Progress in the Pathogenesis of Inflammatory Bowel Disease

short title: Pathogenesis of IBD

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ABSTRACT

Inflammatory bowel disease (IBD) is a group of chronic, non-specific, recurrent inflammatory diseases, and also has a cancerous tendency. IBD mainly includes ulcerative colitis (UC) and Crohn's disease (CD), and the incidence of IBD is increasing year by year. Although IBD is believed to be a result of an imbalanced interaction among genetic susceptibility, environmental factors, intestinal microflora, and the immune system, its etiology and pathogenesis have not been fully clarified. Moreover, IBD is closely associated with the initiation and progression of colorectal cancer (CRC). Therefore, the early detection and prevention of IBD are important to prevent carcinogenesis. This review discusses advances in the pathogenesis of IBD, to provide new ideas for the treatment and further development of the new drugs of IBD.

Keywords: Inflammatory bowel disease, Pathogenesis, Immunity, Environment, Intestinal flora, Genetics

Inflammatory bowel disease (IBD) is a chronic inflammatory disease mainly affecting the gastrointestinal tract. In the past 20 years, in-depth research has been conducted on the mechanism of occurrence, diagnosis, and treatment of IBD. At present, it is believed that the etiology of IBD is complex, it involves interactions between genetic, environmental, microbial factors, and immune responses（Figure 1）. However, the specific etiology and exact mechanism of IBD are still unclear, and there is no effective therapy available that can completely cure IBD.[1] In recent years, the incidence of IBD has increased year by year, not only in Europe, the United States, and Western countries, but also in newly industrialized countries in South America, Asia, Africa, and Eastern Europe.[2] The incidence of IBD in China has been rapidly increasing, which may be related to environmental changes, dietary changes, and accelerated life rhythms. IBD is a lifelong disease and mostly occurs between adolescence and adulthood. It can be life-threatening, disabling, chronic, recurrent and progressive, which poses a significant burden on patients because of detrimental effects on quality of life, growth, and development. IBD is a chronic immune-mediated disease that severely affects the digestive tract and may lead, in the long term, to the irreversible deterioration of their structure and function. At present, IBD has become one of the most common diseases of the digestive system. Therefore, the basic research, clinical diagnosis, and development of novel treatment of IBD is one of the current research hotspots.

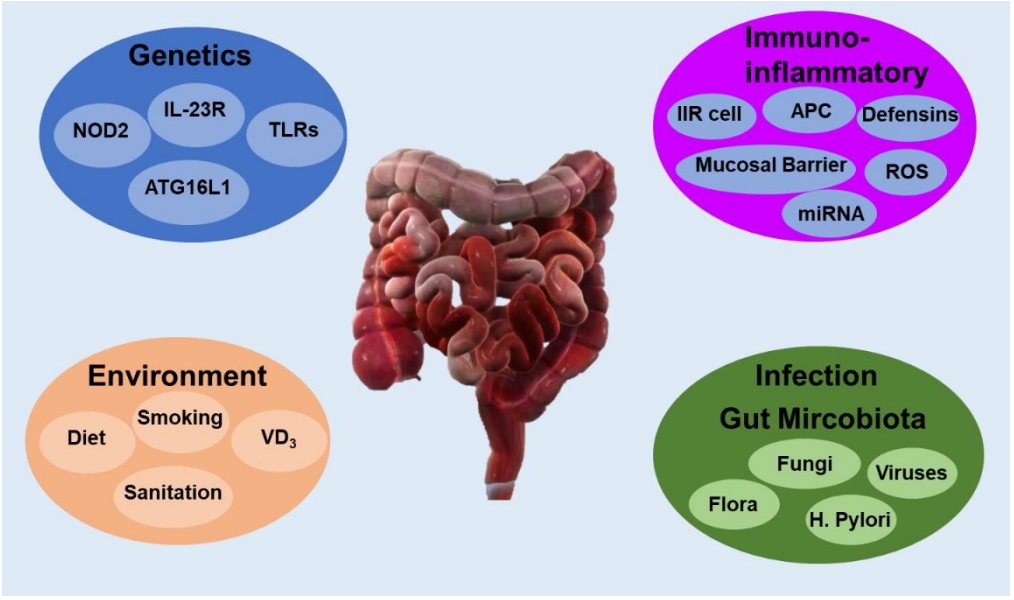

Figure1 Pathogenesis of Inflammatory Bowel Disease

GENETIC FACTORS (HOST SUSCEPTIBILITY)

