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ORIGINAL ARTICLE

Serial Changes of Cytokines in Steroid-Refractory Ulcerative Colitis Patients Treated with Tacrolimus

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ABSTRACT

AIM: We examined cytokine levels in steroid-refractory ulcerative colitis (UC) patients before and after administration of tacrolimus.

METHODS: A total of 124 patients with UC were enrolled, and 13 with steroid-refractory UC were evaluated. Patients were given a 0.025 mg/kg oral dose of tacrolimus twice daily with plasma trough levels of 10 to 15 ng/mL for the first 2 weeks, and doses were maintained to achieve plasma levels between 5 and 10 ng/mL. The Disease Activity Index (DAI) and serum CRP levels were determined before and 12 weeks after treatment. Serum levels of 17 cytokines were simultaneously determined using a Bio-Plex suspension array system before and 12 weeks after treatment.

RESULTS: DAI scores and DAI mucosal appearance subscores after treatment were significantly decreased compared to before treatment ($P<0.05$). CRP levels were significantly decreased after treatment. G-CSF levels were significantly decreased after treatment compared to before treatment in all patients. In the efficacy group, IL-8 and G-CSF levels were significantly decreased after treatment compared to before treatment, and a significant correlation was noted between CRP and IL-8 levels.

CONCLUSION: Treatment with tacrolimus reduced serum IL-8 and G-CSF levels in UC patients, which may be associated with an overall reduction in disease severity.

Key words: Ulcerative colitis; Tacrolimus; Cytokines

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INTRODUCTION

Ulcerative colitis (UC) is a T helper (Th) 2-mediated disease associated with the production of various autoantibodies, immunoglobulin subclasses, and Th2 cytokines[1]. Natural killer T-cells are increased in the lamina propria of an inflamed colon and capable of producing many Th2 cytokines, which seem to have a key role in the pathogenesis of UC[2]. Th17 cells that produce the proinflammatory cytokine interleukin (IL)-17, the levels of Th17 are increased in the mucosa of patients with inflammatory bowel disease (IBD)[3]. Clinical studies have reported the utility of aminosalicylates, immunomodulators, and corticosteroids for the treatment of UC. Derivatives of 5-aminosalicylic acid (mesalamine) inhibit tumor necrosis factor α (TNF-α)-mediated effects on intestinal epithelial cell proliferation and activation of mitogen-activated protein (MAP) kinase and nuclear factor kappa (NF-κ) B[4]. The biological effects of corticosteroids are pluripotent, and include both immunologic and anti-inflammatory properties, including inhibitory effects on NF-κB, and activating protein-1 (AP-1) regulation of proinflammatory cytokines[5,6]. Immunomodulators, such as azathioprine and its metabolites, induce apoptosis in T cells isolated from IBD patients, and this apoptosis induction requires co-stimulation with CD28[7].

Tacrolimus, a calcineurin inhibitor, has a similar mode of action to that of cyclosporine, but is 100 times more potent, has little effect on renal function, and has better intestinal absorption. Tacrolimus inhibits the transcription of IL-2 and interferon (IFN)-γ in T lymphocytes. Tacrolimus has been effective for short-term clinical improvement in patients with refractory UC[8-10]. Oral tacrolimus was well-tolerated and effective in patients with refractory IBD in the short (4 weeks) to medium term (5 months)[11]. The efficacy of tacrolimus for inducing remission of refractory UC has been established, and long-term administration of tacrolimus appears to be an effective and well-tolerated treatment for patients with refractory UC[12,13]. Oral tacrolimus has been used as a possible alternative to parenteral cyclosporine to treat refractory UC[9,14], and maintenance

therapy with tacrolimus for patients with UC could be considered an alternative to thiopurine therapy[10,15].

However, the influence of tacrolimus on serial changes in cytokine levels has not been well characterized in UC. In the present study, we examined cytokine levels in steroid-refractory UC patients before and after tacrolimus administration.

