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Original Article

$CD4^+CD29^+$T cells are blamed for the persistent inflammatory response in ulcerative colitis

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Abstract: Ulcerative colitis (UC) is a chronic gastrointestinal disorder eliciting occurrence of colorectal cancer, the third most common human malignancy. The diagnosis of UC is based on clinical symptoms combined with typical findings on endoscopy, radiology, and ultimately pathology. We investigated the variation trend of $CD4^+CD29^+$T cells together with MPO, VCAM-1 in different periods of rat UC model and UC patients. We also evaluated the relationship between $CD4^+CD29^+$T cells and disease severity. UC model was induced by administering DNCB liquid and acetate solution. We found upregulated expression of $CD4^+CD29^+$T cells in both peripheral blood and colon from rats, and a similar trend for MPO and VCAM-1 in colon ($P < 0.05$); the expression was especially enhanced in UC rats at two weeks after the model was established ($P < 0.01$). Such upregulation was also indicated in active and remission UC patients as compared to the healthy and enteritis groups ($P < 0.05$), with the highest expression level detected in the active UC patients ($P < 0.01$). Pearson correlation analysis showed a positive correlation of $CD4^+CD29^+$T cells in rat and human peripheral blood with DAI score ($r_{rat} = 0.712$, $r_{human} = 0.677$, $P < 0.01$), and MPO in colon ($r_{rat} = 0.514$, $r_{human} = 0.682$, $P < 0.05$). These results suggest that $CD4^+CD29^+$T cells may act as major effector cell subsets in persistent inflammatory responses for UC and that infiltration into colon inflammation may be induced by the combination of VCAM-1 and CD29.

Keywords: Ulcerative colitis, $CD4^+CD29^+$T cells, VCAM-1, persistent inflammatory responses

Introduction

Ulcerative colitis (UC), whose etiology remains unclear, is a chronic relapsing and remitting inflammatory disorder of the gastrointestinal tract [1]. The regulatory T cells in intestinal tract known for their inability to balance the excessive inflammatory responses against intestinal flora and food antigens are considered an important immune mechanism of UC [2, 3]. The role of main effector cell subsets in excessive inflammatory responses for UC, however, remains to be elucidated.

$CD4^+$T cells serve as a major effector cells and are frequently overexpressed in UC [4]. $CD4^+CD29^+$T cells belong to memory effector cells. CD29 is preferentially expressed on activated T cells that respond very well to recall antigens [5-7]. Activation of beta (1) integrins (CD29) has been reported to mediate proliferation and inhibit apoptosis of intestinal CD4-positive lymphocytes, important factors to perpetuate inflammatory process [8]. Existing studies suggested that enhanced expression of adhesion molecules might lead to accumulation of $CD4^+CD29^+$T memory cells in inflamed tissues [9]. The aforementioned data led to the hypothesis that $CD4^+CD29^+$T cells might act as main effector cell subsets in persistent inflammatory responses for UC, which could be migrated to inflamed tissues through VCAM-1, a from of adhesion molecule and the ligand of

CD29. As yet, there are sparse data on association between variation of $CD4^+CD29^+$T cells and UC, highlighting the need for additional investigations to identify the role $CD4^+CD29^+$T cells have on the serious chronic inflammatory disease.

Herein, we analyzed the change trend of $CD4^+CD29^+$T cells, VCAM-1 and myeloperoxidase (a vital indicator of neutrophilic-granulocyte in active UC) at different phases for rat and human UC. In addition, we explored the relationship of $CD4^+CD29^+$T cells to DAI score and MPO.

Methods and materials

Animals and experiment design

Experiments approved by Guangdong Medical Laboratory of Animal Center were performed using 2-month-old female SD rats (200-220 g). Thirty-six rats were maintained under standard conditions for two weeks of acclimatization. Among these, twenty-eight were randomly taken as cases and the remainders as normal controls. The UC model was established as detailed elsewhere [10], with small changes. In brief, all rats were administered 0.25 mL of 20 g/L DNCB acetone liquid (DNCB, Sigma, USA) solution on their bare necks once daily for 14d after depilation with Na_2S (100 g/L). At day 15, 0.25 mL of 20 g/L DNCB acetone liquid was applied to their bare abdomen after depilation with Na_2S. The bare abdomen that turned red and swollen the next day was considered immune sensitization.

At day 16 and 17, 0.25 mL of 0.1% DNCB ethanol solution was infused through a nylon hose inserted into the colon of rat (8 cm in depth). At day 18, 2 mL 8% acetate solution was administered at the same site. Then, the colon was rinsed with 5 mL normal saline after 10s of retention. After that, all rats were housed under standard conditions and the general situations such as diet, hair, and excrement were observed. We obtained 24 survival rats that were randomized into 3 groups. They were killed at day 1, 2 weeks, 6 weeks, respectively, after the model was stable (labeled as 1 day, 2 weeks, 6 weeks). Heparinized peripheral blood was extracted from aorta abdominalis for $CD4^+CD29^+$T cells by flow cytometry. Colon was obtained for HE hemotoxylin and eosin staining, immunohistochemistry and immunofluorescence. Disease activity index (DAI) was determined using the method described by Murthy et al. [11].

