Expression of Inflammatory Cytokines in Patients with Ulcerative Colitis and Its Impact on Prognosis: Postprint

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Abstract

Objective To investigate the expression and clinical significance of cytokines IL-17, IL-23, IL-22, and IL-11 in the intestinal mucosa of patients with ulcerative colitis (UC). Methods Forty patients with active UC, 15 patients with quiescent UC, and 15 healthy controls who visited the Department of Gastroenterology at Nanfang Hospital between January 2008 and January 2015 were enrolled. Immunohistochemistry was employed to detect the expression and distribution of IL-17, IL-23, IL-22, and IL-11 in intestinal mucosal biopsy tissues from these 70 subjects. Furthermore, 40 active UC patients who received standardized treatment and regular follow-up were included. Based on endoscopic mucosal healing status after 2 months of standardized treatment, patients were divided into a good mucosal healing group and a poor prognosis group, and the expression of the aforementioned cytokines in intestinal mucosa before treatment was compared between the two groups. Results The expression levels of IL-17, IL-23, IL-22, and IL-11 in intestinal mucosal tissues of active UC patients were significantly higher than those in the quiescent UC group and healthy control group (0.0727±0.0037 vs 0.0354±0.0243 vs 0.0330±0.0045; 0.1407±0.0068 vs 0.0865±0.0051 vs 0.0442±0.0137; 0.0522±0.0045 vs 0.0259±0.0063 vs 0.0115±0.0061; 0.0479±0.0022 vs 0.0365±0.0024 vs 0.0232±0.0009, respectively) ($P<0.05$). The expression levels of IL-17, IL-23, and IL-22 in intestinal mucosa increased with disease activity (0.0545±0.0072 vs 0.0786±0.0051 vs 0.0847±0.0197; 0.1112±0.0046 vs 0.1480±0.0089 vs 0.1644±0.0190; 0.0307±0.0063 vs 0.0548±0.0071 vs 0.0719±0.0056, respectively) ($P<0.05$). The expression levels of IL-17, IL-23, IL-22, and IL-11 were positively correlated with endoscopic activity grades ($P<0.05$), and the expression levels of IL-17 and IL-22 were also positively correlated with histopathological grades ($P<0.05$). Additionally, the expression levels of these inflammatory cytokines were positively correlated with each other. The rate of poor mucosal healing

in the high IL-17 expression group (66.67%) was significantly higher than that in the low expression group (25.00%) (P<0.05). Conclusion IL-17, IL-23, IL-22, and IL-11 all play certain roles in the pathogenesis and development of ulcerative colitis and can be used to evaluate disease severity to some extent. The expression level of IL-17 may have certain reference value for predicting mucosal healing prognosis after short-term treatment.

Full Text

Preamble

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Abstract

Objective: To detect the expressions of IL-17, IL-23, IL-22, and IL-11 in the intestinal mucosa of patients with ulcerative colitis (UC) and analyze their prognostic values.

Methods: Forty patients with active UC, 15 with UC in remission, and 15 healthy subjects were examined for the expressions and distribution of IL-17, IL-23, IL-22, and IL-11 in the colorectal mucosa using immunohistochemistry. We further collected data from 40 patients with active UC who received standardized treatment and regular follow-up. Based on endoscopic mucosal healing status after 2 months of standardized treatment, patients were divided into good mucosal healing and poor mucosal healing groups, and pretreatment mucosal expression levels of these cytokines were compared between the two groups.

Results: The expressions of all four cytokines in active UC patients were significantly higher than those in both the remission group and healthy controls (IL-17: 0.0727±0.0037 vs 0.0354±0.0243 vs 0.0330±0.0045; IL-23: 0.1407±0.0068 vs 0.0865±0.0051 vs 0.0442±0.0137; IL-22: 0.0522±0.0045 vs 0.0259±0.0063 vs 0.0115±0.0061; IL-11: 0.0479±0.0022 vs 0.0365±0.0024 vs 0.0232±0.0009; P<0.05). The expression levels of IL-17, IL-23, and IL-22 increased significantly with disease activity (IL-17: 0.0545±0.0072 vs 0.0786±0.0051 vs 0.0847±0.0197; IL-23: 0.1112±0.0046 vs 0.1480±0.0089 vs 0.1644±0.0190; IL-22: 0.0307±0.0063 vs 0.0548±0.0071 vs 0.0719±0.0056; P<0.05). In active UC patients, the expression levels of all four cytokines were positively correlated with endoscopic activity grade (P<0.05), and IL-17 and IL-22 expression levels were also positively correlated with histological grade (P<0.05). All four cytokines showed positive intercorrelations. The poor

