**Name of journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO: 30919**

**Manuscript Type: ORIGINAL ARTICLES**

***Basic Study***

**Therapeutic effect of curcumin treated experimental colitis by inhibiting CD8+CD11c+ cells**

Zhao HM *et al*. Curcumin inhibited CD8+CD11c+ Cells in colitis mice

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**Author contributions:** Zhao HM and Han F contributed equally to this work as co-first author; Zhao HM and Liu DY designed the research; Zhao HM, Han F, Xu R, Huang XY, Cheng SM, Huang MF, Yue HY, Wang X and Zou Y performed the research; Liu DY contributed to the new reagents and analytic tools; Zhao HM, Huang XY,Xu HL and Liu DY analyzed the data; Zhao HM and Liu DY wrote the paper.

**Supported by** the Projects of National Natural Science Foundation of China, No. 81260595 and No. 81460679; Chinese Scholarship Council and Jiangxi Province as visiting scholar, No. 201408360106 and No. 201408360110; the Traditional Chinese Medicine project of Health Department of Jiangxi Province, No. 2015B049; and the Project of Jiangxi University of Traditional Chinese Medicine, No. JZYC15S13.

**Institutional animal care and use committee statement:** Mice were purchased from the Animal Center of Peking University Health Science Center (The animal certificate number was SCXK 2012-0001). All animals were housed in a specific pathogen-free environment at the animal facilities [No. SYXK (Gan) 2005-0001] of Jiangxi University of Traditional Chinese Medicine. The experimental protocols (JZ2015-16) were approved by Biomedical Ethics Committee Experimental Animal Ethics Branch of Jiangxi University of Traditional Chinese Medicine.

**Conflict-of-interest statement:** All authors have declared that there is no conflict of interests.

**Data sharing statement:** No additional unpublished data are available.

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**Manuscript source:** Invited manuscript

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**Received:** October 22, 2016

**Peer-review started:** October 25, 2016

**First decision:** December 2, 2016

**Revised:** December 25, 2016

**Accepted:** January 17, 2017

**Article in press:**

**Published online:**

**Abstract**

***AIM***

To verify whether curcumin (Cur) can treat inflammatory bowel disease by regulating CD8+CD11c+ cells.

***METHODS***

We evaluated suppressive effect of Cur on CD8+CD11c+ cells in spleen and Peyer’s patches (PPs) in colitis induced by trinitrobenzene sulfonic acid. Colitis mice were treated by 200 mg/kg of Cur for 7 d. On the 8th day, the therapeutic effect of Cur was evaluated by visual assessment and histological observation, while costimulatory molecules of CD8+CD11c+ cells in the spleen and PPs were measured by flow cytometry. The levels of interleukin-10 (IL-10), interferon-gamma (IFN-γ) and transforming growth factor-β1 (TGF-β1) in spleen and colonic mucosa were analyzed by enzyme-linked immunosorbent assay (ELISA).

***RESULTS***

The disease activity index, colonic weight, weight index and histological score of colonic of experimental colitis were obviously decreased after Cur treatment, while the body weight and colonic length were remarkably recovered. After treatment with Cur, totalities of CD8+CD11c+ cells were decreased in the spleen and PPs, and the expressions of major histocompatibility complex II (MHC II), CD205, CD40, CD40L and intercellular adhesion molecule-1 (ICAM-1) were inhibited. Meanwhile, IL-10, IFN-γ and TGF-β1 levels were increased as compared with colitis mice without treatment.

***CONCLUSION***

Cur effectively treated experimental colitis, which was realized by inhibiting CD8+CD11c+ cells.

**Key words:** Curcumin; Experimental colitis; Therapeutic effect; CD8; CD11c

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**Core tip:** CD11c is a known and specific labeled molecule of dendritic cells (DCs), and high-expressed in DCs, including CD8+ DCs and CD8- DCs. Over-accumulation of CD8+ DCs in colonic mucosa induced inflammatory injury in experimental colitis and human inflammatory bowel disease (IBD) patients. Maybe, CD8+CD11c+ cell is a potential therapeutic strategy to explore the mechanism of medicines treated IBD. As an immune suppressant, curcumin (Cur) plays a therapeutic role to treat various immune disease included IBD and rheumatoid arthritis. However, it is unclear whether Cur can regulate level of CD8+CD11c+ cells to treat IBD. In the present study, we found that therapeutic effect of Cur treated experimental colitis was closely related with the decreased level of CD8+CD11c+ cells.