Patients

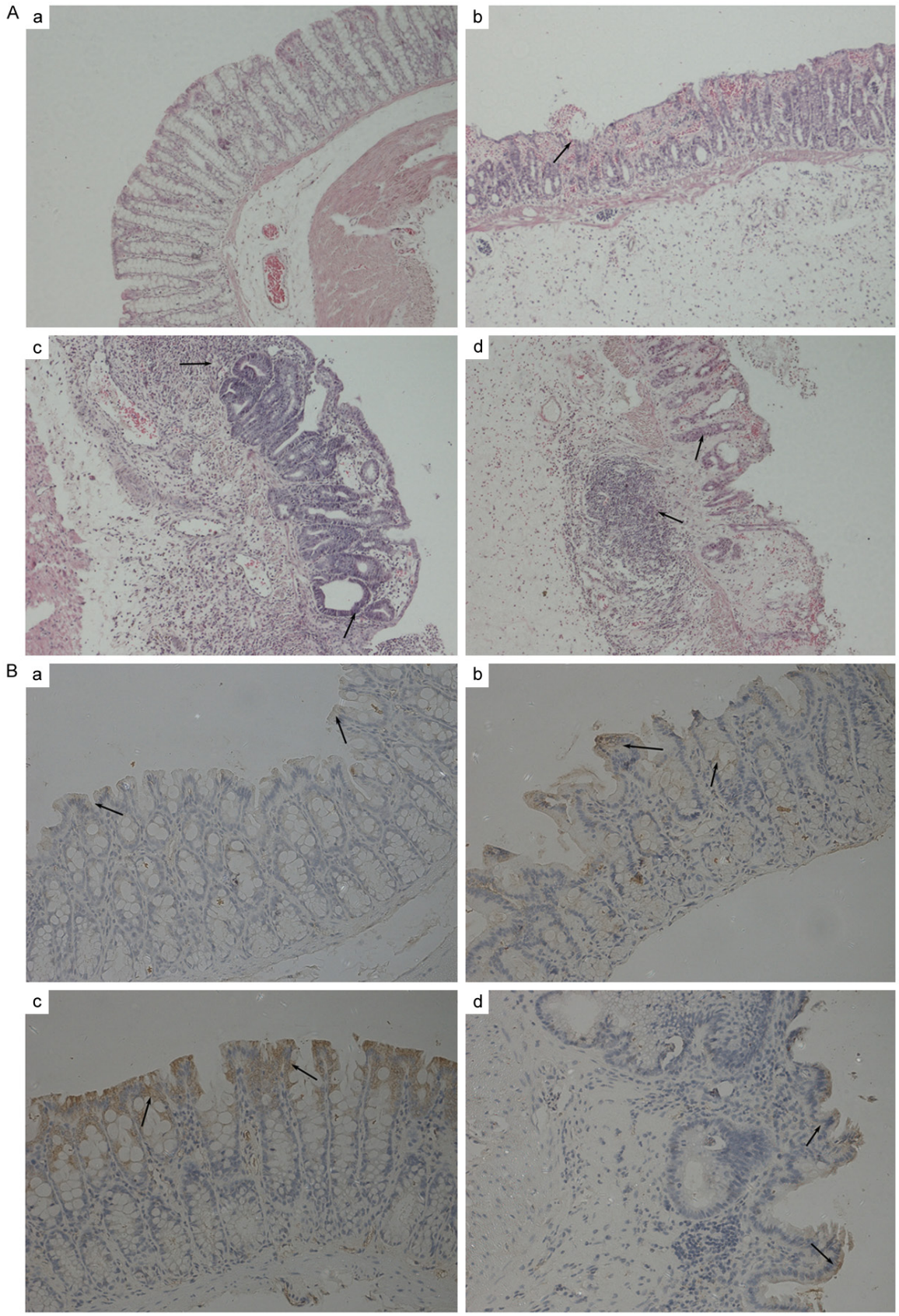
Sixty participants including healthy control individuals (n = 15), enteritis-infected patients (n = 15), active UC patients (n = 15) and remission patients (n = 15) were recruited from Guangzhou, China. Thirty UC patients were selected according to a previous study. Sutherland DAI was used to assess disease activity [12]. UC patients who scored 3-10 were categorized into the active group and those who scored 1-2 were categorized into the remission group. Infective enteritis patients selected in accordance with an earlier study [13] had similar clinical features to UC patients in abdominal pain, diarrhea and mucous stool. Heparinized peripheral blood was collected for $CD4^+CD29^+$T cells by flow cytometry. Moreover, 7, 6, 5, 5 intestinal tissues were obtained from the active UC, remission, infective enteritis and healthy control group, respectively, then fixed by formalin and imbedded by paraffin immediately after excision for HE hemotoxylin and eosin staining and immunohistochemistry of MPO and VCAM-1. All patients provided informed consent.

Flow cytometric analysis

Peripheral mononuclear cells (PBMC) obtained from heparinized peripheral blood were prepared for flow cytometry analysis based on previously described methods [14]. Briefly, 1×10^6 cells per sample were incubated (30 mins, on ice) with PE and APC-conjugated MoAb to antigen CD4 and CD29 (Biolegend, California, USA). PE and APC-labeled isotype-matched control Ig (Biolegend, California,USA) were also incubated. Double fluorescent analysis was conducted using a Facscan flow cytometer (Becton, Dickinson). Results were expressed as percentage of $CD4^+$T cells.