mucosal healing rate was significantly higher in the high IL-17 expression group (66.67%) compared with the low IL-17 expression group (25.00%) ($P<0.05$).

Conclusion: Cytokines IL-17, IL-23, IL-22, and IL-11 all participate in the pathogenesis of UC and may serve as indicators for evaluating disease severity. The expression level of IL-17 may be a valuable predictor for mucosal healing after short-term treatment.

Key words: ulcerative colitis; interleukin 23; interleukin 17; interleukin 22; interleukin 11

Introduction

Immune abnormalities are considered a critical factor in the pathogenesis of ulcerative colitis and represent one of the most active research areas in this field. Cytokines play an indispensable role in this process, particularly the imbalance between pro-inflammatory and anti-inflammatory factors, which has become a major focus of UC research. IL-23 and IL-17, as the principal pro-inflammatory cytokines of the IL-23/IL-17 axis, have attracted considerable attention from scholars worldwide in recent years. Previous studies have suggested that IL-23 and IL-17 may be key pro-inflammatory mediators that trigger intestinal inflammation.

Although multiple studies have demonstrated elevated expression of IL-23 and IL-17 in both intestinal mucosal tissue and plasma of UC patients, the correlation between IL-23 and IL-17 expression and their relationship with prognosis regarding intestinal mucosal healing after treatment remain unclear. IL-22 and IL-11 have been confirmed to participate in the pathogenesis of various inflammatory and autoimmune diseases. Many studies have shown that IL-22 expression is enhanced in the plasma of IBD patients, and while IL-22 is associated with pro-inflammatory factor expression and may have pathogenic effects, recent research has gradually revealed its protective role against intestinal mucosal damage. IL-11, as a pleiotropic cytokine, has demonstrated anti-inflammatory effects in numerous in vivo and in vitro models, including colitis models.

However, the expression of IL-22 and IL-11 in the intestinal mucosa of UC patients, their relationship with disease activity and histopathological grading, and their impact on mucosal healing prognosis remain poorly defined. Therefore, this study investigated the distribution and expression levels of cytokines IL-23, IL-17, IL-22, and IL-11 in the intestinal mucosa of active UC patients to analyze their relationships with disease activity, endoscopic activity grading, histopathological grading, inter-cytokine correlations, and their influence on mucosal healing prognosis after short-term treatment, thereby exploring their roles and interrelationships in UC pathogenesis.

Methods

1.1 General Information

We initially enrolled 40 patients diagnosed with active UC at the Department of Gastroenterology, Nanfang Hospital, Southern Medical University between January 2008 and January 2015. Inclusion criteria were based on the "Consensus on Diagnosis and Treatment of Inflammatory Bowel Disease" established by the Inflammatory Bowel Disease Group of the Chinese Society of Gastroenterology at the 2012 Guangzhou Conference. Exclusion criteria included: (1) severe cardiac, hepatic, pulmonary, or renal disease; (2) severe infection or diabetes; (3) pregnancy or lactation; (4) other autoimmune diseases; and (5) treatment with biological agents.

Additionally, we selected 40 active UC patients who received standardized treatment and underwent colonoscopy re-examination after 2 months. Treatment protocols followed the same 2012 consensus guidelines. Based on endoscopic mucosal healing status after 2 months of treatment, patients were divided into a good mucosal healing group (20 cases) and a poor mucosal healing group (20 cases). Mucosal healing assessment was performed according to the "Therapeutic Endpoints Consensus" established by the American Gastroenterological Association (AGA) in 2007.