METHODS

Patient Selection (Figure 1)

A total of 124 patients with UC (55 men, 69 women; mean age, 44.1 years) in our institution between November 2009 and May 2012 were enrolled, and 43 patients were given prednisolone with moderate to severe active UC in this period. Of the 43 patients, 20 patients with steroid-refractory UC were observed. Then, patients were informed about the potential risks and benefits of tacrolimus therapy and consented to its use. Finally, 13 with steroid-refractory UC were evaluated in this study (8 men, 5 women; mean age, 47.7 years) (Table 1). Patients with steroid-refractory UC were included as steroid-resistant or steroid-dependent; 2 were steroid-dependent and 11 were steroid-resistant. Patients with active UC were defined as steroid-resistant when the disease failed to respond to a systemic daily dose of 1 mg per kg of body weight, or 40 mg or more of prednisolone given over at least 7 days, or the equivalent of a daily dose of prednisolone of 30 mg or more over at least 2 weeks. Steroid-dependent patients were defined as patients with active UC in whom attempts to taper steroids had been unsuccessful. The steroid dosage remained the same for 2 weeks, and only those patients in whom a dose of prednisolone of 60 mg/day or more was effective were permitted to decrease the dosage during this period. Efficacy was based on improvement in the frequency of stools and a decreased amount of blood in the stool.

All patients provided informed consent after being told the study purpose and the nature of the procedures involved. Adherence to the Principle of Good Clinical Practice and the Declaration of Helsinki was maintained at all times.

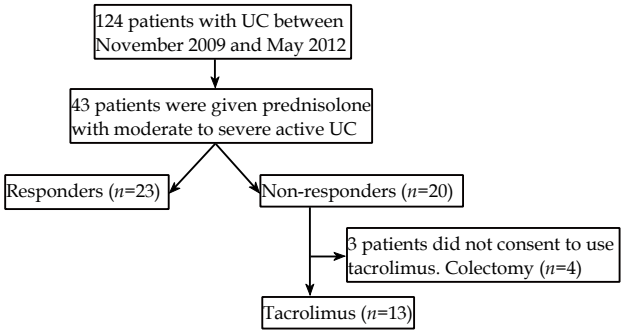

Figure 1 Patient flowchart in this study. A total of 124 patients with UC between November 2009 and May 2012 were enrolled, and 43 patients were given prednisolone with moderate to severe active UC in this period. Of the 43 patients, 20 patients with steroid-refractory UC were observed. Finally, 13 with steroid-refractory UC were evaluated in this study.

Table 1 Characteristics of UC patients.

Age (years) [median (range)]	47.7 (22-74)
Gender (%)	
Men	8 (61.5)
Women	5 (38.5)
Disease duration (years) [median (range)]	4.0 (0.1-16.0)
Disease site (%)	
Total colitis	11 (84.6)
Left sided	2 (15.4)
Degree of severity (moderate/severe)	8/5
Steroid response (%)	
Steroid resistant	11 (84.6)
Steroid dependent	2 (15.4)
Concurrent treatment	
5-aminosalicylic acid	13 (100)
Azathioprine	1 (7.7)
Leukocytapheresis	4 (30.8)

Symptom Assessment

Patients were evaluated using the Disease Activity Index (DAI)[16,17].

The DAI score is a sum of subscores for the following four factors: stool frequency, rectal bleeding, mucosal appearance, and physician's overall assessment. Each factor is graded on a scale from 0 to 3. The total DAI score ranged from 0 to 12; the score increases with severity of disease activity. As an entry criterion, patients were required to have a total DAI score of 6 or more, and mucosal appearance subscore of 2 or 3. The DAI score was determined both before and after 12 weeks of treatment. The efficacy group was defined as a reduction in DAI by at least 4 points and improvements in all categories (stool frequency, rectal bleeding, mucosal appearance, and physician's overall assessment). A worse or unchanged score in any category was considered a treatment failure, even if all other scores improved.

Dosage and Monitoring

The tacrolimus capsules used (Tacrolimus, Astellas Pharma, Japan) contained 0.5 mg or 1 mg of FK506. Patients were given tacrolimus orally in a twice-daily dose of 0.025 mg/kg, with plasma trough levels of 10 to 15 ng/mL for the first 2 weeks. For maintenance therapy, tacrolimus trough levels were maintained at a lower level between 5 and 10 ng/mL.

