ORIGINAL RESEARCH

Correlation of Macrophages with Inflammatory Reaction in Ulcerative Colitis and Influence of Curcumin on Macrophage Chemotaxis

Yalan Chen, MD; Dalei Chen, MD; Chuanhe Zhou, MD; Xiaocang Cao, PhD; Juan He, PhD

ABSTRACT

Objective • Our study aimed to elucidate the correlation of macrophage (mø) with the inflammatory reaction in ulcerative colitis (UC) and the influence of curcumin (Cur) on mø chemotaxis in mice with UC.

Methods • A total of 49 patients with UC (research group; RG) admitted between June 2020 and October 2021 and 56 healthy individuals (control group; CG) who visited concurrently were selected as the study participants. The peripheral blood mononuclear cells (PBMCs) were analyzed, and M1-type/M2-type mø and inflammatory factors (IFs) interleukin (IL)-1, IL-6, IL-10, tumor necrosis factor alpha (TNF-α) and transforming growth factor beta (TGF-β) were detected. In addition, 15 BALB/c mice were purchased and divided into the normal group fed normally, the UC model group established with sodium dextran sulfate (DSS) and the Cur group induced by DSS + Cur feeding. Colon tissue mø was collected from mice to measure mø activity via CCK-8 and to quantify levels of IFs and chemokine CCL2 by polymer chain reaction (PCR)c and Western blotting.

Results • The RG had a higher percentage of peripheral blood M1-type mø and a lower percentage of M2-type mø and M1/M2 mø ratio than the CG ($P < .05$). In the RG, IL-1, IL-6 and TNF-α all increased and were inversely correlated with the ratio of M1/M2 mø, while IL-10 and TGF-β decreased, with a positive connection with the M1/M2 mø ratio. In the UC model mice, mø activity increased, but the apoptosis rate decreased. mø activity was lower in the Cur group than in the model and normal groups; mø apoptosis in the Cur group was higher than in the model group but lower than in the normal group. In addition, proIFs increased and anti-IFs decreased in the model group, and Cur also ameliorated this process. Finally, CCL2 and MCP-1 levels in the model group were also increased, while those in the Cur group were lower compared with the model group.

Conclusion • In UC, the M1/M2 mø ratio is severely misadjusted, activation of M1-type mø is enhanced and pro-IFs are released in large quantities. Cur can ameliorate the abnormal activation of mø in mice with UC, inhibit mø chemotaxis and alleviate the inflammatory reaction, which may make it a new option for UC treatment in the future. (*Altern Ther Health Med.* 2023;29(2):97-103)

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INTRODUCTION

Ulcerative colitis (UC) is a chronic non-specific intestinal inflammatory disease, mainly involving the rectum and colon, with the characteristic of repeated attacks; it is extremely difficult to cure and is considered one of the main pathogenic factors in colon cancer.[1,2] At present, the global average incidence of UC is approximately 8 to 15 per 100 000 population, with an equal risk in all age groups.[3,4] The etiology of UC has not been fully clarified, but it is clinically

believed to result from the interaction of the environment, genetics and intestinal microbes and immunity, among which the inflammatory reaction caused by the imbalance of the intestinal mucosal immune system is considered to be the most important link.[5] Therefore, immunomodulation is a research focus in the field of UC.[6,7]

Macrophages (mø), which are important research targets that are involved in phagocytosis, cellular immunity and molecular immunology in the human body, may be extremely critical in the pathogenesis of UC,[8] and may also provide a breakthrough for treating UC in the future. Therefore, an in-depth understanding of the relationship between mø and UC has great clinical implications. In addition, curcumin (Cur), as a diketone compound extracted from the rhizomes of some plants in the Zingiberaceae and Araceae families, has an extremely high safety profile, and has achieved excellent treatment results in many fields.[9,10] In the medical field, Cur also exhibits excellent anti-inflammatory, antioxidant, anti-tumor and immunomodulatory effects.[11] Moreover, previous studies also confirmed that Cur adjuvant therapy in UC can improve patients' clinical results.[12] It can be seen that Cur may play a potential role in pathological improvement in UC, but further confirmation is still needed.

Consequently, by analyzing the relationship between mø and UC, as well as the pathological improvement benefit of Cur in UC, this study will provide novel references for future clinical diagnosis and treatment, and provide a more reliable guarantee for the quality of life of patients with UC.