Multiple lines of evidence suggest that IBD is caused by genetic and environmental factors that alter intestinal homeostasis in genetically susceptible individuals. The high comorbidity rate among identical twins, family aggregation, and the racial and regional differences all indicate that genetic factors play an important role in the pathogenesis of IBD. Descriptive epidemiologic studies have investigated the prevalence of familial clustering of IBD, the relative risk for developing IBD is estimated to be 5 times higher for first-degree relatives of an IBD patient than in the general population.[3] A study in South Korea showed that the concordance rate in monozygotic twins was 30-58% in CD and 10-15% in UC.[4] Genome-wide association study (GWAS) shows that most IBD have complex genetic polymorphisms.[5] To date, 201 susceptibility loci have been identified for IBD via GWAS and transethnic association studies, of which 110 sites are shared by both CD and UC, whereas 41 and 30 were unique to CD and UC, respectively. Which may lead to different clinical, endoscopic, and histological phenotypic characteristics of IBD patients.[6] Various genes have been identified as the major IBD susceptibility gene, including nucleotide-binding oligomerization domain protein 2 (NOD2), IL-23 receptor (IL-23R), toll-like receptors (TLRs), and autophagy-related protein 16-1 (ATG16L1). These genes may play important roles in barrier function, epithelial repair, microbial defense, innate immune regulation, reactive oxygen species production, autophagy, adaptive immune regulation, endoplasmic reticulum stress, and the pathogenesis of IBD.[7]

NOD2, located on human chromosome 16q12, is the first confirmed genetic susceptibility gene for CD. *NOD2* encodes a protein with homology to plant disease resistance gene products, which mainly expressed in monocytes, but also found in Paneth cells and intestinal epithelial cells. NOD2 impairs the functions of Paneth cells, dendritic cells, macrophages, and absorptive intestinal epithelial cells (IECs) by regulating autophagy, leading to colitis and increasing the risk of CD.[8] NOD2 mutation is related to the decrease of α-defensin level in Paneth cells, which leads to impaired antibacterial function.[9] *NOD2* gene polymorphisms are common in Western CD patients, up to 50% of CD patients carry at least one *NOD2* mutation. Three single

nucleotide polymorphisms (SNPs), Arg702Trp, Gly908Arg, and Leu1007fs have been identified to be associated with CD in Western, and P268S is associated with CD in Chinese population. *NOD2* mutations may be related to CD ileal lesions, intestinal fibrous stenosis, and intestinal penetrating lesions.[10,11]

In the absence of NOD2 or when reintroducing the mutated form of NOD2, the IL-12 family (including IL-12, IL-23, IL-27, and its new member IL-35) not only play an important regulatory role in the process of infection and inflammatory response, but also related to the pathogenesis and treatment of CD. Studies have shown that IL-27p28 mRNA is abnormally expressed in intestinal mucosa, and decreased the negative refutational production stimulated by muramyl dipeptide (MDP), which leads to the occurrence of CD. *IL-23R* gene is located on chromosome 1p31 and its encoded protein, IL-23R, is expressed by Th17 cells and has been associated with chronic autoimmune disorders.[12] Genetic studies have indicated that UC is related to IL-23R, IL-10, and macrophage colony stimulating factor-1 (MCSF-1). Enhanced and sustained activation of IL-12/STAT4 and (or) IL-23/STAT4 signals may exist in UC patients in the active phase, and participate in the chronic inflammatory response process of UC.[13] IL-23 has a pro-inflammatory effect, and this signal transduction pathway might be used as a therapeutic target for IBD.[14]

TLRs are important pattern recognition receptors, as a bridge connecting innate immunity and subsequent adaptive immune responses, could specifically recognize pathogen associated molecular patterns (PAMPs) to initiate immune responses. Dysfunctional TLRs can mediate and maintain chronic inflammation, which is closely related to the onset of IBD. Studies have shown that the expression of *TLR2* mRNA in the intestinal mucosa of IBD patients is significantly higher than that of normal people. TLR1 R80T, TLR2 R735G heterozygous carriers have an increased risk of colitis, and TLR1 S602I is negatively correlated with ileal CD.[15] TLR4 is overexpressed in intestinal epithelial cells of IBD patients, which is mostly expressed on the lumen side of intestinal mucosal epithelial cells in CD patients, and is mostly expressed on the basal side of mucosal epithelial cells in UC patients. In addition, TLR4 D299G and NOD2 mutations have a synergistic effect, leading to earlier disease onset and faster

progression.[16] Moreover, the expression level of TLR5 is related to the pathogenesis of UC, while TLR3 and TLR9 have a protective effect on the intestinal mucosa and can inhibit the pathogenesis of IBD.[17]

