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Interleukin-18 serum levels in inflammatory bowel diseases: correlation with disease activity and inflammatory markers

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Summary

Background: An imbalance of cytokines is believed to contribute to the immunopathogenesis of inflammatory bowel diseases (IBD). Serum cytokine levels may correlate with disease activity and acute phase reactivity.

Aim: To determine the correlation of systemic interleukin-18 (IL-18) levels with disease activity and other markers of inflammation using a cross-sectional pilot study in outpatients with IBD.

Methods: Peripheral venous blood was obtained from 84 patients with Crohn's disease (CD) and from 46 patients with ulcerative colitis (UC). Serum levels of C-reactive protein (CRP), IL-8, IFN-γ, IL-12p70 and IL-18 were assessed by ELISA. Disease activity was expressed by the Crohn's Disease Activity Index (CDAI) and the Clinical Activity Index (CAI), respectively. Statistical analysis comprised correlation coefficients and linear regression analysis.

Results: In CD IL-18 and other cytokine concentrations, CRP levels, leukocyte and platelet counts did not correlate with the CDAI. However, IL-18 as well as IL-8 and platelets positively correlated with CRP levels ($p < 0.001$), while IFN-γ and IL-12p70 did not. In contrast, in UC only the CAI and CRP levels showed a significant positive correlation.

Conclusions: In CD IL-18 lacks significant correlation with the CDAI, as do serum acute phase protein and other cytokine markers of inflammation. As opposed to UC, IL-18 and IL-8 may, however, serve as indicators of acute phase reactivity in CD and should be explored in a larger study.

Key words: inflammatory bowel disease; disease activity index; CRP; cytokines

Introduction

The chronic relapsing inflammatory bowel diseases, Crohn's disease and ulcerative colitis, are typically characterised by episodes of acute flares and remission [1, 2]. Depending on disease location and extent, exacerbation leads to diarrhoea, abdominal pain and systemic symptoms such as fatigue and weight loss [3–5]. Disease activity indices have been developed as outcome measures in clinical trials [6, 7]. They may help to reproducibly and validly assess the patients' status and to support therapeutic decision making [6]. Variables of disease activity indices comprise frequency of bowel movements, severity of abdominal pain, general well-being, occurrence of extraintestinal manifestations and laboratory parameters [8].

The efficacy of biologicals in recent clinical trials has been associated with elevated concentrations of acute phase proteins in serum samples prior to onset of treatment [9, 10]. Vice versa, placebo responses seem to negatively correlate with systemic inflammation. Therefore, objectively assessable acute phase reactivity may present a better correlate of disease activity. This approach may be particularly important in outpatients with inactive or mild disease, because direct endoscopic or radiologic visualisation of the degree of inflammation are rarely performed in this patient subgroup.

Selection of relevant circulating, thus easily obtainable markers of inflammation may focus on substances correlating with inflammation in general, such as leucocytes and C-reactive protein, or on cytokines that may more specifically reflect inflammatory cascades associated with inflammatory bowel disease [11, 12].

Interleukin (IL)-18 is a pleiotropic proinflammatory cytokine with stimulatory effects on both T helper-1 and T helper-2 cell responses [13–18] that have been implied in Crohn's disease and ul-

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cerative colitis, respectively. In patients with Crohn´s disease a markedly enhanced expression of IL-18 was found in infiltrating lamina propria macrophages and intestinal epithelial cells [19, 20]. Several experimental animal models have been linked to an increased expression of IL-18, and inhibition of IL-18 in some of these led to a significantly decreased degree of inflammation [21–24].

Our study aimed to prospectively assess and then to correlate systemic levels of IL-18 in outpatients with inflammatory bowel disease with disease activity and blood-derived standard markers of inflammation as well as additional cytokines associated with the pathogenesis of these conditions.