Zhao HM, Han F, Xu R, Huang XY, Cheng SM, Huang MF, Yue HY, Wang X, Zou Y, Xu HL, Liu DY. Therapeutic effect of curcumin treated experimental colitis by inhibiting CD8+CD11c+ cells. *World J Gastroenterol* 2017; In press

**Introduction**

As one of integrin family, CD11c is a type I transmembrane protein which can mediate adherency between leukocyte and endotheliocyte, participate in exudation and phagocytosis of leukocyte. It is hinted that CD11c maybe induce tissues injury and inflammatory response[1]. Importantly, CD11c is a known and specific labeled molecule of dendritic cells (DCs), and high-expressed in DCs, including CD8+ DCs and CD8- DCs[2].

As professional antigen-presenting cells, DCs precursors capture antigens and promote T cell migration to regions of the draining lymph nodes where they can mature into functional DCs and present antigens to initiate T cell-mediated immune responses[3]. Increasingly, researches have reported that DCs are critical to maintaining intestinal immunity and mucosal immune tolerance to resist the pathogenicity of commensal microorganisms, which is one of the pivotal inflammatory etiologies of induced inflammatory bowel diseases (IBD)[4].

High-expression of costimulatory molecules and MHC II of DCs, which is a known marker of DC maturation, and a “danger signal” of induced inflammatory mucosal damage in the gut, occurred in the colonic mucosa of colitis animal models[5,6]. Moreover, DCs can develop from both myeloid and lymphoid progenitors. In the mouse, CD8+ DCs had been designated as “lymphoid” DCs, and CD8- DCs as “myeloid” DCs[7]. More importantly, CD8+ DCs predominantly stimulate Th1-inducing cytokines like IL-12p70 and IL-12p40, which can lead to Th1 differentiation[8], and have been reported to play a key role in controlling viral infection[3,9,10]. Over-accumulation of CD8+ DCs was also found to induce inflammatory injury in the colonic mucosa when they migrated into PPs of experimental colitis and human IBD patients[11,12]. Thus, CD8+ DCs may be a potential therapeutic target to explore the mechanisms of clinical treatment of IBD.

Many previous studies had indicated that CD11c expressed in DCs can promote maturity and activation of DCs, and present antigen for CD4+ or CD8+ T cells, and accelerate T cell activation and proliferation, and produce kinds of cytokines[13-15]. While CD11c+ DCs were depleted by diphtheria toxin administration during Oligodeoxynucleotides (ODNs) containing unmethylated cytosine–guanosine (CpG) (CpG-ODN) treated experimental colitis induced indirectly by CD4+ CD62L+ T cells[16]. These results hinted that CD11c+ DCs play an important role in pathogenetic process of IBD.

Curcumin (Cur) is the major constituent of turmeric powder that is extracted from the rhizomes of *Curcuma longa* L., and known as such in China, India and Southeast Asia. Cur has a long history of effectively treating chronic colitis by blocking NF-κB signaling in human IBD and experimental colitis, including trinitrobenzene sulfonic acid (TNBS)-induced and dextran sulfate sodium (DSS)-induced experimental colitis[17-19].Multifunctional Cur has exhibited anti-oxidant, anti-inflammatory, anti-mutagenic, and anti-carcinogenic activities, as well as anti-platelet, hypoglycemic, cholesterol lowering, anti-bacterial, wound-healing and anti-fungal effects[17,20-22]. In addition, Shirley *et al*[23]*,* had previously shown that Cur prevented DCs from responding to immune-stimulants and DC-mediated induction of CD4+ T cell proliferation by blocking maturation marker expression, cytokine and chemokine expression, and reducing both migration and endocytosis. Shirley *et al*[23]*,* concluded that Cur might play a therapeutic role as an immune suppressant in the treatment of various immune disease including IBD and rheumatoid arthritis. In our previous study, we found that Cur repaired colonic structure, decreased colonic weigh and histological injury score, recovered colonic length, indicating that Cur effectively restored damaged colonic mucosa in mice with TNBS-induced colitis[24]. However, it is unclear whether Cur can regulate the expression levels of CD8+CD11c+ cells to treat IBD.

In the present study, we investigated the effects of Cur on CD8+CD11c+ cells in the spleen and PPs in a murine model of colitis that was induced by TNBS to explore the possible mechanisms of Cur in the treatment of experimentally-induced IBD.