The T300A mutation for ATG16L1 has been previously reported as a risk allele for Crohn's disease, which opened up a new research direction for the study of the pathogenesis of IBD.[18] Studies in IBD patients and animal models have shown that NOD2 and ATG16L1 variants are associated with abnormal Paneth cell phenotypes, causing defects in antibacterial autophagy, and then decreased ability to kill pathogenic microorganisms, thus enhanced inflammation.[18] Studies have found that mouse ileal mucosal Paneth cells with less ATG16L1 protein express more antimicrobial peptide particles. Similarly, CD patients with mutant *ATG16L1* gene also have the same changes, suggesting that ATG16L1 may reduce the secretion of antimicrobial peptides in Paneth cells to maintain the intestinal immune balance.[19]

ENVIRONMENT FACTORS

The incidence of IBD has increased sharply in Western countries since the 20th century. In recent years, with the process of industrialization and westernization of lifestyles in Asian countries, the incidence of IBD has been increasing.[2] The gradual westernization of diet, smoking, excessive use of antibiotics, environmental pollution, improved sanitation, and early microbial exposure can affect the gut microbiota and promote intestinal inflammation in genetically susceptible people. The above mentioned indicate that environmental factors are closely related to the pathogenesis of IBD. Environmental factors that have been studied more at present include smoking, diet, environmental sanitation and so on.

Smoking is one of the first major environmental risk factors identified to be associated with IBD process. Smoking can aggravate the IBD condition, increase the number and duration of acute attacks. In addition, the incidence of extraintestinal manifestations, such as chronic skin diseases and joint symptoms, was significantly increased in IBD patients which associated with smoking. When patients quit smoking, the incidence of extraintestinal manifestations in IBD patients was rapidly reduced to the level of non-

smokers. At the same time, the effective dose of hormones was significantly reduced to improve the efficacy of immunosuppression.[20] The reason may be that carbon monoxide in smoke damages the ability of microvascular dilatation, causing intestinal tissue ischemia and chronic inflammation, and then causing the formation of ulcers and fibrosis. In addition, nicotine in tobacco is another major cause. Nicotine affects nicotinic acetylcholine receptors distributed in intestinal epithelial cells,[21] changes the levels of cytokines such as interleukin-8 (IL-8) and tumor necrosis factor -α (TNF-α), and increases microvascular thrombosis.

Diet, which is the most complicated factor, can affect the intestinal microenvironment that include the composition of intestinal microbes, intestinal barrier and host immune status. Previous studies have shown that high dietary fiber diet can prevent the occurrence of IBD and reduce the risk of CD.[22] This may be related to the ability of soluble fiber to modify and scavenge oxygen free radicals. Dietary fiber is metabolized by intestinal bacteria into light chain fatty acids with anti-inflammatory properties. Meanwhile, dietary fiber can reduce cholesterol absorption, promote intestinal probiotics reproduction and regulate intestinal immunity, etc.[22] In contrast, excessive intake of processed meat or diets rich in saturated fatty acids may induce or aggravate IBD process, as fatty acids may induce inflammatory responses by regulating TLRs in macrophages.[23]

Vitamin D plays an important role in the onset and active period of IBD process that participates in the regulation of intestinal immune function. IBD patients, especially CD patients, have vitamin D deficiency. Studies have shown that the lower the serum vitamin D level, the higher the risk of IBD. Vitamin D can increase the abundance of beneficial bacteria in the intestinal by regulating the composition of intestinal bacteria. Compared with the placebo control group, vitamin D supplementation can reduce the risk of recurrence of CD.[24] 1, 25-dihydroxyvitamin D3 [1,25-$(OH)_2$D3]，the active expression form of vitamin D in vivo, can be induced to secrete antimicrobial peptides in Paneth cells through vitamin D receptor. Sequentially, antimicrobial peptides reduce dendritic cell activity and promote the development of type 2 helper T cells. In addition, Vitamin D can reduce the production of Th17 cells and related cytokines. Meanwhile,

Vitamin D also can affect the function of natural killer T cells,[25] which may be related to the occurrence of intestinal inflammation.