Materials and methods

Patients

Patients were recruited at the outpatient clinic at the Department of Medicine II, University Hospital Mannheim, Germany from January 2001 to June 2003. Ethical approval was obtained from the local ethics committee (#0199/02), and was in accordance with the Helsinki Declaration from 1975. All participants gave their written informed consent. The consecutive recruitment ensured that all patients had the opportunity to participate in the cross-sectional study if they met the following study criteria: 1) diagnosis of Crohn's disease or ulcerative colitis on the basis of clinical symptoms and endoscopic, radiological or histological criteria; 2) age ≥16 years; 3) disease duration of at least 6 months; 4) no ostomy, and 5) no other serious medical condition at the time of inclusion. At their initial presentation the majority of the patients underwent upper endoscopy, ileocolonoscopy and small-bowel follow-through or MRI.

The disease activity of patients with Crohn's disease or ulcerative colitis was determined by the criteria of the Crohn's disease activity index (CDAI) [8] and the clinical activity index (CAI) of Rachmilewitz [25], respectively. Furthermore, concomitant medical conditions and current medication was noted.

Assessment of blood cell count, acute-phase reactants and cytokines

On the last day of the seven-day disease activity index diary, blood was obtained by venepuncture into heparinised tubes. An aliquot was used for determination of the complete blood cell count. Serum was collected after centrifugation of heparinised blood and stored at –70 °C until further processing. Levels of C-reactive protein were assessed with a commercially available assay (Siemens, Germany).

The normal ranges of the complete blood cell count and the C-reactive protein were as follows: White blood cells 4.2–10.2 x 10^9/l; haemoglobin 12.3–15.2 g/dl (females), 13.2–16.7 g/dl (males); haematocrit 33.0–41.0% (females), 33.4–46.2% (males); platelets 165–387x10^9/l (females), 145–348x10^9/l (males); C-reactive protein 0–5 mg/l. Historical cytokine levels in a healthy control population were as follows: IL-18 165.2 ± 43.6 ng/l; IL-12p70 14.7 ± 6.8 ng/l; IFN-γ 34.4 ± 18.0 ng/l) [26]; IL-8 11.5 ± 5.3 ng/l (unpublished observation).

Levels of IL-18 were determined in paired supernatant samples by a specific sandwich enzyme immunoassay (ELISA) with minor modifications as described previously [27]. Assessment of IL-12p70, IL-8 and IFN-γ levels was performed with commercially available ELISAs (OptEIA Set, BD Biosciences Pharmingen, San Diego, USA).

Outcomes and statistical analysis

Primarily, correlation of IL-18 with disease activity was assessed. Secondarily, correlation of IL-18 with other laboratory parameters and cytokines was tested. Further, correlations between other parameters were considered. Statistical analysis was performed with the SAS software version 8.2 and 9.1. Descriptive statistics included mean, median and standard deviation. Differences between groups were determined with a T-test for two independent samples. The Wilcoxon test was applied for T-test confirmation. For correlation analysis Pearson's correlation coefficient r and the determination coefficient R^2 were determined. A p-value ≤0.05 was considered to be significant.

Results

Patients' characteristics

Eighty-four patients with Crohn's disease and 46 patients with ulcerative colitis were included in the study. Selected characteristics of the participants are summarised in table 1. Patient age ranged from 16 to 72 years with a mean of 42.75 and a median of 43. Accumulation of very young or very old patients with inflammatory bowel diseases was not present in our cohort. The mean duration of illness was 146 months. There was no significant difference in the mean duration of illness between the two patient groups (141.67 months in Crohn's disease vs 151.83 months in ulcerative colitis [p = 0.653]). At the time of testing, a minority of patients was on systemically active anti-inflammatory drugs such as corticosteroids or immunosuppressants. None of the patients were receiving anti-tumour necrosis factor-α antibodies. The rate of extraintestinal manifestations was comparably high with a predominance of arthralgia.

Disease activity

Disease activity among patients with Crohn's disease and ulcerative colitis as assessed with the CDAI and CAI, respectively, was as follows (table 1):

Crohn's disease (N = 59): 136.49 ± 90.82 (8.80–464.70); ulcerative colitis (N = 35) 2.37 ± 2.33 (0–7). Accordingly, the mean CDAI reflected clinical remission. However, patients with severely active disease were also part of the cohort. Further, the mean CAI also mirrored clinical remission. Overall disease activity in ulcerative colitis was low.