**Materials and Methods**

***Mice***

Nine-twelve week-old male C57BL/6 mice (20–24 g), were purchased from the Animal Center of Peking University Health Science Center (animal certificate number: SCXK 2012-0001). Mice were housed in a special room with a humidity of 50% ± 5%, and an equal 12 h light / dark cycle at 20 ± 2 °C throughout the experimental period. Animals were allowed free access to a commercial diet and clean water *ad libitum*. All animals were allowed to acclimatize for 4 days before the start of the experiment. The experimental protocols (JZ2015-016) were approved by the Biomedical Ethics Committee Experimental Animal Ethics Branch of Jiangxi University of Traditional Chinese Medicine.

***Induction of experimental colitis***

Colitis was induced according to the procedure described previously by Sałaga *et al*[25-28]*.* Briefly, 2, 4, 6-trinitrobenzene sulfonic acid (TNBS) (batch number: p2297, Sigma, USA) was administered as follows: mice were fasted for 12 hours before colitis induction. Each mouse was anesthetized with pentobarbital sodium (40 mg/kg), following which, 100 mg/kg TNBS (Sigma-Aldrich, St. Louis, MO, 100 g / L TNBS dissolved in 0.3 mL 50% ethanol) was then instilled *via* a rubber catheter that was inserted approximately 4 cm into the colon *via* the anus. The rubber catheter was modified with numerous holes posited over the final 4 cm of its length. The instillation procedure required only a few seconds, following which the mice were maintained in a head-down position for 5 min to prevent solution leakage. Mice in the Normal group received 50 % ethanol of the same volume that was delivered using the same technique as described above.

***Treatment protocols***

To explore the effect of curcumin (Cur) (purity ≥ 95 % by HPLC, batch number: GR-133-140421, GANGRUN Biotechnology, Nanjing, China) on CD8+CD11c+ cells in colitis mice, C57BL/6 mice (20–24g) were randomized into four groups of 8 animals with comparable average body weight in each group. The four groups were respectively grouped: Normal group (*n =* 8, receiving ethanol only, and not treated), the TNBS group (TNBS, *n =* 8, which received TNBS and were not treated), the TNBS + Cur group (TNBS + Cur, *n =* 8 that received 100 mg/kg Cur per day intragastrically (ig) and the TNBS + Mesalazine (Mes) group (TNBS + Mes *n =* 8 that received mesalazine at 300 mg/kg per day ig).

Before administration, curcumin was dissolved in 5% dimethyl sulfoxide (DMSO) in physiological saline, which was used as vehicle. Twenty-four hours after colitis was induced, mice in the TNBS + Cur group were administered Cur, and in the TNBS + Mes group were administered Mes for 7 d until the mice were sacrificed. Mice of both the Normal and TNBS groups received the same volume of 5% DMSO in physiological saline daily (which was the vehicle for Cur) until the end of the experiment.

***Assessment of the severity of colitis: disease activity index***

Disease activity index (DAI) is analyzed according to the previous study[29,30], which is the combined score of weight loss, stool consistency, and bleeding. The criterion of DAI scores was described in the Table 1. As shown in Table 1, the changes in growth rate, stool consistency and presence of gross bleeding or occult blood in feces were scored daily from 0-4 for each animal after TNBS treatment.

***Evaluation of colonic damage***

On the 8th day, all mice were sacrificed after having been anesthetized with pentobarbital sodium (40 mg/kg) by intraperitoneal injection. The colon was removed rapidly and its length was measured, opened longitudinally, rinsed with phosphate buffered saline (PBS), assessed immediately for weight (*n =* 8 for each group), and the weight index of colon was computed (*n =* 8) (colonic weight/body weight × 100%). Then, segments of the colon were fixed in 4% polyformaldehyde solution for at least 7 d. Subsequently, colon tissues were dehydrated, embedded in paraffin, sectioned at 5 μm and mounted onto slides. These sections were stained with hematoxylin and eosin (*n =* 8).

A histological damage score (*n =* 8) was determined according to the criteria described by Nicole and Alexander *et al*[31]. The histological score included inflammatory cell infiltration and tissue damage. Scores for infiltration were as follows: 0: no infiltration; 1: an increased number of inflammatory cells in the lamina propria; 2: inflammatory cells extending into the submucosa; and 3: transmural inflammatory cell infiltration. The scores of tissue damage were as follows: 0: no mucosal damage; 1: discrete epithelial lesions; 2: erosions or focal ulcerations; and 3: severe mucosal damage with extensive ulceration extending into the bowel wall.