IMMUNOINFLAMMATORY FACTORS

The role of immune factors in the pathogenesis of IBD has been affirmed and research is increasingly deepened until to now. The intestinal mucosal immune system mainly includes intestinal epithelial cells, innate cells (lymphocytes, macrophages/monocytes, neutrophils, dendritic cells), various mediators (cytokines, chemokines, natural Killer cells factors) secreted from the cells mentioned above and so on. Environmental factors, intestinal flora and pathogen infection can break the balance of the intestinal mucosal immune system in directly or indirectly. These changes destroy the intestinal epithelial barrier and damage the intestinal mucosa that mediates the intestinal tissue to be exposed to a large number of antigens for a long time caused by the permeability of the mucosa. The overreaction and misrecognition of the intestinal immune system causes the activation of macrophages and lymphocytes that stimulates the production of a series of cytokines and inflammatory mediators which activates the immune response in vivo. With the progressive amplification of inflammatory response, tissue damage and IBD-related pathological changes were eventually caused.[26]

Intestinal Immune Regulatory Cell

Naive T cells can differentiate into diverse T cell subpopulations after antigen priming, including helper T cells (Th cells) and regulatory T cells (Tregs cells) and so on. The disturbed balance between Th cells and Treg cells contribute to the pathogenesis of IBD. Various studies about either spontaneous colitis or induced colitis mouse models suggest that T cells play a crucial role in intestinal inflammation.[27] Both Th1 and Th2 cells can cause chronic inflammation of the intestine. Th1 cells mainly secrete IFN-γ, TNF-α and IL-2. The occurrence of Crohn's disease (CD) is related to a Th1 cytokine profile. Microbial antigens in the intestinal lumen stimulate the differentiation of intestinal mucosal T cells into Th1 cells, forming Th1-type mucosal inflammation with elevated IL-12, IFN-γ, and TNF-α, which are involved in the pathogenesis of CD.[28] Th2 cells mainly secrete IL-4, IL-5, IL-10 and IL-13, thereby regulating B cell

differentiation, assisting B cells to produce antibodies, and participating in mucosal defense responses to intestinal parasite infections. Traditionally, it is reported that Th2 cytokines modulate ulcerative colitis, which is characterized as infiltrating by IL4, IL-5, and IL-13.[29] However, there are also studies reporting that UC is the results of the combination of Th1 and Th2. In the early stage, the Th1 response may be enhanced, while the Th2 response is dominant in the late stage.

Treg cells are a type of T cell subtype with negative regulation function in immune system, which maybe have potential preventive and therapeutic effects on UC. The failure of Treg cell function or reduced number of Treg can lead to the occurrence of IBD. Activating by IL-2, Treg can secret anti-inflammatory cytokines, including IL-10 and TGF-β. Various clinical studies have shown that the number of Treg cells in the peripheral blood of patients with IBD and the expression of Foxp3, a specific transcription factor that maintains the immunosuppressive function of Treg cells, are significantly lower than those of healthy people. During the remission phase, the expression levels of cytokines IL-10 increased, and gradually returned to normal levels. This may be caused by the recruitment of Treg cells to the inflammation site to perform the unction of immune regulation.[30]

Th17, as a newly discovered CD4-assisted T-cell subtypes, secreting inflammation-promoting cytokine IL-17. The onset of IBD was initially thought to be associated with Th1 and Th2, but recent studies have shown that the occurrence of IBD is related to Th17.[31] Th17 cells secrete IL-17, IL-21, IL-22, and granulocyte-colony stimulating factor (G-CSF). These cytokines will stimulate other cells in the intestine, including macrophage, intestinal epithelial cell and fibroblast to release TNF-α, IFN-γ, IL-10, IL-6, matrix metalloproteinase (MMPs) and so on. Various studies showed that the expression of IL-17A, IL-21 and IL-23 was significantly increased in the intestinal mucosa of IBD patients.[32,33]

In patients with IBD, the proportion of Treg cells in the surrounding blood decreased, whereas the proportion of Th17 cell cells increased. And the ratio of Treg/Th1 decreased significantly. The level of mRNA and protein expression of anti-inflammatory cytokines IL-10 and TGFβ1 decreased significantly in the peripheral

blood, and Th17 cytokine IL-17 and IL-23 increased significantly.[34-35]

Cytokines

Cytokines play a key role in the onset of IBD. Cytokines are bioactive small molecular proteins produced mainly by activated immune cell stimulation, regulating the immune response process. Breakdown of cytokine networks can lead to IBD, and there are also interactions between a variety of cytokines, including pro-inflammatory and anti-inflammatory cytokines. Pro-inflammatory cytokines, including IL-1, IL-2, IL-6, IL-8, IL-12, IL-17, IL-23, IL-27, TNF-α and IFN-β γ, were mainly produced by monocytes and macrophages, and participate in cellular immune response. Anti-inflammatory cytokines, including IL-4, IL-5, IL-10, IL-13 and TGF-β, were mainly produced by T cells, and participate in the body fluid immune response. The imbalance between pro-inflammatory and anti-inflammatory cytokines was an important factor leading to the damage of intestinal mucosa.

