp. 862–866, 2013.

C.-H. Bao, L.-Y. Wu, H.-G. Wu et al., “Moxibustion inhibits apoptosis and tumor necrosis factor-alpha/tumor necrosis factor receptor 1 in the colonic epithelium of crohn’s disease model rats,” *Digestive Diseases and Sciences*, vol. 57, no. 9, pp. 2286–2295, 2012.

R. Nowarski, R. Jackson, N. Gagliani et al., “Epithelial IL-18 equilibrium controls barrier function in colitis,” *Cell*, vol. 163, no. 6, pp. 1444–1456, 2015.

M. Levy, C. A. Thaiss, D. Zeevi et al., “Microbiota-modulated metabolites shape the intestinal microenvironment by regulating NLRP6 inflammasome signaling,” *Cell*, vol. 163, no. 6, pp. 1428–1443, 2015.

K. Ray, “Inflammation: maintaining the mucosal barrier in intestinal inflammation,” *Nature Reviews Gastroenterology and Hepatology*, vol. 13, no. 1, p. 5, 2015.

N. Kaur, C.-C. Chen, J. Luther, and J. Y. Kao, “Intestinal dysbiosis in inflammatory bowel disease,” *Gut Microbes*, vol. 2, no. 4, pp. 211–216, 2011.

C. P. Tamboli, C. Neut, P. Desreumaux, and J. F. Colombel, “Dysbiosis in inflammatory bowel disease,” *Gut*, vol. 53, no. 1, pp. 1–4, 2004.

J. L. Round and S. K. Mazmanian, “The gut microbiota shapes intestinal immune responses during health and disease,” *Nature Reviews Immunology*, vol. 9, no. 5, pp. 313–323, 2009.

Y. Liu, N. Y. Fatherree, N. Mangalat, and J. M. Rhoads, “Lactobacillus reuteri strains reduce incidence and severity of experimental necrotizing enterocolitis via modulation of TLR4 and NF-κB signaling in the intestine,” *American Journal of Physiology-Gastrointestinal and Liver Physiology*, vol. 302, no. 6, pp. G6608–G6617, 2012.

R. K. Lisnenski, X. W. Huijsdens, P. H. M. Savelkoul, C. M. J. E. Vandenbroucke-Grauls, and S. G. M. Meuwissen, “The bacterial flora in inflammatory bowel disease: current insights in pathogenesis and the influence of antibiotics and probiotics,” *Scandinavian Journal of Gastroenterology*, Supplement, vol. 36, no. 234, pp. 29–40, 2001.

P. D. Cani, R. Bibiloni, C. Knauf et al., “Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice,” *Diabetes*, vol. 57, no. 6, pp. 1470–1481, 2008.
[65] L. Fuccio and A. Guido, “Probiotics supplementation for the prevention of gastrointestinal radiation-induced side effects: the time is now,” *The American Journal of Gastroenterology*, vol. 108, no. 2, p. 277, 2013.

[66] R. Toumi, K. Abdelouhab, H. Rafa et al., “Beneficial role of the probiotic mixture Ultrabiotique on maintaining the integrity of intestinal mucosal barrier in DSS-induced experimental colitis,” *Immunopharmacology and Immunotoxicology*, vol. 35, no. 3, pp. 403–409, 2013.

[67] I. Koboziev, W. C. Reinoso, K. L. Furr, and M. B. Grisham, “Role of the enteric microbiota in intestinal homeostasis and inflammation,” *Free Radical Biology and Medicine*, vol. 68, pp. 122–133, 2014.

[68] H.-M. Zhao, X.-Y. Huang, Z.-Q. Zuo et al., “Probiotics increase T regulatory cells and reduce severity of experimental colitis in mice,” *World Journal of Gastroenterology*, vol. 19, no. 5, pp. 742–749, 2013.

[69] H.-K. Kwon, C.-G. Lee, J.-S. So et al., “Generation of regulatory dendritic cells and CD4+Foxp3 + T cells by probiotics administration suppresses immune disorders,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 5, pp. 2159–2164, 2010.