***Isolation of lymphocyte from spleen and PPs***

PPs (*n =* 8) was separated and collected from the small intestine to the terminal rectum. To prepare single-cell suspensions, spleens or PPs were minced and digested in 2 mg/mL collagenase D (Roche Diagnostics) in 1% FCS/RPMI 1640 for 15 min at 37 °C. Next, 10 mM EDTA was added for the last 5 min, and the cell suspensions were then pipetted up and down several times and filtered through a fine mesh sieve. The cell suspensions were centrifuged at 1500 rpm/min at 4 °C for 5 min and suspended at a density of 1 × 106-107/mL in 3 % FCS/PBS buffer. Remnant supernate of spleen and PPs were separately used to analyze the levels of cytokines by enzyme-linked immunosorbent assay.

***Assay of*** ***CD8+CD11c+ cells by flow cytometry***

After removal of RBC, splenic (*n =* 8) and PPs cells (*n =* 8) were labeled respectively with V450-anti-mouse CD8a+Ab (at a concentration of 0.125 μg/100 μL, BD bioscience) and APC/Cy7 anti-mouse CD11c (eBioscience, San Diego, CA) at 37 °C in the dark. Cells were centrifuged at 1500 rpm/min at 4 °C for 5 min, and then fixed in 1% paraformaldehyde/PBS buffer. In addition, fluorescence-activated cell sorting analysis was performed on a FACSCalibur (BD Biosciences).

***Measurement of costimulatory molecules of CD8+CD11c+ cells by flow cytometry***

Cell suspensions (𝑛 = 8) were stained in according to an appropriate isotypic control antibody match of different fluorochromes and then incubated for 30 min with V450-anti-mouse CD8a+ Ab (at a concentration of 0.125 μg/100μL, BD bioscience), APC/Cy7 anti-mouse CD11c (eBioscience, San Diego, CA, USA), PerCP/™ Cy5.5 anti-mouse I-A / I-E (MHC II), PE anti-Mouse CD40, APC anti-mouse CD154 (*i.e*., CD40 ligand) , FITC anti-mouse CD54, and PerCP/™ Cy5.5 anti-mouse CD205. Limits for the quadrant markers were always set based on negative populations and isotype controls.

***Enzyme-linked immunosorbent assay***

The levels of IL-10, IFN-γ and TGF-β1 in spleen and colonic mucosa supernatants were measured using enzyme linked immunosorbent assay (ELISA) kits (eBioscience) according to the manufacturer’s instructions.

***Statistical analysis***

Data were expressed as mean ± standard error of mean (SEM). The statistical significance was evaluated by analysis of variance (ANOVA) followed by the Tukey test for multiple comparisons by Prism 5.0 (GraphPad Software, La Jolla, CA, USA). Nonparametric data were analyzed with Mann-Whitney U test. *P* values< 0.05 were considered statistically significant.

**Results**

***Curcumin attenuated TNBS-induced colitis***

As shown in Figure 1B, the body weight of mice in the TNBS group was significantly decreased compared with the Normal group, the TNBS + Cur and TNBS + Mesalazine (Mes) groups. While disease activity index of experimental colitis kept similar change with the body weight of mice (Figure 1C). Colonic weight and the weight index of the colon from the TNBS groups were higher than those in the Normal group, but they were lower than the TNBS + curcumin (Cur) and TNBS + Mes groups (Figure 1D and E). However, the colonic length of colitis mice was shortened in the TNBS group compared with the Normal, TNBS + Cur, and TNBS + Mes groups (Figure 1A and G). Histological evaluation of colonic sections from untreated animals with colitis showed that TNBS-induced colitis was characterized by a loss of mucosal architecture, thickening of the colon wall, cryptic abscesses, the formation of ulcers and extensive inflammatory cell infiltration in the colonic mucosa (Figure 1F). Treatment with Cur and Mes restrained these pathological symptoms and histo-progressive restoration, reduced inflammatory cell infiltration in the mucosa and submucosa, and maintained the integrity of colonic mucosa (Figure 1F). While ulceration, hyperaemia and edema in local colonic mucosa in colitis mice without treatment were observed by visual assessment, they were ameliorated in colitis mice treated with Cur and mesalazin (Figure 1A). Moreover, the histological scores in the colon of mice from the Normal, TNBS + Cur, and TNBS + Mes groups were significantly lower than those in untreated mice with colitis (Figure 1F and H). All results demonstrated that Cur effectively treated experimental colitis.