Immunofluorescence

To analyze the expression of $CD4^+CD29^+$T cells in colon, intestinal tissues were frozen for immunofluorescence referring to the previous literature [15], with minor changes. The frozen sections were fixed in cold acetone for 10 min,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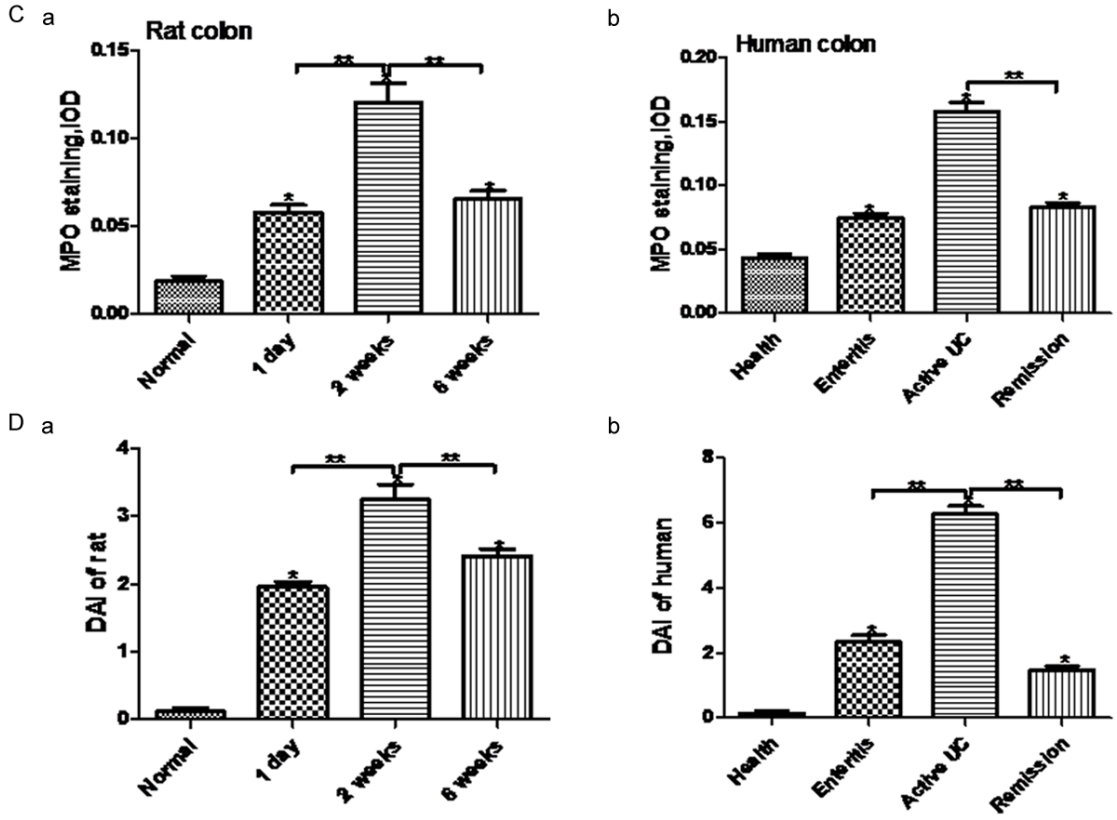

Figure 1. A. Pathology performance of normal and UC rats, (a-d) × 200. (b) We observed infiltration of inflammatory cells and slight ulcer in epithelial mucosae and submucosa 1 day after UC model was established. (c) After 2 weeks, diffuse inflammatory cellular infiltration, erosion and ulcers in epithelial mucosae and submucosa. Much mucous gland bodies were seriously destroyed. (d) After 6 weeks, Inflammatory cells became sparse, with lymphocytes infiltration in mesenchyme and fiber hyperplasia. B. Immunohistochemistry of MPO expression in rats, (a-d) × 200. (c) MPO strong immunoreactivity in mucosa and submucosa 2 weeks after UC model was established. C. IOD of MPO in rats and human colon, analyzed using Image Pro Plus version 6.0 (Maryland, USA) (results were presented as a ratio). $^{*}P < 0.05$, compared with normal or health control group, $^{**}P < 0.05$, compared with 2 weeks group or active UC group. D. DAI of rat and human, using the method described by Murthy et al.

and sealed in 10% of normal goat serum. Then the mixture was incubated in a cocktail containing non-conjugated MoAb to antigen CD4 (Rabbit IgG, Gene Tex) and CD29 (mouse IgG, BD) at 4°C for 12 h, soaked in a cocktail containing secondary Goat anti-rabbit-488 and Goat anti-mouse-594 (Shanghai research set) at room temperature without light for 1 h, and finally blotted and mounted with DAPI-contained anti-fade medium under a coverslip. Images were acquired by Image-Pro Plus microscope and processed with Emage-Pro Express software.

Immunohistochemistry

In order to explore the possible correlative factors for CD4+CD29+T cells, MPO and VCAM-1 in rat and human colon were measured by immunohistochemistry staining as previously detailed [16]. 5-μm paraffin-embedded sections were dehydrated. Antigen retrieval was completed in microwave oven and then incubated with mouse anti-rat and mouse anti-human MPO (1:100) and VCAM-1 (1:100) antibody at 4°C overnight, followed by treatment with biotinylated goat anti-mouse immunoglobulin (1:300 dilution) for 30 min, and staining with DAB. Immunostaining density was assessed by counting five randomly selected microscope fields and analyzed using Image Pro Plus version 6.0 (Maryland, USA) (results were presented as ratio).

Statistical analysis

All data were analyzed using SPSS16.0 and expressed as means ± standard error (SE). The

