Cytokine mucosal expression in ulcerative colitis, the relationship between cytokine release and disease activity☆,☆☆

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Colitis; Ulcerative; Tumour necrosis factor-alpha; Transforming growth factor beta; Interleukin-8; Interferon-gamma

Abstract

Background: Ulcerative colitis (UC) is an inflammatory bowel disease with conflicting evidence from studies on the roles of TNFβ, IL-8, TGFβ and other cytokines and characterised by neutrophil infiltration and tissue destruction.

Aim: To compare cytokine profiles of inflamed and non-inflamed mucosa in patients with distal UC, and matched controls.

Methods: Patients were prospectively recruited, mucosal biopsies at flexible sigmoidoscopy (FS) were taken from UC patients within macroscopically inflamed and non-inflamed proximal mucosa, and from age–sex matched controls undergoing FS. Endoscopic and histological inflammation was graded. Quantitative cytokine analysis for IL-4, TNFα, IL-17A, IL-8, IL-10, TGFβ and IFNγ was carried out on tissue homogenates. Statistical comparison was by Wilcoxon signed rank pair analysis, Mann–Whitney U test and Spearman’s correlation.

Results: 69 active UC patients (54 paired non-inflamed/inflamed mucosa) and 69 controls were compared. In inflamed mucosa, elevation in IL-8 and reduction in TGFβ was measured compared with non-inflamed mucosa (p<0.001; p<0.001) and from age–sex matched controls undergoing FS. Endoscopic and histological inflammation was graded. Quantitative cytokine analysis for IL-4, TNFα, IL-17A, IL-8, IL-10, TGFβ and IFNγ was carried out on tissue homogenates. Statistical comparison was by Wilcoxon signed rank pair analysis, Mann–Whitney U test and Spearman’s correlation.

Comparisons of inflamed with non-inflamed mucosa also demonstrate significant reduction in concentration of IFNγ (p<0.001), IL-4 (p<0.005) and IL-17A (p<0.002).
1. Introduction

Ulcerative colitis (UC) is a chronic, relapsing intestinal inflammatory disorder of the colon, with variable distribution but limited to the distal bowel (distal colitis and proctitis) in 60% of cases. UC has an unconfirmed but likely multifactorial aetiology that may reflect either a primary immunological dysfunction or an inappropriate and pathological immunological response to an environmental factor, e.g. commensal intestinal microorganisms. Mucosal inflammation in UC is characterised by an infiltrate of plasma cells, eosinophils and neutrophils which correlate with disease severity.

In distal UC, there often exists a clear demarcation between actively inflamed distal mucosa and apparently normal proximal mucosa. This demarcation is often well preserved and may persist for years; however, occasionally the disease will extend or regress. The pathogenesis of this clinical presentation remains poorly defined but suggests localised dysregulation of the inflammatory response.

Current pharmacological interventions in UC demonstrate variable efficacy in disease control with approximately half of cases associated with chronic active or intermittent symptoms or requiring colectomy, leading to a significant impact on patient lifestyle. Immunologically targeted interventions including anti-tumour necrosis factor-α (TNFα) antibodies have demonstrated clinical efficacy in UC patients with moderate to severe disease, including in distal colitis. This suggests a contributory role of cytokines towards activation of the inflammatory cascade in UC and their value as a potential therapeutic target; however, there may be alternative immunopharmacological targets in this group of patients.

However, there remains uncertainty over the comparative profiles of different cytokine families during the mucosal inflammatory response, with conflicting results of published studies. Reports from experimental animal models and human IBD colonic resection suggest that UC represents a non-classical TH2 response characterised by the release of interleukin (IL)-4, 5 that contrasts with the proposed Th1/Th17 cell response characteristic of Crohn’s disease associated with production of interferon-γ (IFN-γ), IL-12, IL-17 and IL-23. However, comparative studies of cytokine profiles in patients with active disease and compared to healthy controls suggest a mixed or even predominantly Th1 immunophenotype. Furthermore, there is also evidence to suggest involvement of inappropriate mediator release by T-regulatory (Treg) cells. This has limited our understanding of the relationship of cytokine expression to mucosal inflammation, which is necessary for the introduction of new cytokine modifying strategies.

A number of factors may have contributed to the inconsistent findings in the published literature, including the natural heterogeneity of baseline cytokine profiles confounding comparison between subjects, and the influence of patient characteristics including drug regimens, or epidemiological factors. We hypothesised that comparing cytokine concentration above and below the demarcation line in between endoscopically active and healthy colonic mucosa in active UC patients would reduce the influence of such confounding factors and allow an examination of the relationship between cytokine profiles and mucosal inflammation.