MATERIALS AND METHODS

Study Participants

This study included 49 patients with UC (the research group; RG) and 56 healthy controls (control group; CG) admitted between June 2020 and October 2021. Signed informed consent was obtained from all study participants.

Inclusion criteria. Patients: (1) were age 18 to 70 years; (2) exhibited the pathological manifestations of UC[13] and were diagnosed after examination in our hospital (3) had active symptoms.

Exclusion criteria. Patients: (had: infectious enteritis, radiation enteritis, ischemic colitis, Crohn's disease or other types of colon diseases; (2) had cardio-cerebrovascular, autoimmune dysfunction or neoplastic diseases; (3) had abnormal liver and kidney function; (4) were pregnant or lactating.

Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

At admission, 10 mL of peripheral blood from both groups of patients was drawn into anticoagulation tubes and centrifuged (700 × g, 4°C) for 10 min to separate the plasma and placed in cryopreservation tubes. After the remaining blood was diluted 1:1 with normal saline and added to the PBMC separation solution, the mixture was slowly added along the wall of the centrifugal tube (1:1) and centrifuged (400 × g; 4°C) for 30 minutes. The second annular milky white PBMC layer was placed in a centrifuge tube containing 15 mL of normal saline, centrifuged (700×g; 4°C) for 15 minutes, and the PBMCs were isolated after supernatant removal.

mø Detection

Flow cytometry (FCM) detected M1-type/M2-type mø in the PBMCs of the patients. The M1 cell marker antibodies were clusters of differentiation antigen 68 (CD68) and chemokine-C receptor-2 (CCR2), while the M2 cell marker antibodies were clusters of differentiation antigen 163 (CD163) and chemokine-X3C receptor-1 (CX3CR1).

ELISA Detection

A 5 ml-sample of peripheral blood was taken from both patient cohorts at the time of admission, placed in coagulation promoting tubes and centrifuged (1505 × g; 4°C) for 20 min after standing for 60 minutes in order to obtain serum. Interleukin (IL)-1, IL-6, IL-10, tumor necrosis factor alpha (TNF-α) and growth transforming factor beta (TGF-β) were evaluated according to the ELISA kit instructions (Hanzhou MultiSciences Biotech, Lianke, China).

Animal Data

15 BALB/c mice of SPF grade were ordered from Jiangsu Rec-Biotech (SYXK [Su] 2021-0062), with a body weight of 20 to 25 g and age 6 to 8 weeks. Every 2 mice were placed in a cage, and all mice were allowed to eat and drink freely and raised in an environment of 20°C to 26°C and 40% to 70% humidity under a 12:12-hour light-dark regime. Approval from the Animal Ethics Committee of our hospital was obtained before conducting the animal experiments.

Grouping and Intervention

After 7 days of adaptive feeding, mice were randomly assigned to one of three groups, with 5 mice in each group. One group was fed normally without treatment (normal group); the second group, based on the modeling method of Cui, et al.[14] used 3% dextran sodium sulfate (DSS) to establish a UC model (model group); the third group was given 0.4 mL Cur (50 mg/kg) by gavage daily while UC modeling was performed. After 7 days of continuous treatment, all mice were sacrificed under anesthesia.

mø Screening and Separation

Single cell suspensions prepared from mouse colon tissue were detected by FCM, and a negative control and fluorescence supplement were set. Mouse colon tissue mø was screened and collected according to mø surface markers F4/80 and CD68/CD11b.

mø Activity Assay

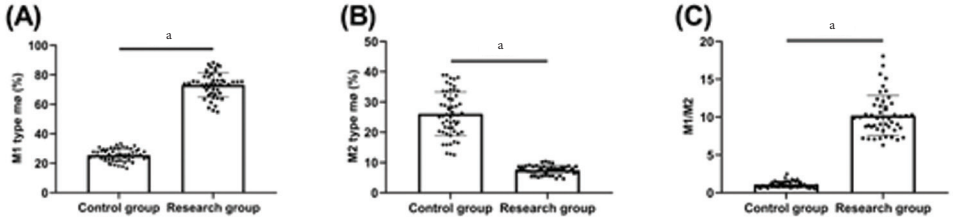
After being inoculated into 96-well plates at 100 μL/well, the cell suspension was cultured in a 5% CO_2 incubator at 37°C for 48 hours, followed by the addition of 10 μL CCK-8 solution into the wells. The absorbance in each well was detected by a microplate reader (450 nm) after another 4 hours of cultivation.