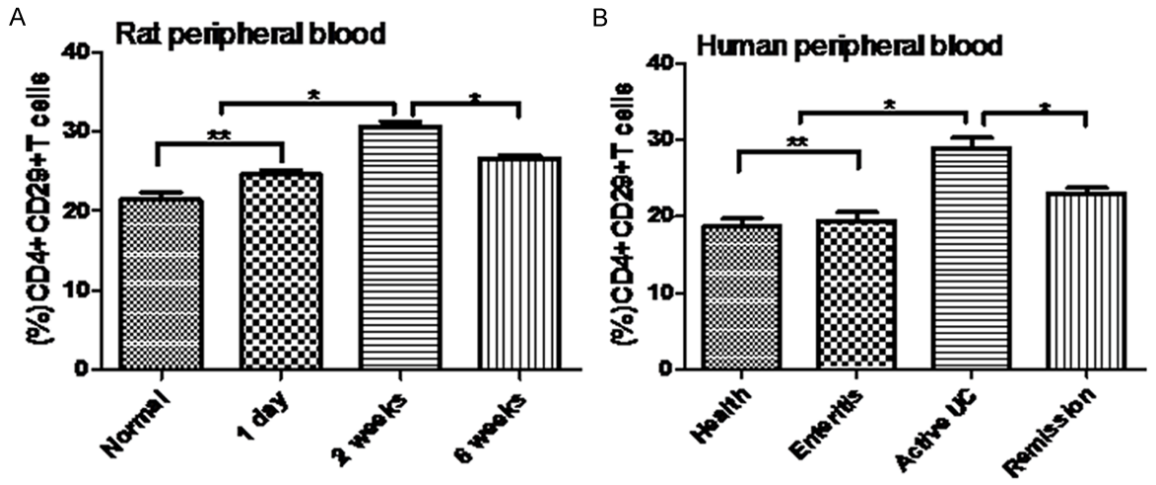

Figure 2. Percentage of $CD4^+CD29^+$T cells in rat and human peripheral blood, analyzed with flow cytometry. A. In rat UC model, no statistically difference between normal and 1 day group ($^{**}P = 0.401$), but $CD4^+CD29^+$T cells in 2 weeks group showed high rise compared with normal and 1 day rat groups ($P < 0.01$) and a significant decrease in the next four weeks ($^{*}P < 0.01$). B. In human, no statistically difference between health and enteritis group ($^{**}P = 0.630$). UC patients showed high percentage compared with the health and enteritis control group ($^{*}P < 0.05$) and the active UC group was higher than remission group ($^{*}P < 0.05$).

one-way analysis of variance was used for statistical analyses to compare the differences among multiple groups. Pearson's method was used to analyze the correlations of DAI and MPO with $CD4^+CD29^+$T cells in rat and human peripheral blood. Statistical significance was defined as $P < 0.05$.

Results

DAI, pathology performance and MPO status

DAI, pathology performance and MPO are important indicators of symptoms, histology and immune reactions to assess activity or severity of UC [11, 12, 17]. DAI was determined by scoring the extent of body weight loss, stool haemoccult positivity or gross bleeding, and stool consistency [11]. We observed higher DAI in UC model groups than in the normal group ($P < 0.01$), with the highest DAI detected in 2 weeks group and relatively lower in 6 weeks group ($P < 0.05$) (**Figure 1D**). A similar variation trend was shown in pathology performance. At 2 weeks, diffuse inflammatory cellular infiltration, erosion and ulcers were present in epithelial mucosae and submucosa. At 6 weeks, the number of inflammatory cells sharply decreased, with lymphocytes infiltration in mesenchyme and fiber hyperplasia (**Figure 1A**). MPO was used as a sensitive marker to assess disease activity [17], and the 2 weeks group showed the highest expression levels among all groups ($P < 0.05$). The 6 weeks group again showed significantly lower levels than the 2 weeks group ($P < 0.05$). Immunohistochemistry was performed under the same experimental conditions in order to evaluate the expression status. We observed significantly upregulated MPO in enteritis and UC patients compared to healthy controls, with more obvious upregulation seen in the active UC patients in comparison to the enteritis and remission group ($P < 0.05$) (**Figure 1B**, **1C**).

Upregulated expression of $CD4^+CD29^+$T cells in peripheral blood

To test the hypothesis that $CD4^+CD29^+$T cells may act as main effector cell subsets in persistent inflammatory responses for UC, we analyzed $CD4^+CD29^+$T cells in peripheral blood of UC rats and patients. As shown in **Figure 2A**, we did not find a significant rise at day 1 after model was stable ($P = 0.401$), but we did observe a notable increase at 2 weeks ($P < 0.01$), and a sharp decrease in the next four weeks ($P < 0.01$).

In human peripheral blood, we observed a higher percentage of $CD4^+CD29^+$T cells as compared with the healthy and enteritis group ($P < 0.05$). We also noted a significantly larger number in the active UC group when compared to

