Analysis of expression of cytokines in intestinal mucosa of patients with inflammatory bowel disease

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Abstract: Inflammatory bowel disease has been common in developed countries in the past, but in recent years, with the continuous improvement of material living conditions in China, the incidence of IBD in China is on the rise. In this paper, the sample was selected and tested to analyze the expression of cytokines in the intestinal mucosa of patients with inflammatory bowel disease. The biggest feature of this paper is not only the analysis of the expression of cytokines, but also the analysis of the symptoms and hazards of IBD. At the same time, based on the classification study of the specimen patients, the expression patterns of cytokines under different genders and different clinical manifestations were investigated, and it is hoped that the related research will be helpful.

1. Introduction
In the past 20 years, with the improvement of national living standards and awareness and diagnosis of IBD, the incidence rate in China has increased year by year, and reports on the disease have also increased significantly. Currently, the prevalence of UC in China is estimated to be 11.6/100,000. The disease rate is 2.29/100,000, and IBD has become a hot spot in the field of enteropathy. The clinical manifestations of inflammatory bowel disease are mainly persistent or recurrent abdominal pain and bloody diarrhea, often accompanied by different degrees of systemic symptoms. In severe cases, some extraintestinal manifestations can be combined.

Due to its long course of disease, repeated remission and episodes, it is difficult to cure, and is associated with the pathogenesis of colon cancer. It has been listed as one of the modern refractory diseases by the World Health Organization. Studies have shown that different cytokines exhibit different expression patterns in inflammatory bowel disease, and cytokines play an important role in the pathogenesis, progression and outcome of inflammatory bowel disease (IBD). Therefore, by selecting certain experimental specimens and testing the expression patterns of different cells, it is helpful to master and treat inflammatory bowel disease.

2. Inflammatory bowel disease

2.1 Concept
Ulcerative colitis is a continuous inflammation of the colonic mucosa and submucosa. The disease usually involves the rectum and gradually spreads to the whole colon. Crohn's disease can affect the whole digestive tract. It is a non-continuous whole-layer inflammation. The most common part is End ileum, colon and perianal. Inflammatory bowel disease (IBD) is a group of chronic non-specific
inflammatory diseases involving the intestine. It is a chronic non-specific intestinal inflammatory disease whose cause is not clear, based on symptoms, signs, extent of lesions, and microscopic mucosal manifestations. It is divided into ulcerative colitis, Crohn's disease, and some patients with colitis who are difficult or indistinguishable from UC or CD, ie, colitis of uncertainty. The main clinical manifestations are recurrent abdominal pain, diarrhea, and pus and bloody stools.

2.2 Symptoms
Epidemiological data show that the incidence of IBD in the world is increasing year by year, and the age of onset is gradually decreasing. Its etiology and pathogenesis are not fully understood at present, and are generally considered to be related to various factors such as environmental, genetic, microbial infection and immunity. Among them, immune factors are particularly important in the pathogenesis of inflammatory bowel disease. There is no cure for this disease, the clinical manifestations are different, easy to relapse, the complications are heavier, and the extraintestinal manifestations are complex and diverse. Studies have shown that nearly 30-60% of patients with IBD must undergo surgical treatment during the course of disease development, and the quality of life of patients is greatly affected. Moreover, IBD has become one of the most common causes of lower gastrointestinal bleeding in China, seriously affecting the lives of patients. In addition, IBD still has a certain risk of cancer, so IBD has always been one of the research hotspots of digestive diseases.

2.3 Mechanism
At present, the etiology and pathogenesis of IBD are still unclear. It is currently considered to be mainly mediated by immune response, and is closely related to genetic factors and environmental factors, and is a kind of autoimmune disease. A large number of facts indicate that there are increased accumulation and activity of immune cells in blood and tissues in IBD. These inflammatory cells are rapidly stimulated to synthesize and secrete cytokines, and many cytokines participate in intestinal mucosal inflammation during IBD. The mechanism of immune injury to the onset of IBD can be summarized as: under the combined effects of various risk factors (including genetics, intestinal microbes, use of non-steroidal anti-inflammatory drugs, etc., intestinal epithelial barrier destruction, increased permeability of the membrane, intestinal Long-term exposure of tissues to a large number of antigens leads to overreaction and misidentification of the intestinal immune system, causing activation of macrophages and lymphocytes, releasing a series of cytokines and inflammatory mediators, activating the body's immune response, and stimulating the inflammatory response step by step. Eventually lead to tissue damage. Excessive activation and imbalance of immune responses may be the most important cause of disease.
3. Cytokines
Cytokine (CK) is a low-molecular-weight soluble protein produced by various cells induced by immunogens, mitogens or other stimulators. It regulates innate and adaptive immunity, hematopoiesis, cell growth, and damaged tissue repair. Cytokines can be divided into interleukins, interferons, tumor necrosis factor superfamily, colony stimulating factors, chemokines, growth factors and the like. Numerous cytokines play a role in the body through paracrine, autocrine or endocrine, and have various physiological characteristics such as pleiotropic, overlapping, antagonistic, and synergistic, forming a very complex network of cytokine regulation and participating in many human bodies.

Cytokines play an important role in the body's immune response. Cytokines with inflammatory-mediated and immunomodulatory activities are associated with inflammatory bowel disease, such as IL-1, IL-6, IL-8, TNF, etc., most of which are produced by T cells and mononuclear macrophages. In recent years, studies have found that a variety of cytokines play a role in regulating the interaction between immune cells and non-immune cells in the formation and development of inflammatory bowel disease, and may be related to the severity of inflammatory bowel disease. Cytokines such as TN-a, IL-17, IFN-y and chemokines can dynamically regulate the tight junction of epithelial cells.