Table 1. Primer Sequence

	Forward (5'-3')	Reverse (5'-3')
IL-1	TTCGAGGCACAAGGCACAA	CCATCATTTCACTGGCGAGC
IL-6	AGGGCTCTTCGGCAAATGT	GAAGAAGGAATGCCCATTAACAAC
IL-10	CTGCTCTGTTGCCTGGTCCTC	TCTCGAAGCATGTTAGGCAGGT
TNF-α	CTCATCTACTCCCAGGTCCTCTTC	CGATGCGGCTGATGGTGTG
TGF-β	CTCCACCTGCAAGAC	CTGGCGAGCCTTAGTTTGGA
GAPDH	GCCTTCCGTGTCCCCACTGC	CAATGCCAGCCCCAGCGTCA

Abbreviations: IL, interleukin; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha.

Figure 1. Comparison of peripheral blood M1/M2 mø ratio. (**1A**) Comparison of M1-type mø between the research group and the control group. (**1B**) Comparison of M2-type mø between the research group and the control group. (**1C**) Comparison of M1/M2 mø ratio between the research group and the control group.



[a]$P < .001$

Abbreviations: mø, microphage.

mø Apoptosis Rate Detection

Cells were digested by 0.25% trypsin, and the cell suspension was collected after complete shedding of the cells. An appropriate amount of incubation buffer was then added to the centrifuge tube. AnnexinV-FITC was added after the cells were suspended, and the cells were incubated in a dark room for 10 minutes. Following addition and thorough mixing of propidium iodide (PI) staining solution, buffer solution and cell solution, the apoptosis rate was measured via FCM.

mø Inflammatory Factor Detection

Trizol® extracted total RNA from cells, and after verifying the purity, reverse transcription was carried out according to the reverse transcription kit manual to obtain template cDNA. The quantitative polymerase chain reaction (qPCR) kit amplified the target genes with GAPDH as internal reference (the primer sequences were constructed and synthesized by Tsingke Biotech; see Table). Relative expression of target genes was computed by $2^{-\Delta\Delta CT}$.

mø Chemokine Assay

The total protein was extracted by adding cell lysate to the cells, and the protein concentration was determined by Pierce™ BCA Protein Assay Kit (Thermo Fisher, Waltham, Massachusetts, USA). After membrane transfer by SDS-PAGE, the proteins were immersed in primary antibodies and incubated at 4°C overnight. The second antibody was added the next day, following which enhanced chemiluminescence (ECL) was used for development. ImageJ (National Institutes of Health, USA) was used to analyze target bands' gray values.

Statistical Analysis

Statistical analysis was performed with IBM® SPSS version 22.0 software. Categorical data was expressed as [n(%)] and compared between groups with the chi-square test. Quantitative data, expressed as ($\bar{\chi} \pm s$), were analyzed using the single factor analysis of variance and LSD post hoc test among multiple groups. The Pearson correlation coefficient was responsible for correlation analysis. $P < .05$ indicated statistical significance.

RESULTS

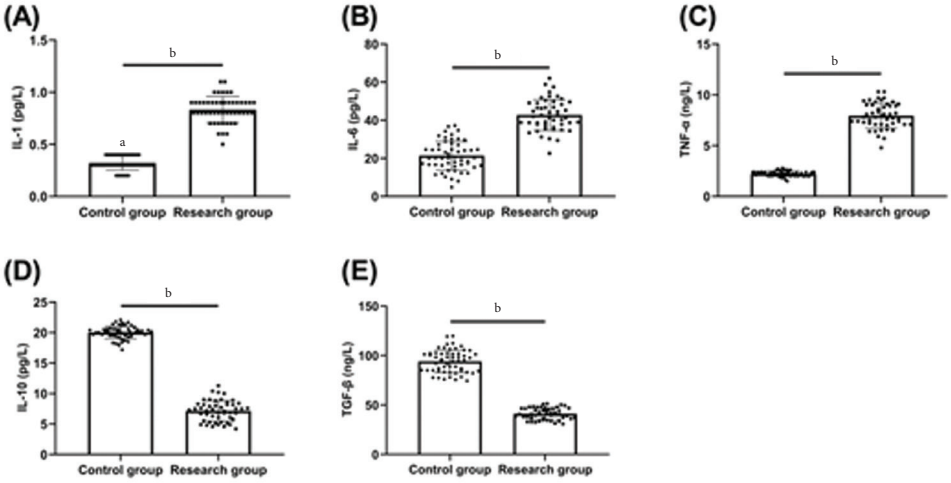
Comparison of Peripheral Blood M1/M2 mø Ratio