Inflammatory markers and cytokine levels

Single laboratory parameters such as maximum, minimum value, median and standard deviation were determined and analysed by means of descriptive statistics (table 2). In some patients, not all of the parameters could be determined, as indicated in the subsequent tables by the number of subjects analysed. The mean and median cy-

Table 1

Patients' disease characteristics and medication at baseline.

	CD (n = 84)	UC (n = 46)
Age, in years mean ± SD (range)	40.35 ± 12.92 (16–68)	45.48 ± 14.36 (21–66)
Gender		
Women, n (%)	40 (47.62)	44 (52.38)
Men, n (%)	26 (56.52)	20 (43.48)
Duration of disease, in months mean ± SD	151.83 ± 121.63	141.67 ± 121.25
Disease location at time of testing, n (%)		
Small bowel only	9 (10.7)	
Large bowel only	9 (10.7)	
Small/large bowel	43 (51.2)	
N.a.	23 (27.3)	
Proctitis		0 (0)
Left sided colitis		16 (34.8)
Extensive and pancolitis		19 (41.3)
N.a.		11 (23.9)
Disease activity, CDAI (CD) and CAI (UC)		
Mean ± SD	136.49 ± 90.82	2.37 ± 2.33
(range)	(8.80–464.70)	(0–7)
Prednisolone equivalent ≥7.5 mg/day, n (%)	13 (10)	5 (10.87)
Immunosuppressant, azathioprine n (%)	21(16.15)	10 (21.74)
Extraintestinal manifestation, n (%)		
All sites	44 (52.39)	22 (47.83)
Arthralgia	55 (42.31)	14 (30.44)
Eye manifestation	16 (19.05)	3 (6.52)
Skin manifestation	16 (19.05)	9 (19.56)

CD: Crohn's disease; UC: ulcerative colitis;
N.a. Not assessed at time of study inclusion

Table 2

Laboratory results (mean, std, range) in patients with Crohn's disease (CD; n = 81) and ulcerative colitis (UC; n = 43).

	CD	UC
CRP (mg/l)	8.33 ± 11.4 (0.5 – 72.7)	4.81 ± 5.21 (0.5 – 24.7)
WBC ($\times 10^9$/l)	8.11 ± 2.85 (3.3 – 17.5)	7.78 ± 3.16 (3.3 – 18.7)
Hb (g/dl)	13.96 ± 1.39 (10.6 – 17.1)	14.09 ± 1.58 (7.8 – 16.2)
Hct (%)	41.35 ± 3.76 (31 – 49.4)	41.93 ± 3.53 (33.9 – 45.2)
Plt ($\times 10^9$/l)	333.48 ± 95.45 (141 – 728)	314 ± 92.1 (170 – 666)

CRP: C-reactive protein; WBC: white blood count; Hb: haemoglobin; Hct: haematocrit; Plt: platelets

Table 3

Cytokines (ng/L) in patients with Crohn's disease (CD; n = 81) and ulcerative colitis (UC; n = 43) (mean, Std, range).

	CD	UC
IL-18 (ng/l)	387.76 ± 170.45 (125.8 – 1011.35)	274.09 ± 173.68 (118.35 – 873.8)
IL-8	45.96 ± 243.35 (3.16 – 2175)	82.59 ± 293.28 (3.97 – 2594)
IL12p70	14.45 ± 6.77 (7.96 – 51.70)	12.61 ± 3.46 (8.92 – 24.51)
IFN-γ	31.27 ± 35.65 (9.18 – 220.2)	33.1 ± 29.2 (9.83 – 179.35)

tokine levels in the whole cohort as well as in the two patient groups were determined (table 3). In Crohn's disease the mean C-reactive protein level was above the upper level of normal. As compared with historical data of a control group, mean serum levels of IL-18 and IL-8 were higher in Crohn's disease, while IL-12p70 and IFN-γ levels were not different. In ulcerative colitis, the mean serum level of IL-18 was lower than in Crohn's disease but still higher than in the historical control group. By contrast, the mean IL-8 serum level was higher in ulcerative colitis than in Crohn's disease. In the former, mean serum levels of IL-12p70 and IFN-γ were not different from historical controls. The data indicated suitability of both IL-18 and IL-8 serum levels for disease activity assessment in inflammatory bowel diseases.