***Cur inhibited levels of CD8+CD11c+ cells in spleen and PPs in colitis mice***

The total numbers of CD8+CD11c+ cells in the spleen and PPs of mice with colitis were analyzed (Figure 2). Data clearly indicated a significantly increased tendency in this parameter in the spleen (Figure 2A and D) and PPs (Figure 2A and C) in colitis mice in the TNBS group as compared with the normal group. Significantly, after 7 d treatment with Cur, the numbers of CD8+CD11c+ cells in the spleen and PPs in colitis mice in the TNBS + Cur, and TNBS + Mes groups were decreased dramatically as compared with the TNBS group.

***Cur increased IL-10, IFN-γ and TGF-β1 secretion in spleen and colonic mucosa in colitis mice***

To understand the effects of activated CD8+CD11c+ cells in the development of murine colitis, the secretion of IL-10, IFN-γ and TGF-β1 were determined (Figure 3). As shown in Figure 3B, a significantly increased expression of TGF-β1 was observed in the colonic mucosa of mice that were not treated. In addition, the secretions of TGF-β1 in the colonic mucosa in the TNBS + Cur, and the TNBS + Mes groups were lower than that seen in the TNBS group.

However, the expression of TGF-β1 in the spleen of mice treated with Cur and Mes were higher than that found in the TNBS group. Interestingly, when comparing with the normal groups, all levels of IFN-γ (Figure 3B and E) and IL-10 (Figure 3C and F) in the spleen and colonic mucosa in untreated colitis mice were decreased 7 d after TNBS-induced colitis. Both in the colonic mucosa and spleen, the expression of both IL-10 and IFN-γ were increased in colitis mice that had been treated with Cur and Mes as compared with untreated colitis mice.

***Cur suppressed expression of costimulatory molecules of CD8+CD11c+ cells in colitis mice***

Expression of costimulatory molecules of CD8+CD11c+ cells including CD40 (Figure 4), CD40L (Figure 5), CD54 (Figure 6), CD205 (Figure 7) and MHC II (Figure 8) were detected in normal spleen and PPs (please see Figures 4-8), and their levels were increased after TNBS-induced colitis. However, treatment with Cur decreased the expression of CD40 (Figure 4), CD40L (Figure 5), CD54 (Figure 6), CD205 (Figure 7) and MHC II (Figure 8) in the spleen and PPs in mice from the TNBS + Cur group as compared with the TNBS group.

**Discussion**

In the present study, the DAI, colonic weight, weight index and histological score of colonic of experimental colitis were significantly decreased after Cur treatment, while the body weight and colonic length were remarkably recovered. The results had indicated that Cur can effectively treat experimental colitis. With that, the levels of CD8+CD11C+ cells in spleen and PPL were inhibited. It showed that the therapeutic effect of Cur treated colitis was related with the level of CD8+CD11C+ cells.

As a positive regulatory factor, CD11c, which is one of adhesion molecule CD11/CD18 family, participates in conglutination, migration, antigenic recognition and present of DCs, and activate CD4+, CD8+ T cells to regulate immune response. CD11c can promote DCs activation and maturity by elevating expression of costimulatory molecules[32,33]. Activated CD11c+ DCs secrete a large number of inflammatory factors (including IL-1β, IL-6, IL-12 and IL-20, *etc*.) and promote CD4+ T cells transform into Th 1 cells, induce inflammatory injury[34]. In the present study, the total number of CD8+CD11c+ cells in spleen and PPL were increased in colitis mice, which is decreased in colitis mice with Cur treatment. While our other paper had indicated that Cur effectively alleviated inflammatory injury in colonic mucosa from colitis mice which are the same model with the present study[24]. So these results were shown that Cur inhibited the level of CD8+CD11c+ cells to treat TNBS-induced colitis.