The most representative cytokine is TNFα, which has developed to be a key target of IBD therapy. TNF-α can induce inflammatory reaction in the inherent layer of intestine, thereafter the neutrophils gather and stimulate lymphocytes to express IL 6 and IL 8.[36] The tight junctions formed by intestinal epithelial cells destroys, which results in the damage of intestinal mucosa barrier.[37]

IL17 was the main effect molecule secreted by Th17 cells. IL-17 can act alone or in synergy with TNF-α on intestinal mucosa epithelial cells, fibroblasts and neutrophils, etc., to promote the secretion of inflammatory media, chemokines and proteases to induce inflammatory cascading reactions, resulting in intestinal mucosa damage. The expression level of IL-17 was significantly increased in peripheral blood and inflammatory mucosa in patients with IBD, and the number of Thl7 cells increased in inflammatory mucosa, especially in patients with active CDs and UC.[38] In the mouse colitis model induced by sodium glucose sulphate (dextran sulfate, DSS), IL-17F knock-out reduced the symptoms of colitis, while IL-17A knock-out increased inflammation.[39]

IL-23 plays an important role in the amplification and maintenance of Th17 cells. IL-

23 and IL-23R receptor complexes regulate the production of IL-17A, IL-17F, and IL-22 in differentiated Th17 cells by activating JAK2 and STAT3.[40] IL-25, known as IL-17E, is a member of the IL-17 cytokine family, which inhibits Th1/Thl7 cell differentiation. The expression levels of IL-25 in serum and inflammatory mucosa of active IBD patients are significantly lower than that in healthy people, and are negatively correlated with the activity of IBD.[41] Some animal experiments have shown that exogenous IL-25 can significantly improve colitis symptoms, which provides new ideas for the discovery of new targeted treatment of IBD.

Other important cytokines IL-6, IL-21, IL 10, etc. are also associated with the occurrence of IBD. IL-6 can regulate endothelial molecular expression, promote neutrophils adhesion to vascular endothelial cells and tend to inflammatory sites. The expression of IL-6 and sIL-6R were increased in peripheral blood, intestinal mucosa and inherent layer in patients with IBD. Studies have shown that in DSS-induced UC models, TNF-α and IL-6 levels were also increased.[42] IL-21, when combined with IL-21 receptors, can stimulate intestinal epithelial cells and fibroblasts to secret chemokines, matrix metalloproteinase, etc. and induces specific plasma cell to secrete granules B, which plays a critical role in mucous damage.[43,44] In IBD, compared with normal colonic mucosa, the number of CD4+ T cells expressing IL-22 in the inflammatory lesion area is significantly increased, especially in active UC.[45] IL-10 is an important anti-inflammatory cytokine acting on immune cells and epithelial cells, which inhibits the release of TNF-α and plays an important role in maintaining gastrointestinal immune homeostasis. There was a significant decrease in IL-10 levels in the serum of IBD patients, and the absence of IL-10 can aggravate IBD.[46,47]

Antigen Presenting Cells

Antigen-presenting cells (APC) mainly include monocytes-macrophages, dendritic cells, B cells and endothelial cells, etc., which are considered to connect specific immune response and non-specific immunity and plays a key role in mucosal homeostasis. In recent years, a large number of studies have shown that the colonic mucosal lamina propria mononuclear cells increase significantly in the IBD mouse

model, the interaction between APC and lymphocytes and the local mucosal production of soluble immunoregulatory factors trigger and expand the mucosal inflammatory response.

As an important immunoregulatory cell of APC, dendritic reticulum cells (DCs) is the most representative and most effective one.[48] DC has the dual function of causing immune activation and immune tolerance, and has become a hotspot in the field of IBD in recent years. Studies have shown that the percentages of plasmacytoid dendritic cells (pDCs) and myeloid dendritic cells (mDCs) expressing CD200R1 in IBD patients are significantly reduced. Among them, DCs expressing CD200R1 are significantly positively correlated with Treg, while negatively correlated with Th17. The study showed that the reduction of DCs may lead to the imbalance of Th17 and Treg cells that participate in the pathogenesis of IBD.[49] In addition, IBD stimulates the intestinal microbiota and activates TLR signals through APCs such as lamina propria DC and macrophages to increase pro-inflammatory cytokines such as IL-1, IL-6, and IL-18, TNF, IL-12 family (IL-12, IL-23, IL-27, IL-35), interferon (IFN-α, IFN-β) level.[50]