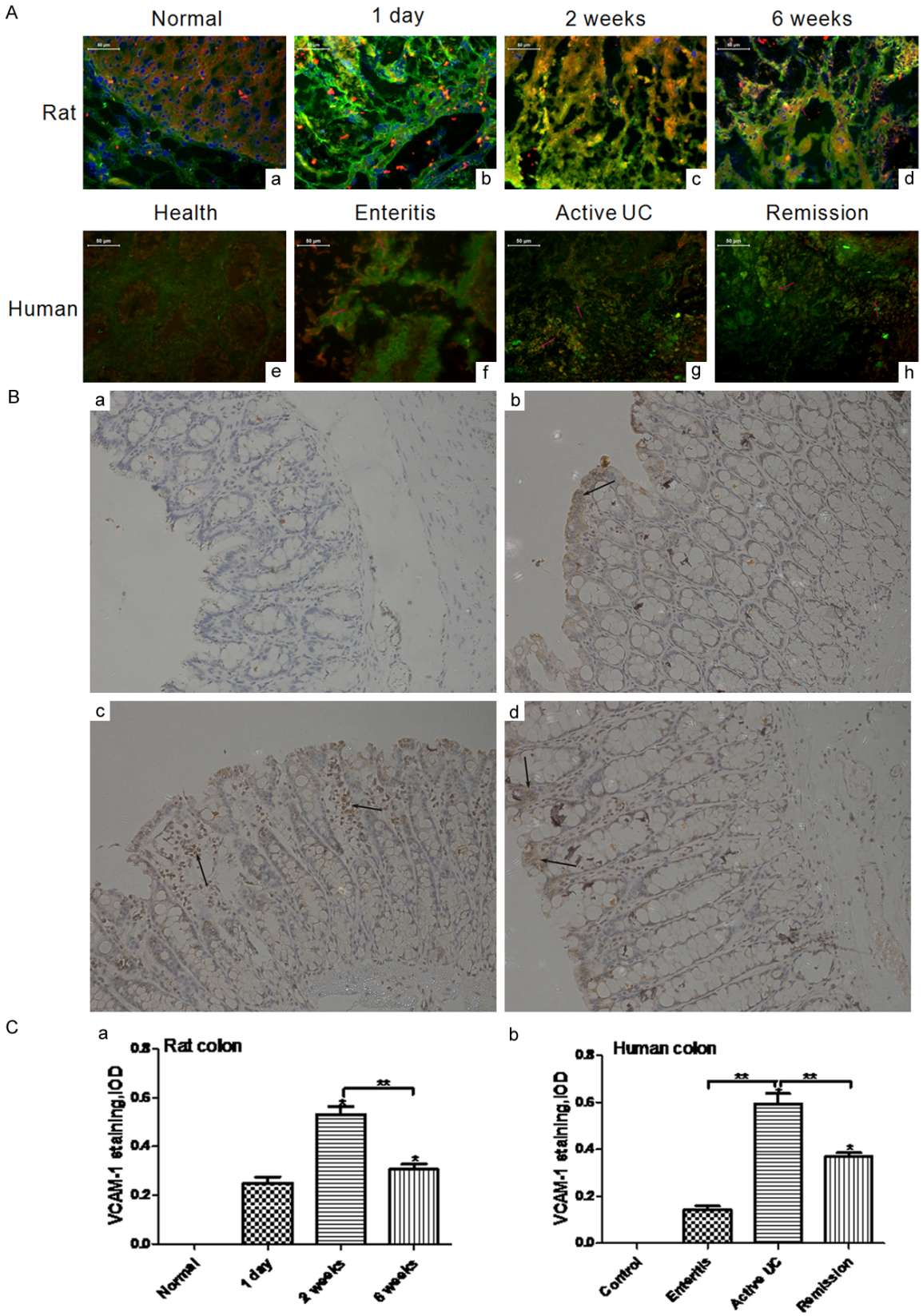

Figure 3. A. Immunofluorescence of CD4+CD29+T cells expression in rat and human colon (the yellow section that arrows point to was expression of CD4+CD29+T cells, (a-d) × 400). (a, e) no obvious expression. (b, f) a little and weak expression. (c, g) strong fluorescence intensity. (d, h) a little and weak expression. B. Immunohistochemistry

of VCAM-1 expression in rat colon, (a-d) × 200. (a) no obvious expression (b) a little expression, mainly in mucosa (c) much more expression than 1 day group, mainly in mucosa and submucosa (d) a little expression, mainly in mucosa. C. IOD of VCAM-1 in rat and human colon, analyzed using Image Pro Plus version 6.0 (Maryland, USA) (results were presented as a ratio). *$P < 0.05$ as compared with the 1 day and enteritis group, **$P < 0.05$ as compared with the 2 weeks and active UC group.