Previous researches had manifested that CD8 + DCs played an important role in the development of experimental colitis and human IBD[11,12]. From the foregoing findings in our study, it was shown that costimulatory molecules of DCs were increased in the spleen and PPs in colitis mice without treatment, and Cur attenuated the expressions of MHC-Ⅱ, CD205, CD40, CD40 L and CD54 (ICAM-1) of CD8+ DCs in the spleen and PPs in colitis mice in the TNBS + Cur group. Our previous and present studies had shown that Cur effectively treated experimental colitis induced by TNBS or DSS[35,36]. Meanwhile, the present study sufficiently proved that Cur can regulate levels of CD8+ DCs to treat TNBS-induced colitis.

Dendritic cells are essential in the activation of the adaptive immune system[37], and can be distinguished into “myeloid” DCs and “lymphoid” DCs based on their cell-surface expression of CD8[8,9], which is one of the most important DC subset markers. Researchers have previously demonstrated that lymphoid DCs express CD8 in the mouse, whereas myeloid DCs do not[38-40]. Thus, DCs in the spleen and PPs of the mouse can be labeled into CD8+ DCs, which were identified in the present study[39,41].

Overwhelming evidence has suggested that the activation of CD8 + DCs is a significant pathway to generate specific CD8 + T cell immune responses[42,43]. The complex pathway included activation of TLR3[44], major histocompatibility complex (MHC) and costimulatory molecule expressions, among others. MHC can promote DCs to migrate into the lymphoid tissues such as the spleen and PPs, and accelerate antigen presentation, activation and maturation of DCs.

DC biologists had previously incorporated MHC II as a phenotypic segregation marker for *ex vivo* analysis of DC under inflammatory settings such as influenza infection[44]. Jason *et al*[45]*,* had shown that many of the CD8+ DC sub-populations underwent a phenotypic change from CD11chigh MHC IIint in naïve mice to CD11cint MHC IIhigh expression in IAV infected mice. According to MHC II and CD11c expression levels, CD8+ DCs, which are a classic migratory DCs phenotype, could be segregated into both lymphoid resident DC subsets and migratory subsets found at inflammatory zones[46-48]. Based on the high-expression of MHC II, CD8+ DCs capture antigens (Ags) and promote T cell migration at regions of the draining lymph nodes where they mature into functional DCs and present Ags to initiate primary immune responses[49,50]. In the process of maturation and activation of CD8+ DCs, costimulatory molecules are highly expressed and include expression of CD205, CD24, CD40, and CD40L, among others[2].

As a symbol of DC maturation and activation, DC expression of co-stimulatory molecules includes members of the TNF/TNF receptor protein family, for example, CD40/CD40L, OX40/OX40L and TNFR/TNF, and members of the immune globulin superfamily including ICAM-1/LAF-1, CD28/CTLA4/B7. Collectively, these cell-surface expressed protein receptors and their cognate ligands regulate the balance between T helper (Th) 1 and Th2 responses, and were found to be highly expressed in human and animal colitis[51]. For example, CD40/CD40L signaling can stimulate DCs to secrete interleukin (IL) 12 (IL-12), and direct the differentiation of CD4+ T cells into Th1 cells. Similar functions were shown in the context of ICAM-1/LFA-1signaling and the B7-1 molecular signaling pathway (*i.e*., the B7/CD28 signal)[52,53].

More importantly, CD8+ DCs predominantly produce Th1 promoting cytokines like IL-12 p70 and IL-12 p40, while CD8+ DCs lead to Th1 differentiation with reduced secretion of IFN-γ and IL-10[8,54,55], and enhanced secretion of the pro-inflammatory cytokine IL-6, which is associated with autoimmunity and chronic inflammatory diseases[56]. These cytokines were previously shown to be closely related to the pathogenesis of inflammatory bowel disease (IBD)[57,58]. Therefore, we had a number of lines of experimental evidence to believe that CD8 + DCs played a critical role in the development of TNBS-induced colitis in our study. This was confirmed in our study by increased numbers of CD8+ DCs in the spleen and PPs in mouse models of untreated colitis. The results showed high expression levels of MHC II, CD205, CD40, CD40L and ICAM-1. These costimulatory molecules and MHC II promoted CD8+ DCs to migrate into the colonic mucosa. Here, CD8+ DCs secreted pro-inflammatory cytokines and suppressed anti-inflammatory cytokine production, where they ultimately induced inflammatory injury in the colonic mucosa.

Seven days after administration of Cur, the total number of CD8+CD11c+ cells was decreased, and the expression of these costimulatory molecules of DCs was inhibited. Although it is uncertain that Cur regulated the function of CD8+CD11c+ cells, Shirley *et al*[23]*,* had previously indicated that Cur prevents DCs from inducing CD4+ T cell proliferation by blocking maturation marker expression, cytokine and chemokine secretion and reduced migration and endocytosis of DCs.