Defensins

As the main antibacterial peptide secreted by Paneth cells at the bottom of the small intestine acinar, defensin is an important part of the natural defense system in the organism. It plays a very important role in maintaining and regulating the mucosal barrier function and immune function of the intestine. In recent years, it has been found that defensins are closely related to the pathogenesis of IBD, and their down-regulation or functional defects play an important role in the pathogenesis of IBD. The expression disorder of intestinal defensin in IBD patients and animal models may be related to the occurrence and prolonged healing of IBD. In addition to directly killing or inhibiting pathogenic microorganisms, defensins also have the function of regulating or expanding the adaptive immune response. Disordered expression of defensins may interfere with the initiation of acquired immunity, expand the local inflammatory response in the intestine, and accelerate IBD. It is reported that humans and mice are mainly α-defensins and β-defensins. Both of these defensins can chemoattract T

lymphocytes, dendritic cells, monocytes, etc., so that they can quickly accumulate in Inflammation reaction site, which plays a role in cellular immune response.[51]

Wehkamp *et al.* found that in patients with ileal CD, the expression of Tcf-4 mRNA, a regulator of Paneth cell differentiation and defensin expression, was significantly decreased, which led to a dual decrease in Paneth cell secretion defensin levels and sterilization activity. Which suggest this may be one of the important mechanisms leading to the pathogenesis of ileal CD.[52] β defensins can induce the migration of CD8 + T cells and naive CD4 + T cells, followed by the recruitment of immature dendritic cells (IDC) by binding to the CCR6 receptor of dendritic cells. In the infected colon, especially UC, β-defensin-2 can be inducibly expressed. In non-infected cases, whether it is UC or CD, the expression of β-defensin-2 in the intestinal tract of patients is very low. This indicates that the occurrence of UC may be related to the overexpression of β-defensin-2, which aggravates the intestinal inflammatory response. There are abundant NOD genes expressed in Paneth cells of human terminal ileum and colonic metaplasia. NOD is mainly involved in the expression of α-defensins in Paneth cells. NOD2 is distributed in the cytoplasm of Paneth cells, next to the small vesicles containing defensins. It is speculated that NOD2 is involved in the secretion of defensins. NOD2 mutations can affect the antibacterial activity of defensins in patients with IBD.[53] Studies about defensins on the pathogenesis of IBD provides new ideas for using defensins as drug targets to relieve IBD intestinal mucosal lesions and treat IBD.

Intestinal Epithelial Cells (Mucosal Barrier)

The intestinal barrier is composed of intestinal epithelial cells (IEcs) and innate immune cells, and maintains the balance between the contents of the cavity and the mucosa. The intestinal mucosal barrier is composed of different types of cells, including intestinal epithelial cells, goblet cells, neuroendocrine cells, Paneth cells and M cells. These cells maintain the balance of the luminal mucosa through different mechanisms.[54] Among them, the intestinal epithelial cells are located between the immune cells of the lamina propria and the microbial zone in the intestinal lumen. The mucosal barrier composed

of tight junctions between epithelial cells can resist the invasion of macromolecular substances and microorganisms. If the integrity and continuity of epithelial cells are destroyed, the intestinal mucosal immune system will be activated and IBD will be triggered. Paneth cells are located at the bottom of the small intestine crypts and are responsible for the homeostasis of the ducts. Several key genetic risk factors for IBD, such as NOD2 and autophagy, can damage the functions of Paneth cells, dendritic cells, macrophages and absorptive IEcs. Thus, causes colitis and increases the risk of CD.[55] After the contaction of intestinal epithelial cells with microorganisms in the intestinal lumen, they can recognize pathogen components through extracellular receptors such as TLR and intracellular receptors such as NOD-like receptors. Once TLR and NOD2 receptors are activated, intestinal epithelial cells and Paneth cells located in the crypts can produce the antimicrobial peptide β defensin, expressing major histocompatibility complex (MHC) molecules to trigger mucosal acquired immune responses.[56] In NOD2 receptor-deficient mice, the permeability of the intestinal epithelium is increased, the secretion of antibacterial components is reduced, the structure and function of the junction complex between epithelial cells are defective, and the susceptibility to colitis is increased.[57]

Intestinal dendritic cells, macrophages, innate lymphoid cells and neutrophils constitute the first line of defense of the intestinal innate immune system. They play important role in producing intestinal inflammatory response and maintaining intestinal immune tolerance under physiological conditions

MicroRNA（miRNA）

Non-coding RNAs (ncRNAs)，play a key regulatory role in the biological processes of inflammation and immunity, are closely related to the occurrence of many diseases. At present, MiRNAs, which are the best studied, become a popular biomarker and potential therapeutic target. At the cellular level, miRNA has a strong ability to regulate gene expression and that participates in the abnormal intestinal immune response by influencing intestinal epithelial cells differentiation or regulating of signal transduction. Many miRNAs have been found to be abnormally expressed in the intestinal mucosa

of UC patients until now. These miRNAs are involved in the pathogenesis of UC which are related to many important mechanisms such as TLR/NF-κB signaling pathway, intestinal epithelial permeability, autophagy, cytokines and chemokines.[58]