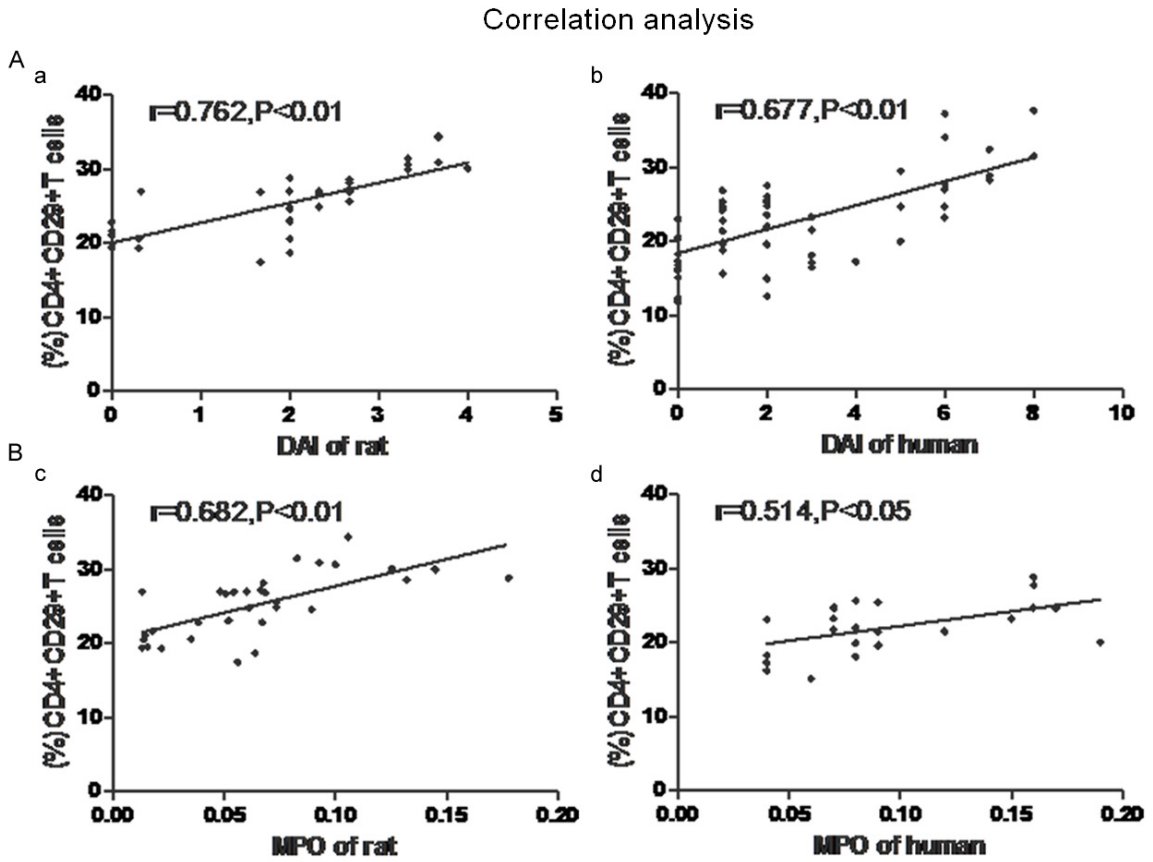

Figure 4. A. Correlation between DAI and CD4+CD29+T cells in peripheral blood of rat and human. B. Correlation between MPO in colon and CD4+CD29+T cells in peripheral blood of rat and human. Pearson's t test was used to analyze the correlation; *P* values are indicated.

the remission group ($P < 0.01$). Nevertheless, no statistically significant difference was indicated between the health group and the enteritis group ($P = 0.630$, **Figure 2A**). CD4+CD29+T cells in peripheral blood showed consistency with UC patients in DAI, pathology performance and MPO, suggesting CD4+CD29+T cells may be closely related to UC activity and severity (**Figure 4**).

Enhanced expression of CD4+CD29+T cells and VCAM-1 in colon

As UC was considered an inflammatory response prone to initiate colon cancer [18], thus it is substantially important to analyze the expression of CD4+CD29+T cells in colon. Immunofluorescence analysis was performed to detect the expression status. We also investigated the expression of VCAM-1, an adhesion molecule and the ligand of CD29, using Image Pro Plus version 6.0. No obvious expression of CD4+CD29+T cells or VCAM-1 was found in reference groups. Interestingly, different degrees of expression were detected in UC groups. The CD4+CD29+T cells were sparsely distributed and predominantly expressed in cell membrane, while VCAM-1 was mainly detected in mucosa and submucosa. We found significantly upregulated VCAM-1 in 2 weeks rat group and the active UC group ($P < 0.05$). In addition, CD4+CD29+T cells and VCAM-1 showed the widest range and the strongest

intensity in 2 weeks rat group and the active UC group (**Figure 3A**, **3B**).

Correlation between CD4+CD29+T cells and DAI

We observed larger percentage of CD4+CD29+T cells in peripheral blood correlated with higher DAI grade and MPO in colon. As shown in **Figure 4**, CD4+CD29+T cells in peripheral blood were positively associated with DAI score (r_R = 0.762, r_H = 0.677, $P < 0.01$), and MPO in colon (r_R = 0.682, r_H = 0.514, $P < 0.05$).

Discussion

UC is one of the autoimmune diseases and thought of as a public health burden due to its persistent inflammatory and repeated attacks. Thus, investigating the molecular mechanism is critical for an increased understanding of the pathogensis, early detection, prevention and treatment. It is believed that UC is a consequence of insufficient immune tolerance, and extensive research has focused on regulatory T cells that play a vital role in immunosuppress and immune tolerance mediation [19]. Over the past years, cellular immune function of CD4+T cells receives little attention in the epidemic filed, except for Sartor et al. indicating that CD4+T cells are main immunocytes resulting in inflammatory UC [4]. Currently, the knowledge about the effects of CD4+T-lymphocyte subsets in the pathopoiesis of human diseases remains limited.

We showed a significantly higher percentage of CD4+CD29+T cells in peripheral blood of UC rats, especially those at two weeks after the model were established. Likewise, a significant rise was indicated in peripheral blood of human patients when compared with healthy controls, and a more notable increase was detected in active UC patients relative to the remission patients.

To our knowledge, this is the first study investigating the role of CD4+CD29+T cells in colon samples. The variation tendency our study demonstrated is in line with previous CD4+CD29+T cell studies focusing on other autoimmune diseases such as multiple sclerosis, and rheumatoid arthritis [20, 21]. It is interesting that a lower percentage of CD4+CD29+T cells was found in patients with atopic diseases and mixed connective tissue disease [7, 22]. A plausible explanation for this inconsistency may relate to the different behaviors of CD4+CD29+T cells in different diseases, whose pathogenesis may vary extensively.

To expand the understanding of CD4+CD29+T cells in UC, we assessed their expression in colon by immunofluorescence. Our results indicated no obvious expression in normal rats and healthy individuals. Interestingly, CD4+CD29+T cells were significantly upregulated in UC rats and patients. A relatively smaller rise also took place in other intestinal inflammatory conditions, such as infective enteritis. We therefore inferred that CD4+CD29+T cells may be the main effector cell subsets resulting in persistent inflammation for UC. In order to evaluate the effects of CD4+CD29+T cells on UC incidence, we evaluated DAI score and MPO, and found a positive association of CD4+CD29+T cells with DAI score and MPO. MPO exists in azurophilic granule of neutrophils primarily recruited into the areas of acute inflammations and subsequent UC [23]. Previous groups have reported that MPO is positively associated with severity of intestinal inflammation, and an ideal indicator of UC activity and severity [17, 24, 25]. Consistently, UC patients showed statistical differences in DAI and MPO in our study, with higher levels in active UC patients than in remission patients. Also, we demonstrated a positive correlation of CD4+CD29+T cells with DAI score and MPO. Taken together, CD4+CD29+T cells might be closely related to the activity and severity of UC.