The peripheral blood M1-type mø in the RG was 73.25 ± 8.29 %, significantly higher than in the CG ($P < .05$; see Figure 1A). The peripheral blood M2-type mø in the RG was 7.49 ± 1.43 %, which was lower than the CG ($P < .05$; see Figure 1B). Intergroup comparison of the M1/M2 mø ratio revealed a lower ratio in the RG compared with the CG ($P < .05$; see Figure 1C).

Comparison of Peripheral Blood Inflammatory Factors (IFs)

The peripheral blood IL-1 content in the RG was 0.83 ± 0.13 pg/L, higher than in the CG (0.32 ± 0.07 pg/L) ($P < .05$; see Figure 2A). The IL-6 content was also higher in the RG (42.78 ± 8.13 pg/L) compared with the CG ($P < .05$; see Figure

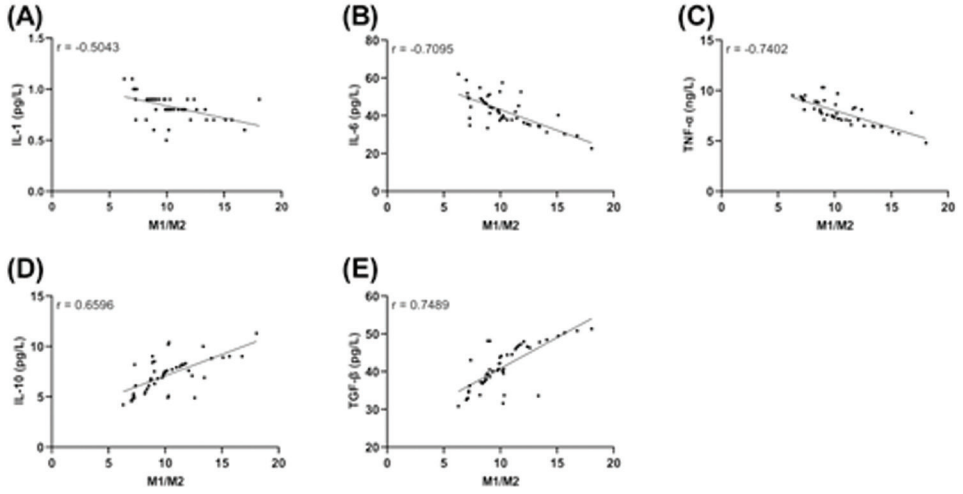
Figure 2. Comparison of peripheral blood IFs. (**2A**) Comparison of IL-1 in the research group and the control group. (**2B**) Comparison of IL-6 in the research group and the control group. (**2C**) Comparison of TNF-α in the research group and the control group. (**2D**) Comparison of IL-10 in the research group and the control group. (**2E**) Comparison of TGF-β in the research group and the control group.



[a]$P < .05$
[b]$P < .001$

Abbreviations: IF, inflammatory factor; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.

Figure 3. Correlation between M1/M2 mø ratio and IFs. (**3A**) Correlation between M1/M2 mø ratio and IL-1. (**3B**) Correlation between M1/M2 mø ratio and IL-6. (**3C**) Correlation between M1/M2 mø ratio and TNF-α. (**3D**) Correlation between M1/M2 mø ratio and IL-10. (**3E**) Correlation between M1/M2 mø ratio and TGF-β.