The increase of pro-inflammatory miRNA expression or the inhibition of protective miRNA expression can lead to the occurrence of IBD receptivity. Previous studies have found that miR-21 has been confirmed to increase expression in UC intestinal tissue that is related to nitric oxide synthase 2 (NOS2) which promotes increased NO secretion. The Abnormally increased of NO concentration activates macrophages which is the mainly cell mediates intestinal inflammation.[59] An abnormally activated macrophage-mediated inflammatory responses can damage intestinal epithelial cells, leading to the incident and development of IBD process. Besides, miR-126, miR-146a, miR-22l and miR-223 have also been confirmed to participate in the regulation of NOS2 in IBD tissues.[60] Moreover, down expression of miR-10a in the intestinal mucosa of patients with IBD can inhibit the intestinal inflammation by inhibiting Th1 and Th17 cells by reducing the production of TNF-α, IFN-γ and IL-17.[61] The expression of miR-301a in the intestinal mucosa of IBD patients is higher than that in remission and healthy patients. The miR-301a inhibitor reduces the proportion of intestinal mucosal lamina propria Th17 cells and IL- in the TNBS-induced acute colitis model in mice.[62] On the other hand, The low-expressed miR-511-3p, which induces the activation of NF-κB signaling pathway by up-regulating TLR4, promotes the expression of inflammatory cytokines TNF-α, IL-1β, IL-6 and IL-8.[63] In addition, miRNA can be used as a diagnostic marker for IBD as it has good stability outside the cell. Whereas the specificity of miRNA as a disease diagnostic marker in existing studies is poor and further research is needed.

Abnormal expression of other ncRNAs such as long non-coding RNAs (10ng non-coding RNAs, lncRNAs) and circular RNAs (circRNAs) are also involved in the induction of inflammation in IBD process.[58] Further exploration of related miRNAs may be expected to make them an important target for the treatment of IBD patients.

Abnormal Immune Regulation of Reactive Oxygen Species

Reactive oxygen species (ROS) regulate cell apoptosis by oxidizing DNA, proteins, lipids and other cellular structures. ROS is not only a harmful byproduct of cell metabolism, but also a key factor in regulating intestinal homeostasis. Low levels of ROS may act as a signal to regulate the interaction between mucosa and microorganisms, and are necessary for certain physiological processes including protein phosphorylation, transcription factor activation, cell differentiation, cell apoptosis, and cellular immunity. However, too much ROS has harmful effects on cellular DNA, proteins and lipids. Continuously high concentrations of ROS can irreversibly destroy or change the function of target molecules, causing DNA damage, lipid peroxidation and protein oxidation. Xanthine oxidase, amine oxidase, aldehyde oxidase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that exist in macrophages in the lamina propria of the intestinal mucosa can all produce ROS. The main source of ROS in gastrointestinal tract is NADPH oxidase. In the process of chronic inflammation and repeated immune response of the intestinal tract, the oxidative reaction begins to dominate. ROS can destroy the smooth muscle, nerve and cell tight junctions of the intestine.

Studies have shown that with the development of the disease, the concentration of reactive oxygen species in the intestinal cells increases during the chronic inflammation and repeated immune responses in the intestine.[64] In order to protect the biological system from excessive ROS damage, the cell's antioxidant system is activated to regulate the production of ROS. Reducing the concentration of ROS in the affected area may be used as a method to prevent and treat IBDs, thereby preventing the occurrence and development of the disease.

INFECTION AND GUT MICROBIOTA

Currently the IBD is thought to develop as a result of interactions between environmental, microbial, and immune-mediated factors in a genetically susceptible host. Several strands of evidence suggest a role for the microbiome in the intestinal biologic barrier, promoting digestion and absorption, immunomodulation, anti-tumor and metabolism. Now, the investigators have found the role for gut microbiome in the

pathogenesis of IBD, including the bacteria, viruses and fungi.