Assay of Serum Cytokines

Serum levels of 17 cytokines were simultaneously determined using a Bio-Plex suspension array system (Bio-Rad Laboratories, Inc.) before and after 12 weeks of treatment. Levels of the following 17 serum cytokines: IL-1β, IL-2, IL-4, IL-5,IL-6, IL-7, IL-8, IL-10, IL-12, IL-13 and IL-17; TNF-α; INF-γ; granulocyte colony-stimulating factor (G-CSF); granulocyte macrophage colony-stimulating factor (GM-CSF); macrophage inflammatory protein-1β (MIP-1β); and macrophage chemoattractant protein (MCP)-1, were determined before and after 12 weeks of treatment using a Bio-Plex human cytokine 17-plex panel (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and a Bio-Plex suspension array system (Bio-Rad Laboratories, Inc.). The range of detection for all targets was 2-32,000 pg/mL. Serum samples (minimum volume, 12 μL) were diluted (1:4) using Bio-Plex human serum diluent kits (Bio-Rad Laboratories, Inc.). Assays were performed according to the manufacturer's instructions. 50 μL of each serum sample, diluted 1:4, were added to a suspension of beads coated with 17 primary antibodies in each well of an assay plate and incubated for 30 minutes at room temperature with shaking at 300 rpm. After incubation, the beads were washed with washing buffer three times and subsequently reacted with a mixture of 17 types of biotin-conjugated secondary antibody. After a 30-minute reaction, the beads were again washed and resuspended in assay buffer containing streptavidin-phycoerythrin (Str-PE). After 10 minutes of agitation at room temperature, the beads were washed and resuspended in assay buffer without Str-PE. Cytokine concentrations were measured using a Bio-Plex Array Reader (Bio-Rad Laboratories, Inc.). Cytokine types were identified on the basis of the luminescence of the beads, and cytokine concentrations

captured by primary antibody on the beads were determined using PE luminescence. Standard curves for cytokines were generated using the reference cytokine concentrations supplied by the manufacturer. Duplicate examination was performed for each sample. Serum levels of CRP were also measured before and after 12 weeks of treatment.

Statistical Analysis

Data are expressed as means (SD) or as median (25^{th}-75^{th} percentiles), as appropriate. Within-group comparisons were analyzed by paired Student's *t*-tests or non-parametric comparisons using the Mann-Whitney U test. Baseline comparisons between groups were performed using one-way analysis of variance. Correlations were expressed using Spearman's rank correlation coefficient. A *P* value <0.05 was considered statistically significant.

RESULTS

Clinical responses

Clinical responses were calculated, and 11 patients achieved clinical remissions (7 men, 4 women; mean age, 44.6 years; 9 with total colitis, 2 with left-sided colitis; 2 with steroid-dependent, 9 with steroid-resistant). No adverse events were noted in this study.

DAI score

DAI scores across all UC patients were 11.3±4.1 before treatment, and 5.5±4.1 after 12 weeks of treatment. DAI scores after 12 weeks of treatment were significantly decreased compared to those before treatment ($P<0.05$) (Figure 2). In total colitis, DAI scores were 8.4±1.4 before treatment, and 3.5±1.9 after 12 weeks of treatment. DAI scores after 12 weeks of treatment were significantly decreased compared to those before treatment in total colitis. In the efficacy group, DAI scores were 7.7±2.3 before treatment, and 3.0±2.1 after 12 weeks of treatment. DAI scores after 12 weeks of treatment were significantly decreased compared to those before treatment in the efficacy group. No significant differences in DAI scores were observed before and after treatment in the left side colitis or the no efficacy group.

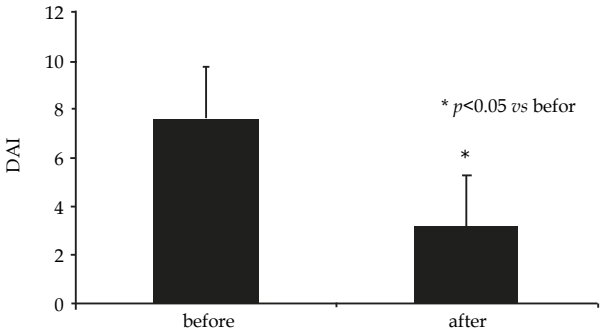

Figure 2 DAI scores after 12 weeks of treatment were significantly decreased compared to those before treatment ($P<0.05$).

DAI mucosal appearance subscores

The DAI mucosal appearance subscore in all UC patients was 2.1±0.6 before treatment, and 1.0±0.7 after 12 weeks of treatment. The DAI mucosal appearance subscore after 12 weeks of treatment was significantly decreased compared to the subscore before treatment (Figure 3). In total colitis patients, the DAI mucosal appearance subscore was 2.1±0.7 before treatment, and 1.0±0.6 after 12 weeks of treatment. The DAI mucosal appearance subscore after 12 weeks of treatment was significantly decreased compared to those before treatment in total colitis. In the efficacy group, DAI scores were 2.3±0.5 before treatment, and 1.0±0.8 after 12 weeks of treatment. The DAI mucosal appearance subscore after 12 weeks of treatment was significantly decreased compared to those before treatment in the efficacy group. No significant differences in the DAI mucosal appearance subscore were observed before and after treatment in the left side colitis or the no efficacy group.