In the present study, these results suggested that Cur restrained the quantity and activation of CD8+CD11c+ cells by down-regulating the expression costimulatory molecule of DCs in an attempt to improve the level of anti-inflammatory cytokines (*i.e*., IL-10, IFN-γ and TGF-β1). These data suggested a therapeutic role of Cur as an immune suppressant in the treatment of IBD. However, the level of TGF-β1 in the colonic mucosa was decreased by Cur, which is contrary to that seen in the spleen. We speculated that over-production of TGF-β1 in the colonic mucosa was related to the chronicity and fibrosis of experimental colitis. Thus, Cur might inhibit fibroplasias at the base of the ulcer. However, the signaling pathway remains unknown under conditions when Cur controls maturation and migration of CD8+ DCs. Future work is very important in this area in an attempt to explore the pathway that regulates the function of CD8+ DCs by TGF-β1 signaling.

In conclusion, we demonstrated that Cur effectively treated experimental colitis, which was realized by inhibiting CD8+CD11c+ cells.

**COMMENTS**

***Background***

CD11c is a known and specific labeled molecule of dendritic cells (DCs), and high-expressed in DCs, including CD8+ DCs and CD8- DCs. Over-accumulation of CD8+ DCs in colonic mucosa induced inflammatory injury in experimental colitis and human inflammatory bowel disease (IBD) patients.

***Research frontiers***

CD8+ DCs predominantly stimulate Th1-inducing cytokines like IL-12p70 and IL-12p40, which can lead to Th1 differentiation, and have been reported to play a key role in controlling viral infection. Over-accumulation of CD8+ DCs was also found to induce inflammatory injury in the colonic mucosa when they migrated into Peyer’s patches of experimental colitis and human IBD patients. Thus, CD8+ DCs may be a potential therapeutic target to explore the mechanisms of clinical treatment of IBD.

***Innovations and breakthroughs***

The present study is firstly shown that Cur effectively treated experimental colitis, which was realized by inhibiting CD8+CD11c+ cells.

***Applications***

It is known that Cur has a long history of effectively treating human IBD and experimental colitis. While Cur prevented DC-mediated induction of CD4+ T cell proliferation by blocking maturation marker expression, cytokine and chemokine expressions. However, it is unclear whether Cur can regulate the expression levels of CD8+CD11c+ cells to treat IBD. In the present study, our results had hinted that Cur effectively treated experimental colitis, which was realized by inhibiting CD8+CD11c+ cells. The results are favorable to explore the mechanism of Cur treated chronic colitis *via* CD8+CD11c+ cells.

***Terminology***

CD11c is a type I transmembrane protein which can mediate adherency between leukocyte and endotheliocyte, participate in exudation and phagocytosis of leukocyte.

***Peer-review***

The manuscript is presented in an easy understandable manner. The topic in the manuscript is very well explained. But it requires substantial corrections for the acceptance. According to detailed experimental data and reliable results, the present study had proved that Cur effectively treated experimental colitis, which was realized by inhibiting CD8+CD11c+ cells.

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**P-Reviewer:** Bolboaca SD, Gopu B, Sharaf IA **S-Editor:** Yu J **L-Editor:** **E-Editor:**

**Specialty type:** Gastroenterology and hepatology

**Country of origin:** China

**Peer-review report classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): C, C

Grade D (Fair): 0

Grade E (Poor): 0

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**Figure 1 Macroscopic and microcosmic observation.**A: Macrography of the opened colon. They respectively represent the Normal, TNBS, TNBS+ Curcumin, and TNBS+ Mesalazine animal groups; B: Body weight; C: DAI score; D: Colonic weight; E: Weight index of the colon; F: Typical histological images stained by HE, f1-4: Bar = 40 μm, f5-8: Bar = 100 μm; G: Colonic length; H: Histological scores. Data were presented as mean ± SEM (*n =* 8). a*P <* 0.05 and c*P <* 0.01 *vs* the Normal group; b*P <* 0.05 and d*P <* 0.01 *vs* the TNBS group.