Table 4

Correlation analysis in patients with Crohn's disease (CRP: C-reactive protein, RBC: red blood cells, HGB: haemoglobin, HCT: haematocrit, PLT: platelets, WBC: white blood cells).

parameters	N	R^2	r	p
IL-12p70/IL-8	83	0.1175	0.34273	0.0015
IL-8/IL-18	83	0.1519	0.38973	0.0003
IL-8/CRP	82	0.2520	0.50204	<0.0001
IL-8/HGB	74	0.0599	–0.24472	0.0356
IL-18/CRP	82	0.1843	0.42930	<0.0001
IL-18/HGB	74	0.0721	–0.26859	0.0207
HCT/IL-18	75	0.0852	–0.29188	0.0111
HCT/CRP	75	0.1278	–0.35745	0.0016
PLT/IL-18	74	0.0729	0.26996	0.0200
PLT/CRP	74	0.3992	0.63182	<0,0001

Table 5

Correlation analysis in patients with ulcerative colitis (CAI: Clinical Activity Index, CRP: C-reactive protein, HGB: haemoglobin, WBC: white blood cells, HCT: haematocrit, PLT: platelets).

parameters	N	R^2	r	p
CAI/CRP	35	0.2694	0.51909	0.0014
CAI/HGB	35	0.2671	–0.51678	0.0015
CRP/WBC	41	0.1453	0.38118	0.0139
HCT/CAI	35	0.2002	–0.44745	0.0070
PLT/CAI	41	0.1120	0.33471	0.0324

Correlation analyses

Pearson's correlation coefficient r, determination coefficient R^2 and p-values (significance) were calculated between all laboratory parameters after descriptive statistics were completed. Parameters were analysed separately for patients with Crohn's disease (table 4) and ulcerative colitis (table 5). Interestingly, no statistically significant correlation between Crohn's disease activity as determined by the CDAI and IL-18 was found. The same was true for the other cytokines that were assessed in the study and the C-reactive protein levels (table 4). However, in contrast to patients with ulcerative colitis, patients with Crohn's disease displayed a highly significant correlation between levels of IL-18 with the C-reactive protein levels as did levels of IL-8 and platelets, respectively. It is of note that the CAI and C-reactive protein level in ulcerative colitis showed a good correlation (table 5). However, no significant correlation was demonstrated between IL-18 and the CAI. Further, in this condition IL-18 did not correlate with levels of other markers of inflammation or cytokines (not shown).

Discussion

Crohn's disease and ulcerative colitis are chronic inflammatory intestinal diseases (IBD). Different pro-inflammatory cytokines and chemokines seem to be responsible for sustained inflammation, causing a continuous invasion of leukocytes from the bloodstream into the intestinal mucosa [11, 12, 28, 29]. Data support the assumption that Crohn's disease is characterised by a cytokine milieu favouring T helper 1 cell activation, while ulcerative colitis has been associated with a T helper 2 cell predominance [19, 30–33].

This cross-sectional pilot study focussed on the systemic presence of T helper 1 cell associated cytokines in IBD and particularly the potential correlation of IL-18 with clinical activity indices and acute phase reactants. Our data suggest that IL-18, although not correlating with clinical disease activity, may serve as an indicator of acute phase reactivity in Crohn's disease but not in ulcerative colitis. The same seems to be true when using IL-8 as a marker. Disease activity was generally low in our outpatient cohort. However, a patient group was thereby enrolled that might more realistically reflect the setting of an outpatient clinic. The results justify a larger study indicating sensitivity, specificity and prediction in patients with inflammatory bowel disease when using these markers.