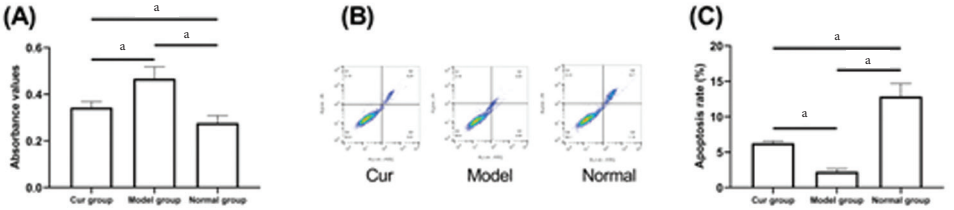
Abbreviations: IF, inflammatory factors; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.

2B). A similar trend was observed in peripheral blood TNF-α content; namely, the RG had a higher TNF-α level than the CG ($P < .05$; see Figure 2C). The peripheral blood IL-10 level in the RG was 7.16 ± 1.69 pg/L, which was lower compared with the CG (20.02 ± 1.04 pg/L) ($P < .05$; see Figure 2D). Finally, the TGF-β level was determined to be lower in the RG (41.14 ± 5.77 ng/L) compared with the CG ($P < .05$; see Figure 2E).

Correlation of M1/M2 mø Ratio with IFs in Patients with UC

According to Pearson correlation coefficient analysis, the M1/M2 mø ratio in the RG was negatively correlated with IL-1, IL-6, and TNF-α ($P < .05$; see Figure 3A-3C), but positively correlated with IL-10 and TGF-β ($P < .05$; see Figure 3D and 3E). It is suggested that the increase in the M1/M2 mø ratio also predicts the decrease of pro-IFs and the increase of anti-IFs.

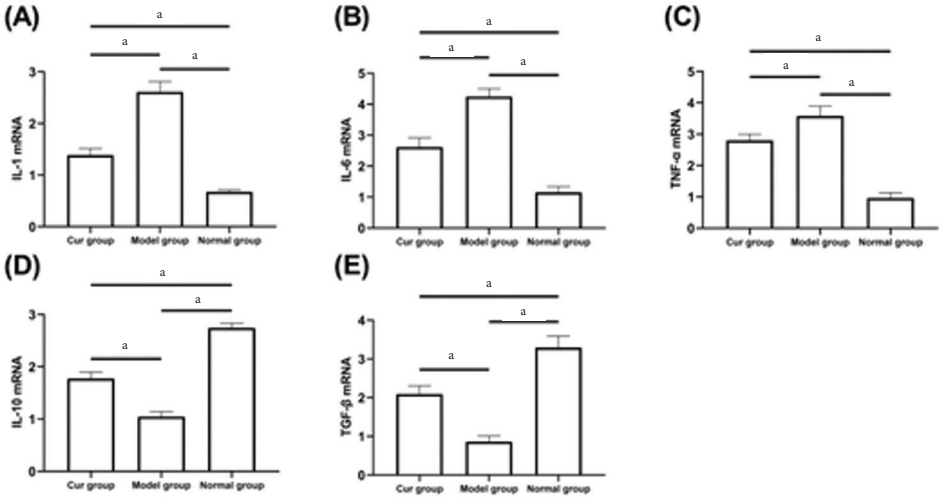
Figure 4. Influence of Cur on mø in mice with UC. (**4A**) Effect of Cur on mø activity in mice with UC. (**4B**) Flow cytometry detection of mø apoptotic rate. (**4C**) Effect of Cur on the apoptosis rate of mø in mice with UC.



[a]P <.05.

Abbreviations: Cur, curcumin; mø, microphage; UC, ulcerative colitis.

Figure 5. Impact of Cur on IFs in mø of mice with UC. (**5A**) Impact of Cur on IL-1 in mø of mice with UC. (**5B**) Impact of Cur on IL-6 in mø of mice with UC. (**5C**) Impact of Cur on TNF-α in mø of mice with UC. (**5D**) Impact of Cur on IL-10 in mø of mice with UC. (**5E**) Impact of Cur on TGF-β in mø of mice with UC.



[a]P <.05.

Abbreviations: Cur, curcumin; IFs, inflammatory factors; IL, interleukin; mø, microphage; TGF, transforming growth factor; TNF, tumor necrosis factor; UC, ulcerative colitis.