1.2 Reagents

Primary antibodies against IL-23, IL-17, IL-22, and IL-11 were purchased from GeneTex. The two-step immunohistochemistry detection kit (PV6001) was purchased from Beijing Zhongshan Golden Bridge Biotechnology.

1.3 Experimental Methods

1.3.1 Immunohistochemical Detection All specimens were fixed in 10% formalin and routinely embedded in paraffin. All paraffin blocks were sectioned into 4 m thick consecutive slices. After routine deparaffinization and hydration, endogenous peroxidase activity was blocked, antigens were retrieved by microwave, and sections were blocked before incubation with primary antibodies (rabbit anti-human IL-17, rabbit anti-human IL-23, rabbit anti-human IL-22, rabbit anti-human IL-11) overnight at 4°C. Secondary antibody (goat anti-rabbit IgG) was then applied, followed by DAB color development, hematoxylin counterstaining, neutral resin mounting, and microscopic examination after drying.

1.3.2 Result Determination Qualitative Immunohistochemical Assessment: Positive results were defined as brown or brownish-yellow granules in the cytoplasm. Immunohistochemical scores were calculated as the product of the percentage of positively stained cells and staining intensity, based on previous literature. The percentage of positively stained cells was scored as: <5%

= 0, 6%-25% = 1, 26%-50% = 2, 51%-75% = 3, and 76%-100% = 4. Staining intensity was scored as: no staining = 0, light brownish-yellow = 1, brownish-yellow = 2, and brown = 3. The final score was the product of these two values: 0-1 = negative (-), 2-4 = weakly positive (1+), 5-8 = moderately positive (2+), and 9-12 = strongly positive (3+). Scores of (2+) to (3+) were considered strongly positive. All results were examined twice, and discrepancies were resolved by re-examination. Negative and weakly positive results were classified as low expression, while strongly positive results were classified as high expression.

Semi-quantitative Immunohistochemical Assessment: Five non-overlapping high-power fields (×400) were selected from each section and analyzed using Image-Pro Plus 6.0 software to calculate mean absorbance values.

1.4 Statistical Methods

Data were analyzed using SPSS 20.0 software. Measurement data are expressed as mean ± standard deviation. One-way ANOVA was used for comparisons among active UC, remission UC, and healthy control groups. Pearson correlation analysis was used to assess correlations among IL-23, IL-17, IL-22, and IL-11 expression levels. Spearman correlation analysis was used to evaluate correlations between cytokine expression levels and endoscopic activity grading or histological grading in active UC. Chi-square test was used to compare pretreatment mucosal expression levels between good and poor mucosal healing groups. $P<0.05$ was considered statistically significant for all tests.

Results

2.1 General Data and Clinicopathological Characteristics of Enrolled Patients

In the first part of the study, 40 active UC patients were enrolled (23 males, 17 females; mean age 45.8±13.66 years), including 12 with mild, 19 with moderate, and 9 with severe disease activity. The remission UC group included 15 patients (9 males, 6 females; mean age 42.7±13.40 years). The healthy control group included 15 subjects (8 males, 7 females; mean age 43.5±14.27 years). There were no statistically significant differences in age or gender distribution among the three groups ($P>0.05$).

In the second part of the study, 40 active UC patients were enrolled, with 20 cases each in the good mucosal healing and poor mucosal healing groups. The pretreatment clinical characteristics of the two groups are shown in . There were no statistically significant differences between the two groups in terms of gender, age distribution, disease extent, disease activity, endoscopic activity grading, histological grading, or treatment modality ($P>0.05$), indicating comparability between groups.

2.2 Expression of IL-17, IL-23, IL-22, and IL-11 in Active UC Intestinal Mucosa

Positive expression of all inflammatory cytokines was observed to varying degrees in active UC mucosa, remission UC mucosa, and healthy control mucosa, with gradually decreasing intensity. IL-17, IL-23, IL-22, and IL-11 were primarily expressed in the cytoplasm of colonic epithelial cells and lamina propria mononuclear cells, appearing brownish-yellow.