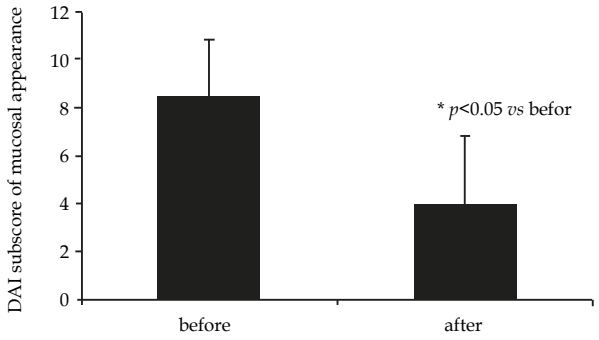

Figure 3 The DAI mucosal appearance subscore after 12 weeks of treatment was significantly decreased compared to before treatment.

Serum CRP levels

Serum CRP levels in all UC patients were 2.5±2.3 mg/dL before treatment, and 0.3±0.3 mg/dL after 12 weeks of treatment. CRP levels after treatment were significantly decreased compared to those before treatment ($P<0.05$). In total colitis, serum CRP levels were 3.0 ±2.1 mg/dL before treatment, and 0.3±0.3 mg/dL after 12 weeks of treatment. Serum CRP levels after 12 weeks of treatment were significantly decreased compared to those before treatment in total colitis patients. In the efficacy group, serum CRP levels were 2.6±2.3 mg/dL before treatment, and 0.2±0.2 mg/dL after 12 weeks of treatment. Serum CRP levels after 12 weeks of treatment were significantly decreased compared to those before treatment in the efficacy group. No significant differences in serum CRP levels were observed before or after treatment in the left side colitis or the no efficacy group.

Cytokine levels

Serum G-CSF levels were significantly decreased after 12 weeks of treatment compared to those before treatment in all UC patients ($P<0.05$) (Table 2). No significant differences in serum levels of other cytokines were observed before or after treatment in all UC patients. In the total colitis group, no significant differences in serum levels of cytokines were observed before or after treatment in total colitis.

In the efficacy group, serum IL-8 levels were 20.6 (8.8-26.6) pg/mL before treatment, and 9.0 (5.1-11.9) pg/mL after 12 weeks of

Table 2 Cytokine levels before and after tacrolimus: All patients.

(pg/mL)	Before	After	P value
IL-1β	2.0 (2.0-2.0)	2.0 (2.0-2.0)	NS
IL-2	2.0 (2.0-2.0)	2.0 (2.0-2.0)	NS
IL-4	2.0 (2.0-2.0)	2.0 (2.0-2.0)	NS
IL-5	2.0 (2.0-2.0)	2.0 (2.0-2.2)	NS
IL-6	3.0 (2.0–7.9)	2.0 (2.0-2.0)	NS
IL-7	6.7 (3.4–10.0)	6.1 (3.9-8.0)	NS
IL-8	22.7 (8.9-31.7)	9.7 (6.2-21.0)	NS
IL-10	2.0 (2.0-2.0)	2.0 (2.0-2.0)	NS
IL-12	2.7 (2.0-8.5)	3.5 (2.0-7.1)	NS
IL-13	2.0 (2.0-2.2)	2.0 (2.0-2.0)	NS
IL-17	2.0 (2.0-7.5)	2.0 (2.0-5.6)	NS
G-CSF	7.4 (2.1-16.0)	2.0 (2.0-4.9)	<0.05
GM-CSF	2.0 (2.0-2.0)	2.0 (2.0-2.0)	NS
IFN-γ	2.0 (2.0-8.7)	2.0 (2.0-2.0)	NS
MCP-1	17.0 (5.7-24.7)	17.7 (3.9-26.1)	NS
MIP-1β	126.3 (113.0-146.5)	115.1 (96.5-142.9)	NS
TNF-α	2.0 (2.0-9.6)	3.2 (2.0-6.6)	NS

treatment, and serum G-CSF levels were 6.8 (2.6-14.3) pg/mL before treatment, and 2.0 (1.5-4.7) pg/mL after 12 weeks of treatment. In the efficacy group, both serum IL-8 and G-CSF levels were significantly decreased after 12 weeks of treatment compared to those before treatment (Figure 4). No significant differences in serum levels of other cytokines were observed before and after treatment in the efficacy group. No significant differences in serum levels of 17 cytokines were observed before or after treatment in the left side colitis group and in the no efficacy group.