We additionally investigated the potential mechanisms by evaluating the expression of VCAM-1 via immunohistochemistry. Existing literature has well documented that recruitment of circulating leukocytes, especially the T-effector and T-memory cell to the intestinal mucosa, is a pivotal step in initiation and progression of UC [26]. CD4+CD29+T cells have been shown to have greater ability in adhering to vascular endothelium than other CD29- counterparts [27]. Enhanced expression of adhesion molecules possibly lead to CD4+CD29+T cell accumulation in inflamed tissues [9]. VCAM-1 as an adhesion molecule is markedly linked with CD29. Collective data have demonstrated that VCAM-1 plays a central role in leukocyte recruitment in colitis and blockade of adhesion molecule and has thera-

peutic effects on DSS colitis [28, 29]. According to Lundberg et al., CD49b/CD29 can regulate neutrophil recruitment and inflammatory responses in dextran sodium sulfate-induced colitis [30]. Therefore, we believe that CD4+CD29+T cells infiltrate to inflamed intestinal tissues most likely through combination with VCAM-1, triggering neutrophil recruitment and inflammatory activity in UC.

Our study showed no obvious expression of VCAM-1 in normal rats and healthy humans, but did indicate different degrees of expression in UC rats and patients. Concordant with CD4+CD29+T cells, VCAM-1 was highly expressed in 2 weeks rat group and the active UC group. Our findings are supported by a recent study conducted by Gulubova et al., who found higher expression levels of VCAM-1 and D4+CD29+T cells in active UC patients when compared to the remission patients, and no expression in healthy controls [31]. Also, Soriano et al. identified low constitutive expression of VCAM-1, but notably increased after induction of colitis [28]. We demonstrated evidence of a prominent rise in the number of CD4+CD29+T cells at two weeks, an observation consistent with an earlier experimental study [32]. It is widely accepted that chronic gut inflammation in UC may arise from an unregulated immune response to components of the normal gut flora [33, 34]. Recruitment of T-effector and T-memory cells that recognize antigens in gut lumens is markedly associated with disease state such as acute intestinal inflammation [26]. CD29 could be detected in cellular membrane of lymphocytes, monocytes, and neutrophils under certain conditions [35]. CD4+CD29+T cells as memory T cells differentiated from CD4+T cells may function significantly in repeated response to antigen.

In summary, we found notably upregulated expression of CD4+CD29+T cells in peripheral blood and colon of UC rats and patients. Pearson correlation analysis indicated a positive association of CD4+CD29+T cells with disease activity and severity. We also noted CD4+CD29+T cell migration to the inflamed colon tissues via combination with VCAM-1. These results support the hypothesis that CD4+CD29+T cells may act as effector cell subsets leading to persistent inflammation and also blamed for the repeated inflammation attacks of UC. Considering the limited sample, our findings should be treated as preliminary and additional studies are warranted to validate these findings possibly in a larger number.

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Disclosure of conflict of interest

None.

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References

[1] Silverberg MS, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, Colombel JF, Gasche C, Geboes K, Jewell DP, Karban A, Loftus EV Jr, Pena AS, Riddell RH, Sachar DB, Schreiber S, Steinhart AH, Targan SR, Vermeire S and Warren BF. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Can J Gastroenterol 2005; 19 Suppl A: 5A-36A.

[2] Pandey S, Luther G, Umanskiy K, Malhotra G, Rubin MA, Hurst RD and Fichera A. Minimally invasive pouch surgery for ulcerative colitis: is there a benefit in staging? Dis Colon Rectum 2011; 54: 306-310.

[3] Kucharzik T, Maaser C, Lugering A, Kagnoff M, Mayer L, Targan S and Domschke W. Recent understanding of IBD pathogenesis: implications for future therapies. Inflamm Bowel Dis 2006; 12: 1068-1083.

[4] Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. Nat Clin Pract Gastroenterol Hepatol 2006; 3: 390-407.

[5] Morimoto C, Letvin NL, Boyd AW, Hagan M, Brown HM, Kornacki MM and Schlossman SF. The isolation and characterization of the human helper inducer T cell subset. J Immunol 1985; 134: 3762-3769.