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**Figure 2 Typical histograms and levels of CD8+CD11c+cells in the spleen and PPs.** A: Typical graphs and mean fluorescence intensity (MFI) levels of CD8+CD11c+ cells in the spleen; B: Typical graphs and MFI levels of CD8+CD11c+ cells in PPs; C: MFI levels of CD8+CD11c+ cells in the spleen; D: MFI levels of CD8+CD11c+ cells in PPs. Data are shown as the mean ± SEM (*n =* 8). a*P <* 0.05 and c*P <* 0.01 *vs* the Normal group; b*P <* 0.05 and d*P <* 0.01 *vs* the TNBS group.



**Figure 3 Levels of TGF-β1, IFN-γ and IL-10 in spleen and colonic mucosal supernatants.** A-C: Concentration of TGF-β1, IFN-γ and IL-10 respectively in the spleen from different groups; D-F: Concentration of TGF-β1, IFN-γ and IL-10 respectively in the colonic mucosa from different groups. Data are shown as the mean ± SEM (*n =* 8). a*P <* 0.05 and c*P <* 0.01 *vs* Normal group; b*P <* 0.05 and d*P <* 0.01 *vs* TNBS group.



**Figure 4** **Typical histograms and levels of CD11c+CD40+ cells in spleen and PPs.** A: Typical graphs of CD11c+CD40**+** cells in the spleen; B: Typical graphs of CD11c+CD40**+** cells in the PPs; C: MFI levels of CD11c+CD40**+** cells in the spleen; D: MFI levels of CD11c+CD40**+** cells in the PPs. Data are described as the mean ± SEM (*n =* 8). a*P <* 0.05 *vs* the Normal control group; b*P <* 0.05 *vs* the TNBS group.



**Figure 5** **Typical histograms and levels of CD11c+CD40L+ cell in spleen and PPs.** A: Typical graphs of CD11c+CD40L**+** cells in the spleen; B: Typical graphs of CD11c + CD40L **+** cells in PPs; C: Levels (MFI) of CD11c+CD40L**+** cells in the spleen; D: Levels (MFI) of CD11c+CD40L**+** cells in PPs. Data are described as the mean ± SEM (*n =* 8). a*P <* 0.05 *vs* the Normal control group; b*P <* 0.05 *vs* the TNBS group.



**Figure 6 Typical histograms and levels of CD11c+ ICAM-1+** **cells in spleen and PPs.** A: Typical graphs of CD11c+ICAM-1+ cells in the spleen**;** B: Typical graphs of CD11c+ICAM-1+ cells in the PPs; C: Levels (MFI) of CD11c+ ICAM-1+ cells in the spleen; D: Levels (MFI) of CD11c+ICAM-1+ cells in the PPs. Data are shown as the mean ± SEM (*n =* 8). a*P <* 0.05 *vs* the Normal control group; b*P <* 0.05 *vs* the TNBS group.



**Figure 7 Typical histograms and levels of CD11c+CD205+ cells in the spleen and PPs.** A: Typical graphs of CD11c + CD205 + cells in the spleen; B: Typical graphs of CD11c+CD205+ cells in the PPs; C: Levels (MFI) of CD11c+CD205+ cells in the spleen; D: Levels (MFI) of CD11c+CD205+ cells in PPs. Data were mean ± SEM (*n =* 8). a*P <* 0.05 and c*P <* 0.01 *vs* the Normal group; b*P <* 0.05 *vs* the TNBS group.



**Figure 8 Typical histograms and levels of CD11c+MHC II+ cells in the spleen and PPs.** A: Typical graphs of CD11c + MHC II + cells in the spleen; B: Typical graphs of CD11c+ MHC II+ cells in the PPs; C: Levels (MFI) of CD11c+ MHC II+ cells in the spleen; D: Levels (MFI) of CD11c+ MHC II+ cells in the PPs. Data are shown as the mean ± SEM (*n =* 8). a*P <* 0.05 *vs* the Normal control group; b*P <* 0.05 and d*P <* 0.01 *vs* the TNBS group.

**Table 1 Scoring of the disease activity index**

| **Score** | **Decrease in growth****or weight loss (%)** | **Stool consistency** | **Occult/gross rectal bleeding** |
| --- | --- | --- | --- |
| 0 | 0 | Normal | Normal |
| 1 | 1-5 | Normal | Occult blood+ |
| 2 | 5-10 | Loose stools | Occult blood++ |
| 3 | 10-15 | Loose stools | Occult blood+++ |
| 4 | > 15 | Diarrhea | Gross bleeding |