Intestinal Flora

Bacteria are the most striking components among the normal microbiota. And there are more than 1,000 kinds of bacteria in the gastrointestinal tract. Imbalance of gastrointestinal flora could change microorganism-host cell interaction and the metabolic process. Besides it could cause diseases through changing the counts, metabolites and components of the flora.[65] Intestinal flora could change from symbiotic microbiota to potentially pathogenic microbiota. Intestinal flora imbalance and symbiotic disruption between host-microbiota played a decisive role in the development of IBD. When compared with the microbiota of healthy subjects, microbiota samples from patients with IBD demonstrate an increased abundance of proinflammatory taxa, a decrease in overall diversity and a reduced abundance of anti-inflammatory taxa.[66] To active CD patients, the number of the invasive Escherichia coli increased by about 38%, while to the heathy controls, it was 6%.[67] Using antibiotics and probiotics to regulate the components of intestinal flora, and then reducing inflammation and improving symptoms has now become an important assistant mean to treat IBD.[68] Faecal microbiota transplantation (FMT) could change the components of intestinal flora within a short time, and has already been applied to treat IBD.[69-70]

H. Pylori

H. pylori was associated with many gastric and non-gastric diseases. There was an inverse relationship between H. pylori infection and IBD.[71-72] Some epidemiological studies showed that the incidence of IBD in Western countries and Asian areas had increased every year, while the H. pylori infection rate had decreased.[73] Possible mechanisms of the potential protective role of H. pylori infection against the development of IBD may be associated with the immunomodulatory properties of H. pylori and other confounding factors such as drug factors (antibiotics, Sulfathiazole) and environmental factors. The DNA of the H. pylori could play a role in immunoregulation by promoting the release of INF-α and IL-12 and down-regulating

the systematic immune response, and then reducing the inflammation of intestinal mucosa.[74] Other studies suggested that H. pylori infection could down-regulate the DC-SIGN expression of intestinal epithelial cell, thereby reducing the latter mediated proinflammation and intestinal mucosa injury.[75] Engler *et al*. demonstrated that experimental infection with H. pylori and administration of regular doses of H. pylori extract both alleviate the clinical and histopathological features of dextran sodium sulfate–induced chronic colitis, which could be attributed to transcriptional activation of the mucin 2 gene that induced the production of large quantities of protective mucus.[76] While others suggested another possible mechanism. The gastrointestinal tract produces specific antibodies after H. pylori infection, which could be immunologically protected against other bacterial infection, and then inhibiting the immune response and reducing intestinal injury. These results suggested the existence of cross-reacting antigen between bacteria and the antibodies of H. pylori were cross-reactive.[72] Moreover the H. pylori related products could improve the chronic intestinal inflammation. Neutrophil activating protein secreted by H. pylori could reduce the inflammatory response through regulating the TLRs and increasing the IL-10 production.[77]

Intestinal Viruses

Cytomegalovirus (CMV) is a virus that could disrupt immune response, and cause symptoms in different target organs, such as CMV colitis. More than 50 years ago, scholars began to explore the association of CMV with IBD. If the CMV persistently present in the UC patients after infection, patients in remission stage will have an acute attack. CMV positive IBD patients were more likely to happen systemic vasculitis, which manifested as necrosis of intestinal mucosa, toxic megacolon and increased risk of perforation.[78] Persistent measles virus infection of the mesenteric microvascular endothelial cells could cause chronic granulomatous vasculitis, which then resulted in CD. To CD patients, there were measles viruses in the vascular endothelial cells, lymphocytes and macrophages in the foci of inflammation.[79]

Intestinal Fungi

The gut fungi have recently been suggested to play roles in intestinal microecological balance. IBD patients had distinctive fungal communities. Through characterizing the fungal communities by deep sequencing of rRNA gene segments, Chehoud *et al.* found Candida was significantly more abundant in IBD patients while Cladosporium was more abundant in healthy subjects.[80] The change in abundance of gut fungi is not only in the faecal samples, but also in the pathological intestinal mucosa. Malassezia restricta, a fungus that is commonly found on the skin, is abundant in the intestinal mucosa of patients with CD. Malassezia species elicit inflammatory cytokine production from innate immune cells that have CARD9 gene mutations typically associated with IBD and exacerbates colitis in mouse models of disease.[81]

The IBD generally shows long course and is easily repeated attack. It could increase risk for colorectal cancers, influence the life quality of patient severely and even be life-threatening. The mechanism of IBD is complex. And exploring the etiology and pathogenesis of IBD has been a difficulty. IBD is thought to develop as a result of interactions between immune, inflammatory, infective, genetic and environmental factors. In-depth study of the exact mechanism of the key molecules of these factors in the pathogenesis of IBD will help to thoroughly understand the pathological process of IBD and explore more effective therapeutic targets and specific medications. These will provide new ideas for exploring effective therapeutic strategies, thereby effectively preventing some diseases and delaying the disease progression.

Conflict of Interest

None declared.

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