The imbalance between cytokines is an important factor in causing intestinal membrane damage. The study found that TNF-a is released by macrophages in the first stage of inflammation, and it plays an important role in the model of inflammatory bowel disease induced by trinitrobenzenesulfonic acid. TNF-a is one of the most important pro-inflammatory cytokines that directly affect intestinal epithelial tissue. II, 1 R and II, 6 are also the main mediators in the process of inflammatory bowel disease. Excessive TNF-a secretion leads to intestinal Injury of the epithelial barrier and epithelial cell apoptosis, and the secretion of colonic epithelial chemokines.

![Fig2. Cytokine expression equation](image)

ILK is produced by mononuclear macrophages, dendritic cells and epithelial cells, and is involved in the activation of T cells, NK cells and macrophages. It has a wide range of immunomodulatory effects and can be used as an indicator for judging the severity and efficacy of the disease. ILK is mainly produced by Th1 cells and is a T cell growth factor, which enhances the killing activity of NK cells and macrophages. ILK is an important pro-inflammatory cytokine. Interfering with ILK signaling can effectively prevent autoimmune and play an important role in the pathogenesis of UC. ILK is mainly produced by mononuclear macrophages and Th2 cells, and is composed of ILK receptor and signal transduction protein gp130, which stimulates the growth and differentiation of T cells and B cells, and promotes the proliferation of various cells. ILK induces polarized expression of intercellular adhesion molecules via the STAT pathway. Therefore, ILK is thought to play an important role in chronic...
intestinal inflammation. IL-3 is a pro-inflammatory cytokine that forms a complex with the IL-3 receptor (IL-3R) on the cell membrane surface, stimulating intracellular signaling systems to induce cell activation. The main mechanism of action of IL-3 is to stimulate Th17 cells to secrete IL-17 and induce memory T cell proliferation.

4. Comparison and verification

4.1 Research object
In this paper, 40 patients with IBD admitted to the Second People's Hospital of Guangzhou from March 2017 to March 2018 were selected, including 29 UCs and 11 CDs. The IBD diagnostic criteria refer to the consensus opinion on the diagnosis and treatment of inflammatory bowel disease (Guangzhou) households. The Mayo scoring system and the simplified CD activity index (CDAI) were used to evaluate the activity of UC and CD diseases. The situation was analyzed and several sets of representative data were sorted out. The selected cases excluded other immune diseases, severe cardiopulmonary diseases, and tumors. The research protocol was reviewed and approved by the hospital ethics committee, and the selected persons all signed informed consent.

Table 1. Gender status of 40 patients with IBD

| Sex  | UC | CD |
|------|----|----|
|      | Number | Per% | Number | Per% |
| Male | 15 | 65.2 | 9 | 52.9 |
| Female | 8 | 34.8 | 8 | 47.1 |
| Total | 23 | 100 | 17 | 100 |

4.2 Material preparation
Specimen collection: Intestinal preparation was performed using polyethylene glycol electrolyte before endoscopy. IBD patients were taken biopsy specimens in the intestinal inflammatory lesions. The specimens were placed in the sterilized EP tube immediately after collection, and transported to a 80 °C low temperature refrigerator in a liquid nitrogen tank.

Table 2. Disease statistics of 40 patients with IBD

| Condition distribution | UC | CD |
|-------------------------|----|----|
|                         | Number | Per% | Number | Per% |
| Active period           | 16 | 69.5 | 8 | 47 |
| Relief period           | 7 | 30.5 | 9 | 53 |
| Total                   | 23 | 100 | 17 | 100 |

Total protein extraction: Take appropriate amount of Western and IP cell lysate (Biyuantian Bio), add PMSF and Cocktail a few minutes before use, and make the final concentration of 1 mmol/L. The preserved biopsy tissue was cut into small pieces, and the lysate was added in a ratio of 100-200 L of lysate per 20 mg of tissue, and homogenized on ice (15 000 r/min) until fully lysed. Centrifuge for 1 to 5 min at 11,000 to 13 000 x g and take the supernatant for cytokine detection.

4.3 Cytokine assay: 27 cytokine assays were performed on the extracted intestinal epithelial tissue total protein using Bio-Plex PromTM Human Cytokine 27-lex Assay (Bio-Rad Laboratories Inc.). Including IL-1b, IL-1, IL-K, IL-4, IL-5, IL-7, IL-8, ILK, etoxetin, basic fibroblast growth factor (FGF basic), granulocyte colony-stimulating factor (G-SF), granulocyte macrophage colony-stimulating factor AGM-0SF, IFN-y, IFN-induced protein (IP-10), vascular endothelial growth factor (VEGF) )Wait.
4.4 Statistical analysis
Using SPSS 19.0 statistical software, the count data was expressed as a percentage. The comparison between groups was performed by $\chi^2$ test; the measurement data was expressed by $x$ (Min-Max), and the comparison between groups was analyzed by one-way ANOVA. $P<0.05$ was statistically significant.

5. Conclusion
In summary, this study found that IL-1B, IL-2, IL-15, MCP-1, MIP-1a, MIP-3 expression in the intestinal epithelium of patients with IBD. Compared with normal controls, the expression of IL-8 and PDGF-BB in the intestinal epithelium of CD patients was also significantly down-regulated, suggesting that these cytokines may play a role in the pathogenesis of IBD, providing further exploration of the role of cytokines in IBD.

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