[6] Beverley PC. Human T-cell memory. Curr Top Microbiol Immunol 1990; 159: 111-122.
[7] Schauer U, Jung T, Heymanns J and Rieger CH. Imbalance of $CD4^+CD45R^+$ and $CD4^+CD29^+$T helper cell subsets in patients with atopic diseases. Clin Exp Immunol 1991; 83: 25-29.
[8] Stallmach A, Giese T, Pfister K, Wittig BM, Kunne S, Humphries M, Zeitz M and Meuer SC. Activation of beta(1) integrins mediates proliferation and inhibits apoptosis of intestinal CD4-positive lymphocytes. Eur J Immunol 2001; 31: 1228-1238.
[9] Mackay CR. T-cell memory: the connection between function, phenotype and migration pathways. Immunol Today 1991; 12: 189-192.
[10] Fan H, Qiu MY, Mei JJ, Shen GX, Liu SL and Chen R. Effects of four regulating-intestine prescriptions on pathology and ultrastructure of colon tissue in rats with ulcerative colitis. World J Gastroenterol 2005; 11: 4800-4806.
[11] Murano M, Maemura K, Hirata I, Toshina K, Nishikawa T, Hamamoto N, Sasaki S, Saitoh O and Katsu K. Therapeutic effect of intracolonically administered nuclear factor kappa B (p65) antisense oligonucleotide on mouse dextran sulphate sodium (DSS)-induced colitis. Clin Exp Immunol 2000; 120: 51-58.
[12] Bernstein CN, Fried M, Krabshuis JH, Cohen H, Eliakim R, Fedail S, Gearry R, Goh KL, Hamid S, Khan AG, LeMair AW, Malfertheiner, Ouyang Q, Rey JF, Sood A, Steinwurz F, Thomsen OO, Thomson A and Watermeyer G. World Gastroenterology Organization Practice Guidelines for the diagnosis and management of IBD in 2010. Inflamm Bowel Dis 2010; 16: 112-124.
[13] Schumacher G, Sandstedt B and Kollberg B. A prospective study of first attacks of inflammatory bowel disease and infectious colitis. Clinical findings and early diagnosis. Scand J Gastroenterol 1994; 29: 265-274.
[14] Ladell K, Hellerstein MK, Cesar D, Busch R, Boban D and McCune JM. Central memory CD8+ T cells appear to have a shorter lifespan and reduced abundance as a function of HIV disease progression. J Immunol 2008; 180: 7907-7918.
[15] Tkaczuk KH, Tait NS, Ioffe O, Tan M, Goloubeva OG, Lesko SA, Deamond SF, Zhou D, Lum ZP, Sutula MJ, Van Echo D and Ts'o PO. Computer assisted quantitative immunofluorescence of tumor tissue marker expression and clinical outcome to chemotherapy in advanced breast cancer patients. Discov Med 2011; 12: 33-40.
[16] Yuan SM, Wang YQ, Shen Y and Jing H. Transforming growth factor-beta in graft vessels: histology and immunohistochemistry. Clinics (Sao Paulo) 2011; 66: 895-901.
[17] Garrity-Park M, Loftus EV Jr, Sandborn WJ and Smyrk TC. Myeloperoxidase immunohistochemistry as a measure of disease activity in ulcerative colitis: association with ulcerative colitis-colorectal cancer, tumor necrosis factor polymorphism and RUNX3 methylation. Inflamm Bowel Dis 2012; 18: 275-283.
[18] Costello CM, Mah N, Hasler R, Rosenstiel P, Waetzig GH, Hahn A, Lu T, Gurbuz Y, Nikolaus S, Albrecht M, Hampe J, Lucius R, Kloppel G, Eickhoff H, Lehrach H, Lengauer T and Schreiber S. Dissection of the inflammatory bowel disease transcriptome using genome-wide cDNA microarrays. PLoS Med 2005; 2: e199.
[19] Mottet C, Uhlig HH and Powrie F. Cutting edge: cure of colitis by $CD4^+CD25^+$ regulatory T cells. J Immunol 2003; 170: 3939-3943.
[20] Rosen G. [Facial nerve surgery]. Harefuah 1975; 88: 416-419.
[21] Emery P, Gentry KC, Mackay IR, Muirden KD and Rowley M. Deficiency of the suppressor inducer subset of T lymphocytes in rheumatoid arthritis. Arthritis Rheum 1987; 30: 849-856.
[22] Becker H, Langrock A and Federlin K. Imbalance of $CD4^+$ lymphocyte subsets in patients with mixed connective tissue disease. Clin Exp Immunol 1992; 88: 91-95.
[23] Kumar SD, Krishnamurthy K, Manikandan J, Pakeerappa PN and Pushparaj PN. Deciphering the key molecular and cellular events in neutrophil transmigration during acute inflammation. Bioinformation 2011; 6: 111-114.
[24] Opavsky MA, Martino T, Rabinovitch M, Penninger J, Richardson C, Petric M, Trinidad C, Butcher L, Chan J and Liu PP. Enhanced ERK-1/2 activation in mice susceptible to coxsackievirus-induced myocarditis. J Clin Invest 2002; 109: 1561-1569.
[25] Zaware BB, Nirmal SA, Baheti DG, Patil AN and Mandal SC. Potential of Vitex negundo roots in the treatment of ulcerative colitis in mice. Pharm Biol 2011; 49: 874-878.
[26] van Assche G and Rutgeerts P. Antiadhesion molecule therapy in inflammatory bowel disease. Inflamm Bowel Dis 2002; 8: 291-300.
[27] Damle NK and Doyle LV. Ability of human T lymphocytes to adhere to vascular endothelial cells and to augment endothelial permeability to macromolecules is linked to their state of post-thymic maturation. J Immunol 1990; 144: 1233-1240.
[28] Soriano A, Salas A, Sans M, Gironella M, Elena M, Anderson DC, Pique JM and Panes J. VCAM-1, but not ICAM-1 or MAdCAM-1, immunoblockade ameliorates DSS-induced colitis in mice. Lab Invest 2000; 80: 1541-1551.
[29] Guri AJ, Hontecillas R and Bassaganya-Riera J. Abscisic acid ameliorates experimental IBD by downregulating cellular adhesion molecule expression and suppressing immune cell infiltration. Clin Nutr 2010; 29: 824-831.

[30] Lundberg S, Lindholm J, Lindbom L, Hellstrom PM and Werr J. Integrin alpha2beta1 regulates neutrophil recruitment and inflammatory activity in experimental colitis in mice. Inflamm Bowel Dis 2006; 12: 172-177.
[31] Gulubova MV, Manolova IM, Vlaykova TI, Prodanova M and Jovchev JP. Adhesion molecules in chronic ulcerative colitis. Int J Colorectal Dis 2007; 22: 581-589.
[32] de Oliveira JG, Xavier P, Carvalho E, Ramos JP, Magalhaes MC, Mendes AA, Faria V and Guerra LE. T lymphocyte subsets and cytokine production by graft-infiltrating cells in FSGS recurrence post-transplantation. Nephrol Dial Transplant 1999; 14: 713-716.
[33] Groux H and Powrie F. Regulatory T cells and inflammatory bowel disease. Immunol Today 1999; 20: 442-445.
[34] Magro F, Araujo F, Pereira P, Meireles E, Diniz-Ribeiro M and Velosom FT. Soluble selectins, sICAM, sVCAM, and angiogenic proteins in different activity groups of patients with inflammatory bowel disease. Dig Dis Sci 2004; 49: 1265-1274.
[35] Muller WA. Leukocyte-endothelial cell interactions in the inflammatory response. Lab Invest 2002; 82: 521-533.