Expression of IL-18 has been studied in patients with inflammatory bowel diseases. In a Japanese cohort, IL-18 serum concentrations were higher in patients with Crohn's disease than in patients with ulcerative colitis or in controls. Furthermore, a positive correlation between IL-18 and the CDAI was noted [34]. Furuya and coworkers reported IL-18 serum levels to be higher in five patients with Crohn's disease than in controls [35]. Interestingly, a positive correlation between IL-18 levels and the expression of the human glucocorticoid receptor β was described in steroid-resistant patients [36]. A predominance of an elevation of IL-18 in Crohn's disease as compared to ulcerative colitis was demonstrated in the plasma. In addition, colonic explant cultures from inflamed areas released more IL-18 than those from unaffected segments [37]. Naftali and colleagues reported increased levels of both IL-18 and IL-18 binding protein during exacerbation of

Crohn's disease and in patients with ulcerative colitis [38]. In children, however, elevated serum levels of IL-18 were confined to patients with Crohn's disease [39].

Our data also support the recent notion that clinical activity indices may insufficiently correlate with markers of systemic inflammation. In our cohort, the CDAI used in patients with Crohn's disease did not significantly correlate with the level of the C-reactive protein or the leukocyte and platelet count. In contrast, the CAI used in patients with ulcerative colitis exhibited a significant correlation with the C-reactive protein level, but not with the leukocyte or the platelet count. The CDAI was developed more than 30 years ago. Lone Jørgensen and co-workers stated that nowadays inflammatory parameters were much more specific than the haematocrit, the only laboratory parameter used for calculation of the CDAI [40]. Others agree that the CDAI does not represent a sufficiently reliable index for determination of disease activity in Crohn's disease. Gomes and colleagues could not find any correlation between the CDAI and histological findings in 50 patients with Crohn's colitis, and therefore concluded that this index was no longer reliable [41]. Vermeire and colleagues, however, provided data demonstrating a good correlation between the CDAI and different inflammatory markers such as C-reactive protein [9]. Furthermore, in clinical studies the CDAI has been the most widely used index for disease activity in Crohn's disease. However, it is desirable to develop an index which on the one hand is mostly independent from patient compliance and easier to calculate, and on the other hand includes more objective parameters, such as C-reactive protein or cytokines.

A highly significant correlation between C-reactive protein on the one hand and IL-18, IL-8 and platelets on the other hand was found in our cohort of patients with Crohn's disease. In contrast, no significant correlation between C-reactive protein and tested cytokines was found in patients with ulcerative colitis. Higher values of C-reactive protein in patients with Crohn's disease than patients with ulcerative colitis have been reported, especially in the event of an acute flare. The underlying reasons are widely unknown. Solem and co-workers in their study analysed the correlation between C-reactive protein and the clinical and endoscopic disease activity in patients with chronic inflammatory intestinal diseases. C-reactive protein level in Crohn's disease correlated with the endoscopic and histological inflammation as well as with the clinical inflammatory activity, whereas no correlation with histological findings was found in ulcerative colitis [42]. Most investigators concluded that the inflammatory activity in ulcerative colitis is difficult to appraise by determination of the C-reactive protein level. This could also explain the missing correlation between C-reactive protein and pro-inflammatory cytokines in patients with ulcerative colitis in our study.

In conclusion our report supports the necessity to identify and validate new activity markers in inflammatory bowel disease patients including mediators of the immunological activation in the gut that potentially better reflect the inflammatory burden of the patient. IL-18 and also IL-8 seem to be good candidates, at least in Crohn's disease.