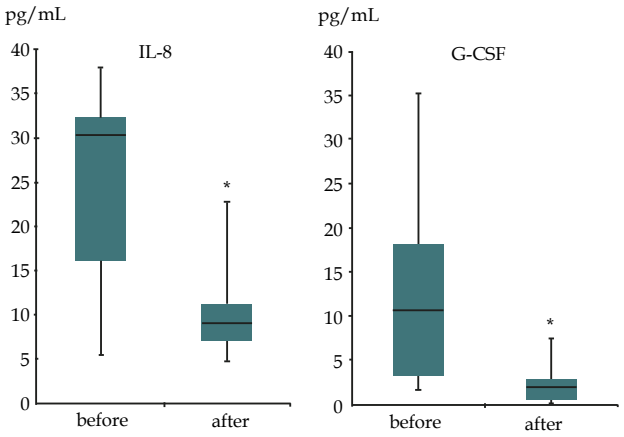

Figure 4 In the efficacy group, serum IL-8 and G-CSF levels were significantly decreased after 12 weeks of treatment compared to those before treatment, * p<0.05 *vs* before.

Relationship between DAI scores and cytokine levels

No significant correlations were observed between DAI scores and the 17 cytokines in all UC patients and in the efficacy group (data not shown).

Relationship between CRP levels and cytokine levels

No significant correlations were observed between CRP levels and the 17 cytokines in all UC patients. Whereas, a significant correlation was noted between CRP levels and serum IL-8 levels in the efficacy group (r=0.60, P<0.05) (Figure 5). No significant correlations were observed between CRP levels and other cytokines in the efficacy group.

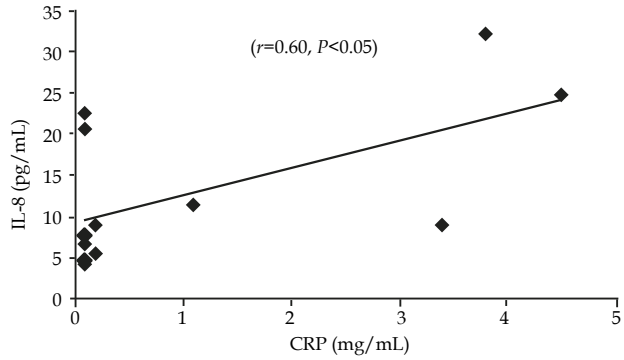

Figure 5 Correlation between changes in CRP levels and serum IL-8 levels before and after treatment in the efficacy group. A significant correlation was noted between CRP levels and serum IL-8 levels (r=0.60; P<0.05).

DISCUSSION

Various studies have reported the role of cytokines in the etiopathogenesis of UC; both humoral and cellular immunologic mechanisms play a role in inflammation in UC patients. Activation of immune cells causes excessive oscillation of both cytokines and inflammatory mediators, which mediates tissue damage and results in increased inflammation. Macrophages in inflamed colons of patients with active UC synthesize IL-1β, TNF-α, and IL-6, which further exacerbates inflammation and an acute phase response. These cytokines may therefore be useful markers for determining prognosis as well as diagnostic, treatment, and follow-up strategies for UC patients[18-25]. Chemokines are excellent candidates for being primarily responsible for the up-regulation, perpetuation, and exacerbation of inflammatory and tissue-destructive processes involved in the immunopathogenesis of IBD.

Tacrolimus is useful for patients with refractory UC to induce remission, and its use improves the quality of life for some UC patients. Tacrolimus is also effective at inducing a clinical improvement in a dose-dependent manner in treatment-resistant UC[26]. The dose-dependent efficacy with a statistically significant benefit for clinical improvement of tacrolimus in trough levels of 10-15 ng/mL was demonstrated previously[27]. Tacrolimus are highly effective for short-term clinical improvement with response rates of about 60-80%[28,29]. However, the use of tacrolimus has been limited by serious adverse events. Then, physicians must know how to use this agent to obtain maximum efficacy while preventing severe adverse effects[30]. Adverse effects that can lead to therapy discontinuation (4%) include: tremor and paresthesias (9%), mild nephrotoxicity (8%), hypertension (2%), and hyperkalemia (2%)[14]. Furthermore, tacrolimus, thiopurine or anti-TNF treatment has a substantial risk of serious adverse events with restricted effectiveness in the long term[31].