Influence of Cur on mø in Mice with UC

In the mouse experiment, we first detected the activity of mouse mø, and found that the absorbance value in the model group was 0.47 ± 0.05, which was the highest among the 3 groups. However, the absorbance in Cur group mouse cells (0.34 ± 0.03) was lower compared with the model group and higher compared with the normal group (P < .05; see Figure 4A). Subsequently, the FCM results (see Figure 4B) showed that the apoptosis rate in the model group was 2.24 ± 0.39%, which was the lowest among the 3 groups, while the apoptosis rate in the Cur group was higher than that in the model group and lower than in the normal group (P < .05; see Figure 4C).

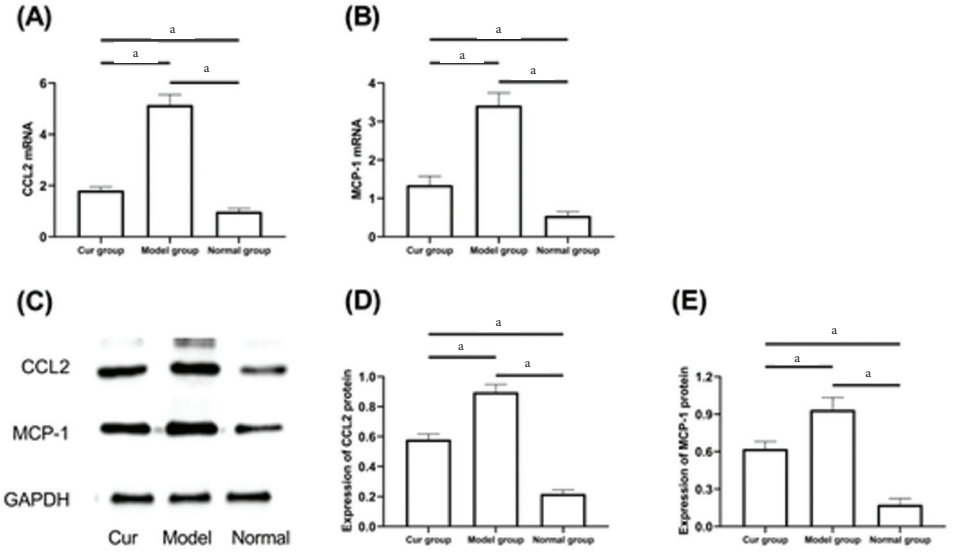
Impact of Cur on IFs in mø of Mice with UC

In a similar fashion, in the detection of IFs in mice with UC mø, IL-1, IL-6 and TNF-α in the model group were 2.61 ± 0.20, 4.25 ± 0.25 and 3.58 ± 0.31, respectively, all of which were the highest among the 3 groups. While the levels in the normal group were 0.68 ± 0.04, 1.15 ± 0.19 and 0.95 ± 0.17, respectively, which were the lowest among the 3 groups (P < .05; Figure 5A-5C). In addition, IL-10 and TGF-β levels in the Cur group were 1.78 ± 0.12 and 2.09 ± 0.22, respectively, higher than in the normal group and lower than in the model group (P < .05; Figure 5D, 5E).

Influence of Cur on mø Chemokines in Mice with UC

Finally, we examined the impact of Cur on mø chemotaxis in mice with UC. PCR results showed that CCL2 and MCP-1 mRNA levels in the model group were 5.14 ± 0.41 and 3.41 ± 0.33, respectively, while those in the normal group were 0.98 ± 0.12 and 0.54 ± 0.11, and the corresponding levels in the Cur group were 1.81 ± 0.14 and 1.34 ± 0.24, respectively. The CCL2 and MCP-1 mRNA levels were highest in the model group, followed in descending order by the Cur group and

Figure 6. Influence of Cur on mø chemokines in mice with UC. (**6A**) CCL2 expression was detected by PCR. (**6B**) MCP-1 expression was detected by PCR. (**6C**) Western blot map of chemokines. (**6D**) Western blot was used to detect CCL2 protein expressions. (**6E**) Western blot was used to detect MCP-1 protein expressions.



[a]P <.05.

Abbreviations: Cur, curcumin; PCR, polymer chain reaction; UC, ulcerative colitis.

the normal group (P < .05; see Figure 6A). Similar findings were determined by Western blot in terms of CCL2 and MCP-1 protein levels; ie, the model group had the highest CCL2 0.90 ± 0.05 and MCP-1 0.93 ± 0.10 protein levels among the 3 groups, while CCL2 and MCP-1 protein levels in the Cur group were 0.58 ± 0.04 and 0.62 ± 0.06, respectively, higher than in the normal group and lower than in the model group (P < .05; Figure 6B and 6C).