2.3 Comparison of Cytokine Expression Levels in Intestinal Mucosa

The mean absorbance values of IL-17, IL-23, IL-22, and IL-11 expression were significantly higher in the active UC group than in both the remission UC group and healthy control group [Figure 1: see original paper] ($P<0.05$). Notably, IL-23 expression in the remission UC group was also significantly higher than in healthy controls ($P<0.05$,).

2.4 Cytokine Expression in Active UC Patients with Different Disease Activity Levels

The mean absorbance values of IL-17, IL-23, and IL-22 in intestinal mucosa increased significantly with disease activity among mild, moderate, and severe active UC groups (IL-17: 0.0545 ± 0.0072 vs 0.0786 ± 0.0051 vs 0.0847 ± 0.0197; IL-23: 0.1112 ± 0.0046 vs 0.1480 ± 0.0089 vs 0.1644 ± 0.0190; IL-22: 0.0307 ± 0.0063 vs 0.0548 ± 0.0071 vs 0.0719 ± 0.0056), with statistically significant differences among all three groups ($P<0.05$). Although IL-11 expression also increased with disease activity (0.0446 ± 0.0042 vs 0.0483 ± 0.0036 vs 0.0519 ± 0.0018), the differences among the three groups were not statistically significant ($P>0.05$, [Figure 2: see original paper]).

2.5 Correlation of IL-17, IL-23, IL-22, and IL-11 Expression with Endoscopic Activity Grading and Histological Grading in Active UC

In active UC patients, the mean absorbance values of IL-17, IL-23, IL-22, and IL-11 expression in intestinal mucosa were positively correlated with endoscopic activity grading ($r=0.613$, $r=0.438$, $r=0.649$, and $r=0.539$, respectively; all $P<0.05$). Additionally, IL-17 and IL-22 expression levels were positively correlated with histological grading ($r=0.426$ and $r=0.374$, respectively; $P<0.05$,).

2.6 Correlation Analysis of Cytokine Expression

The mean absorbance values of inflammatory cytokines IL-17, IL-23, IL-22, and IL-11 in colonic mucosa were normally distributed. Pearson correlation analysis revealed positive correlations among all cytokine expression levels, as detailed in the results.

2.7 Expression of IL-17, IL-23, IL-22, and IL-11 in Pretreatment Active UC Mucosa from Good vs Poor Mucosal Healing Groups

Statistical analysis revealed significant differences in IL-17 expression distribution between the good and poor mucosal healing groups. The poor mucosal healing rate was 66.67% in the high IL-17 expression group compared with 25.00% in the low IL-17 expression group, representing a statistically significant difference ($P<0.05$). In contrast, no significant differences were observed between the two groups in the expression levels of IL-23, IL-22, or IL-11 ($P>0.05$,).

Discussion

Previous immunological research on ulcerative colitis has primarily focused on the imbalance between Th1 and Th2 cell responses. The traditional view holds that UC is mainly associated with Th2 cell-derived cytokines IL-4, IL-5, and IL-13, which participate in humoral immunity. In recent years, novel CD4+ T helper cell subsets and their secreted cytokines have gained increasing attention and have been shown to play important immunomodulatory roles, mediating the development of inflammatory responses, autoimmune diseases, tumors, and transplant rejection. However, the precise roles of these cytokines in ulcerative colitis remain incompletely understood, providing new insights for the diagnosis and treatment of inflammatory bowel disease and representing a current hot topic in UC research.