Tacrolimus inhibits the activation of several pivotal immune cells in the intestinal mucosa[32]. Dose-dependent, inhibitory effects of tacrolimus on C-C motif ligand 2 (CCL2) and C-X-C motif chemokine 10 (CXCL10) expression were observed at the mRNA and protein levels, and tacrolimus strongly inhibited the TNF-α-induced phosphorylation of the p38 mitogen-activated protein (MAP) kinase in human colonic myofibroblasts[33]. However, the changes in serum cytokine levels in UC patients before and after tacrolimus therapy have not been well characterized. We analyzed many serum cytokines simultaneously using a multiplex assay system to identify those cytokines that are clinically and statistically associated with tacrolimus therapy efficacy in patients with active UC. In the present study, we simultaneously analyzed 17 serum cytokines using a multiplex assay system to elucidate the serial changes in their levels, which are clinically and statistically associated with the efficacy of tacrolimus therapy in patients with active UC. We also demonstrated that IL-8 and G-CSF levels decreased following tacrolimus administration, especially in the efficacy group, which suggests that IL-8 and G-CSF would be more crucial marker for the subgroup of patients with UC belonging to the efficacy group with tacrolimus treatment.

IL-8 is an α-chemokine that recruits and activates neutrophils, which are abundant in the intestinal lesions of UC patients. IL-8 expression is significantly increased in active versus inactive UC mucosa, and is therefore thought to play an important role in UC pathogenesis[34-40]. Furthermore, our results showed that following tacrolimus treatment, changes of serum IL-8 levels showed a positive and significant correlation with CRP levels, although similar relationships were not identified for the other cytokines. Therefore, at present, serum IL-8 level is considered to be a potentially useful marker of the efficacy of tacrolimus treatment in UC patients.

Th17-related factors, including G-CSF, were identified as predictive serum biomarkers for chronic colitis[41]. G-CSF has emerged as a potential tool for the modulation of intestinal inflammation and repair. G-CSF may be an alternative treatment for IBD[42]. G-CSF appears to improve intestinal inflammation and immunodeficiency

status in an experimental model of colitis[43]. The ability of epithelial cells to produce chemokines could play an important initiating role in the immunopathophysiologic events associated with the upregulation and perception of inflammation in UC and CD[44]. However, we showed no significant correlations were observed between CRP levels and G-CSF levels of tacrolimus treatment in UC patients. Therefore, a future study should investigate the impact of tacrolimus on the mucosal m-RNA levels in patients with UC. Furthermore, in this study, the numbers of steroid-refractory UC patients were small and we measured only serum cytokine levels, a future study should also investigate to confirm the validity and significance of these findings including mucosal m-RNA levels.

In conclusion, treatment with tacrolimus reduced serum IL-8 and G-CSF levels in UC patients, which may be associated with an overall reduction in disease severity. An additional study is required to see the impact of tacrolimus on the mucosal levels of these cytokines.