DISCUSSION

Previous studies have found that the maintenance of normal mucosal immune defense and homeostasis by mø is disrupted in UC.[15] The change in mø balance can not only stimulate the massive release of IFs, but also induce local PBMCs to release a large amount of interferon.[16,17] Thus, mø may play a crucial role in the pathogenesis of UC.

In this study, we found that M1-type mø in the RG was evidently higher compared with the CG, while the M2-type mø and M1/M2 ratio were statistically lower, indicating that mø was in an obviously imbalanced state during the course of UC. This is also consistent with previous research results,[18] which further proves that mø is strongly linked to the occurrence and development of UC. As we all know, mø, as phagocytes, can kill foreign pathogens through phagocytosis; they can also release pro-IFs, activate other immune cells and initiate the inflammatory response, and the type of mø that can promote inflammation is M1.[19] After the elimination of pathogens, M2-type mø helps inhibit the recruitment of inflammatory cells, and promotes angiogenesis and tissue repair, thus restoring the tissue to its original state.[20] Once the ratio of M1/M2 is unbalanced, continuous M1-type mø activation can cause tissue damage and lead to inflammatory diseases, while M2-type mø overactivation may result in fibrosis due to excessive tissue repair, and even promote tumor growth through immunosuppression.[21] After further detecting the expression of IFs in the peripheral blood in both groups, we also found that the pro-IFs IL-6, IL-8 and TNF-α in the RG were all increased and negatively correlated with the M1/M2 ratio, while the anti-IFs IL-10 and TGF-β were decreased and positively correlated with the M1/M2 ratio. Therefore, we can confirm that the pathological process of UC is strongly linked to M1-type mø overactivation.

In addition, Cur is shown to inhibit the pathological changes of UC,[22] but the specific mechanism is still unclear. This study revealed that compared with normal mice, mø activity in the UC model mice was increased, while apoptosis was reduced, indicating that the life cycle of mø in UC also underwent abnormal changes and its activity cycle was significantly prolonged. However, after the use of Cur, mø activity in the Cur group decreased and apoptosis increased, which tended to the state of the normal group, suggesting that Cur can effectively regulate the activation state of mø. Furthermore, further detection of the inflammatory status of mø in each group of mice was consistent with previous clinical trial results, that is, the pro-IFs in the model group increased, while the anti-IFs decreased, which also suggested that M1-type mø activation was also more obvious in mice with UC.

Cur also ameliorated this process, inhibited the expression of IFs and promoted the release of anti-IFs. In previous studies, the regulatory effect of Cur on the

inflammatory reaction has been repeatedly confirmed with a high safety profile,[23,24] and the alleviation of inflammatory responses in UC also indicates the therapeutic potential of Cur. In the anti-inflammatory studies on Cur, cell chemotaxis has always been a research focus. For example, Cur blocks the chemotaxis of central granulocytes by inhibiting the signal transduction of the IL-8 receptor,[25] and reduces the secretion of chemokines by suppressing the activation of Kupffer cells.[26] Therefore, for mø, cell chemotaxis is one of the important processes in modulating the inflammatory response.[27] We speculated that the impact of Cur on mø may also be related to cell chemotaxis. Therefore, we finally detected the expression of mø chemokines CCL2 and MCP-1 in mice in each group, and the results were consistent with our expectation; that is, CCL2 and MCP-1 in the model group were significantly increased, while those in the Cur group were decreased, suggesting that Cur can inhibit mø chemotaxis.

We need to conduct *in vitro* experiments in a follow-up study to further confirm the specific regulatory mechanism of Cur on mø. Meanwhile, clinical research should be carried out as soon as possible to confirm the therapeutic effect of Cur in UC, so as to provide more reliable information for its clinical use.

CONCLUSION

In UC, the M1/M2 mø ratio is severely misadjusted, the activation of M1-type mø is enhanced and large quantities of pro-IFs are released. Cur can ameliorate the abnormal activation of mø in mice with UC, inhibit mø chemotaxis and alleviate the inflammatory reaction, and may become a new choice for UC treatment in the future.

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