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References

1 Nikolaus S, Schreiber S. Diagnostics of inflammatory bowel disease. Gastroenterology. 2007;133(5):1670–89.
2 Baumgart DC, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. Lancet. 2007;369(9573):1641–57.
3 Stange EF, Travis SP, Vermeire S, Beglinger C, Kupcinkas L, Geboes K, et al. European evidence based consensus on the diagnosis and management of Crohn's disease: definitions and diagnosis. Gut. 2006;55(Suppl 1):i1–15.
4 Caprilli R, Viscido A, Latella G. Current management of severe ulcerative colitis. Nat Clin Pract Gastroenterol Hepatol. 2007;4(2):92–101.
5 Kornbluth A, Sachar DB. Ulcerative colitis practice guidelines in adults (update): American College of Gastroenterology, Practice Parameters Committee. Am J Gastroenterol. 2004;99 (7):1371–85.
6 Sands BE, Abreu MT, Ferry GD, Griffiths AM, Hanauer SB, Isaacs KL, et al. Design issues and outcomes in IBD clinical trials. Inflamm Bowel Dis. 2005;11(Suppl 1):S22–28.
7 Freeman HJ. Use of the Crohn's disease activity index in clinical trials of biological agents. World J Gastroenterol. 2008;14(26):4127–30.
8 Best WR, Becktel JM, Singleton JW, Kern F Jr. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. Gastroenterology. 1976;70(3):439–44.
9 Vermeire S, Van Assche G, Rutgeerts P. C-reactive protein as a marker for inflammatory bowel disease. Inflamm Bowel Dis. 2004;10(5):661–5.
10 Sandborn WJ, Colombel JF, Enns R, Feagan BG, Hanauer SB, Lawrance IC, et al. Natalizumab induction and maintenance therapy for Crohn's disease. N Engl J Med. 2005;353(18): 1912–25.