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REFERENCES

1 Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. *Nat Rev Immunol* 2003; **3**: 521-533
2 Ordás I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. *Lancet* 2012; **380**: 1606-1619
3 Danese S, Fiocchi C. Ulcerative Colitis. *N Engl J Med* 2011; **365**: 1713-1725.
4 Kaiser GC, Yan F, Polk DB. Mesalamine blocks tumor necrosis factor growth inhibition and nuclear factor kappa B activation in mouse colonocytes. *Gastroenterology* 1999; **116**: 602-609
5 Refojo D, Liberman A, Holsboer F, Arzt E. Transcription factor-mediated molecular mechanisms involved in the functional cross-talk between cytokines and glucocorticoids. *Immunol Cell Biol* 2001; **79**: 385-394
6 Yamamoto Y, Gaynor RB. Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer. *J Clin Invest* 2001; **107**: 135-142
7 Tiede I, Fritz G, Strand S, Poppe D, Dvorsky R, Strand D, Lehr HA, Wirtz S, Becker C, Atreya R, Mudter J, Hildner K, Bartsch B, Holtmann M, Blumberg R, Walczak H, Iven H, Galle PR, Ahmadian MR, Neurath MF. CD28-dependent Rac1 activation is the molecular target of azathioprine in primary human CD4+T lymphocytes. *J Clin Invest* 2003; **111**: 1133-1145
8 Vavricka SR, Rogler G. Treatment of severe ulcerative colitis: differences in elderly patients? *Dig Dis* 2009; **27**: 315-321
9 Ogata H, Matsui T, Nakamura M, Iida N, TakazoeM, Suzuki Y, Hibi T. A randomised dose finding study of oral tacrolimus (FK506) therapy in refractory ulcerative colitis. *Gut* 2006; **55**: 1255-1262
10 Ogata H, Kato J, Hirai F, Hida N, Matsui T, Matsumoto T, Koyanagi K, Hibi T. Double-blind, placebo-controlled trial of oral tacrolimus (FK506) in the management of hospitalized patients with steroid-refractory ulcerative colitis. *Inflamm Bowel Dis* 2012; **18**: 803-808
11 Ng SC, Arebi N, Kamm MA. Medium-term results of oral tacrolimus treatment in refractory inflammatory bowel disease. *Inflamm Bowel Dis* 2007; **13**: 129-134
12 Yamamoto S, Nakase H, Mikami S, Inoue S, Yoshino T, Takeda Y, Kasahara K, Ueno S, Uza N, Kitamura H, Tamaki H, Matsuura M, Inui K, Chiba T. Long-term effect of tacrolimus therapy in patients with refractory ulcerative colitis. *Aliment Pharmacol Ther* 2008; **28**: 589-597
13 Miyoshi J, Matsuoka K, Inoue N, Hisamatsu T, Ichikawa R, Yajima T, Okamoto S, Naganuma M, Sato T, Kanai T, Ogata H, Iwao Y, Hibi T. Mucosal healing with oral tacrolimus is associated with favorable medium- and long-term prognosis in steroid-refractory/dependent ulcerative colitis patients. *J Crohns Colitis* 2013 May 13. pii: S1873-9946(13)00172-4
14 Baumgart DC, Pintoffl JP, Sturm A, Wiedenmann B, Dignass AU. Tacrolimus is safe and effective in patients with severe steroid-refractory or steroid-dependent inflammatory bowel disease--a long-term follow-up. *Am J Gastroenterol* 2006; **101**: 1048-1056
15 Yamamoto S, Nakase H, Matsuura M, Masuda S, Inui K, Chiba T. Tacrolimus therapy as an alternative to thiopurines for maintaining remission in patients with refractory ulcerative colitis. *J Clin Gastroenterol* 2011; **45**: 526-530
16 Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis, a randomized study. *N Engl J Med* 1987; **317**: 1625-1629
17 Sutherland LR, Martin F, Greer S, Robinson M, Greenberger N, Saibil F, Martin T, Sparr J, Prokipchuk E, Borgen L. 5-Aminosalicylic acid enema in the treatment of distal ulcerative colitis, proctosigmoiditis, and proctitis. *Gastroenterology* 1987; **92**: 1894-1898
18 Funakoshi K, Sugimura K, Sasakawa T, Bannai H, Anezaki K, Ishizuka K, Yoshida K, Narisawa R, Asakura H. Study of cytokines in ulcerative colitis. *J Gastroenterol* 1995; **30**: 61-63
19 Guimbaud R, Bertrand V, Chauvelot-Moachon L, Quartier G, Vidon N, Giroud JP, Couturier D, Chaussade S. Network of inflammatory cytokines and correlation with disease activity in ulcerative colitis. *Am J Gastroenterol* 1998; **93**: 2397-2404
20 Melgar S, Yeung MM, Bas A, Forsberg G, Suhr O, Oberg A, Hammarstrom S, Danielsson A, Hammarstrom ML. Over-expression of interleukin 10 in mucosal T cells of patients with active ulcerative colitis. *J Clin Exp Immunol* 2003; **134**: 127-137
21 Murata Y, Ishiguro Y, Itoh J, Munakata A, Yoshida Y. The role of proinflammatory and immunoregulatory cytokines in the pathogenesis of ulcerative colitis. *J Gastroenterol* 1995; **30**: 56-60
22 Funakoshi K, Sugimura K, Anezaki K, Bannai H, Ishizuka K, Asakura H. Spectrum of cytokines gene expression in intestinal mucosal lesions of Crohn's disease and ulcerative colitis. *Digestion* 1998; **59**: 73-78
23 Masuda H, Iwai S, Tanaka T, Hayakawa S. Expression of IL-8, TNF-alpha and IFN-gamma m-RNA in ulcerative colitis, particularly in patients with inactive phase. *J Clin Lab Immunol* 1995; **46**: 111-123
24 Dolar ME. Clinical consideration of the cytokines in inflammatory bowel disease. *Am J Gastroenterol* 1995; **90**: 520-521
25 Sato K, Chiba T, Ohkusa T. Serial changes of cytokines in active ulcerative colitis: effects of antibiotic combination therapy. *Hepatogastroenterology* 2009; **56**: 1016-1021
26 Waters OR, Lawrance IC. Understanding the use of immunosuppressive agents in the clinical management of IBD. *Curr Drug Targets* 2011; **12**: 1364-1371
27 Bruns T, Stallmach A. Drug monitoring in inflammatory