In this study, we found that IL-23 and IL-17 were positively expressed in healthy controls, remission UC, and active UC intestinal mucosa, with gradually increasing expression levels. Previous studies have also detected low-level expression of IL-23 and IL-17 in normal intestinal mucosa, which may be related to their role in maintaining intestinal epithelial barrier integrity and inhibiting bacterial proliferation. However, expression levels of IL-23 and IL-17 in the lesioned mucosa of active UC patients were significantly higher than in both the remission UC group and healthy controls, consistent with previous reports. We hypothesize that under normal intestinal mucosal immunity, IL-23 and IL-17 primarily exert protective functions in the intestinal mucosa. When intestinal immune function becomes abnormal, increased secretion of pro-inflammatory factors such as IL-23 and IL-17 triggers inflammatory cascades that mediate excessive secretion by other inflammatory cells, disrupting the intercellular regulatory network within the immune system and leading to strong and persistent immune responses that cause UC onset and chronicity. The specific roles of IL-23 and IL-17 depending on other environmental factors require further investigation. Notably, we found that IL-23 expression remained elevated in remission UC compared with healthy controls, suggesting that mucosa may remain in a low-grade inflammatory state during clinical remission. Previous studies have shown that even after achieving endoscopic mucosal healing, 50% of remission UC patients still exhibit varying degrees of inflammatory changes in intestinal mucosal tissue. Whether IL-23 expression in remission UC is associated with low-grade mucosal inflammation

and whether it promotes tissue repair or induces inflammatory exacerbation leading to relapse remains unclear and warrants further investigation.

We found that IL-22 expression was higher in active UC intestinal mucosa than in remission UC and healthy controls, consistent with previous reports of elevated IL-22 in UC patient blood samples. Furthermore, we confirmed for the first time that IL-11 expression is significantly increased in active UC compared with healthy controls, suggesting its involvement in UC pathogenesis.

Additionally, we observed that IL-23, IL-17, and IL-22 expression gradually increased with disease activity index. These cytokines were positively correlated with endoscopic activity grading, and IL-17 and IL-22 were also positively correlated with histological grading. We infer that IL-23, IL-17, and IL-22 are closely related to disease activity and mucosal damage severity, and their expression levels in intestinal mucosa may reflect the severity of UC inflammation to some extent. Although IL-11 expression in active UC was also significantly higher than in remission UC and healthy controls, the differences among mild, moderate, and severe active UC groups were not statistically significant, possibly due to the small sample size and requiring validation through expanded cohorts.

In recent years, mucosal healing has gradually replaced clinical remission as the primary therapeutic endpoint in ulcerative colitis. Repairing the damaged intestinal mucosal barrier to achieve and maintain complete mucosal healing is crucial for UC treatment. Early studies have shown that mucosal healing after short-term treatment predicts disease recurrence, with patients achieving mucosal healing having higher long-term remission rates and lower relapse risks. Additionally, achievement of mucosal healing has been associated with reduced cancer risk, lower surgical rates, and improved quality of life, and its importance is widely recognized despite ongoing controversies. Therefore, early prediction of mucosal healing status would facilitate better treatment strategy development. In clinical practice, we observe that some UC patients achieve clinical remission and endoscopic mucosal healing after treatment, while others remain endoscopically active despite clinical improvement. The factors underlying these individual differences in mucosal response to therapy remain unclear, and few studies have addressed predictive indicators for mucosal healing.

This study is the first to explore the relationship between pretreatment inflammatory cytokine expression levels and mucosal healing status. We found that poor mucosal healing was significantly more common in the high IL-17 expression group than in the low expression group. Using chi-square analysis, we excluded potential confounding effects of gender, age distribution, disease extent, disease activity, endoscopic grading, histological grading, and treatment modality. Therefore, we propose that high IL-17 expression may have predictive value for mucosal healing prognosis after short-term treatment, though validation in larger cohorts is needed to assess its specific predictive value.

In summary, IL-17, IL-22, IL-23, and IL-11 all play roles in UC pathogenesis and may serve as indicators for evaluating disease severity. IL-17 expression

levels may have predictive value for mucosal healing prognosis after short-term treatment. The precise mechanisms of these cytokines require further in-depth investigation. This preliminary analysis of cytokine expression differences and their relationships with disease activity and mucosal healing prognosis provides a foundation for future research, with the goal of identifying valuable indicators for disease assessment and mucosal healing prediction to guide clinicians in comprehensive disease management and earlier implementation of more aggressive therapeutic strategies to promote mucosal healing.

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Note: Figure translations are in progress. See original paper for figures.