11 Brown SJ, Mayer L. The immune response in inflammatory bowel disease. Am J Gastroenterol. 2007;102(9):2058–69.
12 Bamias G, Cominelli F. Immunopathogenesis of inflammatory bowel disease: current concepts. Curr Opin Gastroenterol. 2007;23(4):365–9.
13 Okamura H, Tsutsi H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, et al. Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature. 1995;378(6552):88–91.
14 Ushio S, Namba M, Okura T, Hattori K, Nukada Y, Akita K, et al. Cloning of the cDNA for human IFN-gamma-inducing factor, expression in Escherichia coli, and studies on the biologic activities of the protein. J Immunol. 1996;156(11): 4274–9.
15 Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. Annu Rev Immunol. 2001;19:423–74.
16 Reddy P. Interleukin-18: recent advances. Curr Opin Hematol. 2004;11(6):405–10.
17 Gracie JA, Robertson SE, McInnes IB. Interleukin-18. J Leukoc Biol. 2003;73(2):213–24.
18 Dinarello CA. Interleukin-18 and the pathogenesis of inflammatory diseases. Semin Nephrol. 2007;27(1):98–114.
19 Monteleone G, Trapasso F, Parrello T, Biancone L, Stella A, Iuliano R, et al. Bioactive IL-18 expression is up-regulated in Crohn's disease. J Immunol. 1999;163(1):143–7.
20 Pizarro TT, Michie MH, Bentz M, Woraratanadharm J, Smith MF Jr, Foley E, et al. IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: expression and localization in intestinal mucosal cells. J Immunol. 1999;162(11): 6829–35.
21 Sivakumar PV, Westrich GM, Kanaly S, Garka K, Born TL, Derry JM, Viney JL. Interleukin 18 is a primary mediator of the inflammation associated with dextran sulphate sodium induced colitis: blocking interleukin 18 attenuates intestinal damage. Gut. 2002;50(6):812–20.
22 Wirtz S, Becker C, Blumberg R, Galle PR, Neurath MF. Treatment of T cell-dependent experimental colitis in SCID mice by local administration of an adenovirus expressing IL-18 antisense mRNA. J Immunol. 2002;168(1):411–20.
23 Ten Hove T, Corbaz A, Amitai H, Aloni S, Belzer I, Graber P, et al. Blockade of endogenous IL-18 ameliorates TNBS-induced colitis by decreasing local TNF-alpha production in mice. Gastroenterology. 2001;121(6):1372–9.
24 Siegmund B, Fantuzzi G, Rieder F, Gamboni-Robertson F, Lehr HA, Hartmann G, et al. Neutralization of interleukin-18 reduces severity in murine colitis and intestinal IFN-gamma and TNF-alpha production. Am J Physiol Regul Integr Comp Physiol. 2001;281(4):R1264–73.
25 Rachmilewitz D. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: a randomised trial. BMJ. 1989;298(6666):82–6.
26 Schneider A, Haas SL, Hildenbrand R, Siegmund S, Reinhard I, Nakovics H, et al. Enhanced expression of interleukin-18 in serum and pancreas of patients with chronic pancreatitis. World J Gastroenterol. 2006; 12(40):6507–14.
27 Hanck C, Manigold T, Bocker U, Kurimoto M, Kolbel CB, Singer MV, et al. Gene expression of interleukin 18 in unstimulated peripheral blood mononuclear cells of patients with alcoholic cirrhosis. Gut. 2001;49(1):106–11.
28 Torres MI, Rios A. Current view of the immunopathogenesis in inflammatory bowel disease and its implications for therapy. World J Gastroenterol. 2008;14(13):1972–80.
29 Ince MN, Elliott DE. Immunologic and molecular mechanisms in inflammatory bowel disease. Surg Clin North Am. 2007;87(3):681–96.
30 Breese E, Braegger CP, Corrigan CJ, Walker-Smith JA, MacDonald TT. Interleukin-2- and interferon-gamma-secreting T cells in normal and diseased human intestinal mucosa. Immunology. 1993;78(1):127–31.
31 Monteleone G, Biancone L, Marasco R, Morrone G, Marasco O, Luzza F, et al. Interleukin 12 is expressed and actively released by Crohn's disease intestinal lamina propria mononuclear cells. Gastroenterology. 1997;112(4):1169–78.
32 Korzenik JR, Podolsky DK. Evolving knowledge and therapy of inflammatory bowel disease. Nat Rev Drug Discov. 2006;5(3):197–209.
33 Shih DQ, Targan SR. Immunopathogenesis of inflammatory bowel disease. World J Gastroenterol. 2008;14(3):390–400.
34 Kanai T, Watanabe M, Okazawa A, Nakamaru K, Okamoto M, Naganuma M, et al. Interleukin 18 is a potent proliferative factor for intestinal mucosal lymphocytes in Crohn's disease. Gastroenterology. 2000;119(6):1514–23.
35 Furuya D, Yagihashi A, Komatsu M, Masashi N, Tsuji N, Kobayashi D, Watanabe N. Serum interleukin-18 concentrations in patients with inflammatory bowel disease. J Immunother. 2002;25(Suppl 1):S65–67.
36 Towers R, Naftali T, Gabay G, Carlebach M, Klein A, Novis B. High levels of glucocorticoid receptors in patients with active Crohn's disease may predict steroid resistance. Clin Exp Immunol. 2005;141(2):357–62.
37 Ludwiczek O, Kaser A, Novick D, Dinarello CA, Rubinstein M, Tilg H. Elevated systemic levels of free interleukin-18 (IL-18) in patients with Crohn's disease. Eur Cytokine Netw. 2005;16(1):27–33.
38 Naftali T, Novick D, Gabay G, Rubinstein M, Novis B. Interleukin-18 and its binding protein in patients with inflammatory bowel disease during remission and exacerbation. Isr Med Assoc J. 2007;9(7):504–8.
39 Leach ST, Messina I, Lemberg DA, Novick D, Rubenstein M, Day AS. Local and systemic interleukin-18 and interleukin-18-binding protein in children with inflammatory bowel disease. Inflamm Bowel Dis. 2008;14(1):68–74.
40 Jorgensen LG, Fredholm L, Hyltoft Petersen P, Hey H, Munkholm P, Brandslund I. How accurate are clinical activity indices for scoring of disease activity in inflammatory bowel disease (IBD)? Clin Chem Lab Med. 2005;43(4):403–11.
41 Gomes P, du Boulay C, Smith CL, Holdstock G. Relationship between disease activity indices and colonoscopic findings in patients with colonic inflammatory bowel disease. Gut. 1986;27(1):92–5.
42 Solem CA, Loftus EV Jr, Tremaine WJ, Harmsen WS, Zinsmeister AR, Sandborn WJ. Correlation of C-reactive protein with clinical, endoscopic, histological, and radiographic activity in inflammatory bowel disease. Inflamm Bowel Dis. 2005; 11(8):707–12.