bowel disease: helpful or dispensable? *Dig Dis* 2009; **27**: 394-403.
28 Lichtiger S, Present DH, Kornbluth A, Gelernt I, Bauer J, Galler G, Michelassi F, Hanauer S. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. *N Engl J Med* 1994; **330**: 1841-1845
29 Baumgart DC, Macdonald JK, Feagan B. Tacrolimus (FK506) for induction of remission in refractory ulcerative colitis. *Cochrane Database Syst Rev* 2008; **3**: CD007216
30 Naganuma M, Fujii T, Watanabe M. The use of traditional and newer calcineurin inhibitors in inflammatory bowel disease. *J Gastroenterol* 2011; **46**: 129-137
31 Maser EA, Deconda D, Lichtiger S, Ullman T, Daniel H, Kornbluthet A. Cyclosporine and infliximab as rescue therapy for each other in patients with steroid-refractory ulcerative colitis. *Clin Gastroenterol Hepatol* 2008; **6**: 1112-1116
32 van Dieren JM, Lambers ME, Kuipers EJ, Samsom JN, van der Woude CJ, Nieuwenhuis EE. Local immune regulation of mucosal inflammation by tacrolimus. *Dig Dis Sci* 2010; **55**: 2514-2519.
33 Aomatsu T, Imaeda H, Takahashi K, Fujimoto T, Kasumi E, Yoden A, Tamai H, Fujiyama Y, Andoh A. Tacrolimus (FK506) suppresses TNF-α-induced CCL2 (MCP-1) and CXCL10 (IP-10) expression via the inhibition of p38 MAP kinase activation in human colonic myofibroblasts. *Int J Mol Med* 2012; **30**: 1152-1158
34 Katsuta T, Lim C, Shimoda K, Shibuta K, Mitra P, Banner BF, Mori M, Barnardet GF. Interleukin-8 and SDF1-α mRNA Expression in Colonic Biopsies from patients with inflammatory bowel disease. *Am J Gastroenterol* 2000; **95**: 3157-3164
35 Mazzucchelli L, Hauser C, Zgraggen K, Wagner H, Hess M, Laissue JA, Mueller C. Expression of IL-8 gene in inflammatory bowel disease is related to histological grade of active inflammation. *Am J Pathol* 1994; **144**: 997-1007
36 Grimm MC, Elsbury SKO, Parli P, Doe WF. Enhanced expression and production of monocyte chemoattractant protein-1 in inflammatory bowel disease mucosa. *J Leukocyte Biol* 1996; **59**: 804-812
37 Izzo RS, Witkon K, Chen AI, Hadjiyane C, Weinstein MI, Pellecchia C. Interleukin 8 and neutrophil makers in colonic mucosa from patients with ulcerative colitis. *Am J Gastroenterol* 1992; **87**: 1447-1452
38 Izzo RS, Witkon K, Chen AI, Hadjiyane C, Weinstein MI, Pellecchia C. Neutrophil activating peptide in colonic mucosa from patients with Crohn's disease. *Scand J Gastroenterol* 1993; **28**: 296-300
39 Mitsuyama K, Toyunaga A, Sasaki E, Watanabe K. IL-8 as an important chemoattractant for neutrophils in ulcerative colitis and Crohn's disease. *Clin Exp Immunol* 1994; **96**: 432-436
40 Nielsen OH, Rudiger N, Gaustadnes M, Horn T. Intestinal interleukin 8 concentration and gene expression in inflammatory bowel disease. *Scand J Gastroenterol* 1997; **32**: 1028-1032
41 McBee ME, Zeng Y, Parry N, Nagler CR, Tannenbaum SR, Schauer DB. Multivariate modeling identifies neutrophil- and Th17-related factors as differential serum biomarkers of chronic murine colitis. PLoS One 2010;5:e13277
42 Barahona-Garrido J, Yamamoto-Furusho JK. New treatment options in the management of IBD - focus on colony stimulating factors. *Biologics* 2008; **2**: 501-504
43 Hommes DW, Meenan J, Dijkhuizen S, Ten Kate FJ, Tytgat GN, Van Deventer SJ. Efficacy of recombinant granulocyte colony-stimulating factor (rhG-CSF) in experimental colitis. *Clin Exp Immunol* 1996; **106**: 529-533
44 Reinecker HC, Loh EY, Ringler DJ, Mehta A, Rombeau JL, MacDermott RP. Monocyte-chemoattractant protein 1 gene expression in intestinal epithelial cells and inflammatory bowel disease mucosa. *Gastroenterology* 1995; **108**: 40-50

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