2. Materials and methods

2.1. Subjects and study design

This was a prospective case-controlled study of UC patients and age and sex-matched healthy controls. Patients were identified during attendance at gastroenterology outpatient clinics at the Royal Haslar and St Mary’s Hospital, Portsmouth Hospitals NHS Trust between November 2008 and July 2009. The diagnosis of UC was based on endoscopic and histological investigation.

All recruited patients underwent a questionnaire based assessment of their demographic characteristics, previous and presenting medical history, and UC history. Clinical disease activity was determined using the UCDAI score.

Patients with clinical evidence of active disease underwent unprepared flexible sigmoidoscopy examination as part of their routine clinical management. An endoscopy assessment was undertaken by two endoscopists blinded to the patient’s clinical presentation using a scoring tool (Sutherland et al.) to stratify patients into active or quiescent UC, or normal mucosa and with photographic evidence obtained. Flexible sigmoidoscopy in patients with active distal UC was followed by grab biopsy in inflamed and non-inflamed mucosa proximal to demarcation between inflamed and non-inflamed mucosa. Biopsies were taken using flexible biopsy forceps according to standard protocol.

A control group of age–sex matched patients undergoing unprepared flexible sigmoidoscopy for other clinical indications but without presenting or previous medical history suggestive of UC were recruited, and with subsequent confirmation of histologically normal colonic mucosa entered into the study as control subjects. Random biopsies were taken and stored as above as external controls.

Exclusion criteria included patients: aged less than 16 or greater than 80 years of age; those declining endoscopic evaluation; with a presumptive diagnosis of Crohn’s, infective or ischaemic colitis; with concurrent use of non-steroidal anti-inflammatory medication or inability to provide consent.

2.2. Sample preparation

Mucosal biopsy samples taken at the time of endoscopy were uniquely identified, and snap frozen in liquid nitrogen. Homogenisation buffer (500 μl) containing phosphate buffer saline (PBS) (Invitrogen, Paisley, UK) without Ca²⁺/Mg²⁺,
detergent (1% Triton X-100 (TX-100) (Sigma-Aldrich, St Louis, USA) and containing double strength protease inhibitor (Calbiochem, USA) was added following thawing and weighing, with homogenisation on ice (13,500 rpm, 15-second gap in between two 15-second bursts) (Ultra-turrax T25-Janke & Kunkel, IVA labortechnik) followed by centrifugation (5 minutes at 13,000 rpm) (Jensons MIKRO 22R). The supernatant was then stored at −80 °C until analysis.

### 2.3. Cytokine ELISA analysis

Cytokines were analysed using ELISA kits as per manufacturer’s instructions (Peprotech EC, London, UK) for IL-4, TNFα, IFNγ, IL-8, IL-17A, IL-10 and TGFβ1. O-phenylene diamine substrate (OPD) (Sigma-Aldrich, St Louis, USA) containing 0.01 g OPD+25 ml PBS with Ca2+/Mg2+ +40 μl H2O2(30%) was added, followed by 100 μl of working reagent, mixing and incubation at 37 °C for 30 minutes, prior to plate reading using a Dynex Technology plate reader at 490 nm.

### 2.4. BCA total protein assay (Pierce assay)

An Albumin standard (2.0 mg/ml) calibration curve was constructed through serial dilutions (1 × PBS [without Ca2+/ Mg2+]). A working reagent (WR) was prepared by admixture of BCA reagent A (20 ml) and B (0.4 ml). 25 μl of standard and sample in duplicate were added to each microplate well, followed by the addition of 200 μl of working reagent, mixing and incubation at 37 °C for 30 minutes, prior to plate reading using a Dynex Technology plate reader at 570 nm.

### 2.5. Histological analysis

Assessment of paraffin embedded serial haematoxylin and eosin (H&E) stained sections was by a histopathologist blinded to patients’ identities including clinical data and using a validated histopathological scoring tool (Gomes et al.).

### 2.6. Statistical analysis

Formal statistical assistance was obtained; SPSS 18 (SPSS Inc., Chicago, IL) was used for data input and analysis. Cytokine assay results were analysed adjusted for protein content. Units are pg/mcg protein except TGFβ1 (fg/mcg protein). Comparisons of cytokine concentrations in active UC patients were expressed as ratios within inflamed compared with non-inflamed mucosa, values above 1 indicate higher cytokine concentration in inflamed mucosa.

Values are quoted as median±inter-quartile range for non-parametric data. The primary outcome variables were analysed using Wilcoxon signed rank pair analysis and Mann–Whitney U test for non-parametric variables. Correlations were tested using Spearman’s rank correlation. To allow for multiple testing, a significance level of 0.01 was chosen to indicate significance.

### 2.7. Ethical considerations

The study was approved by the Isle of Wight, Portsmouth and South East Hampshire research ethics committee (project number 08/H0501/82). All subjects recruited to the study provided informed consent.

### 3. Results

#### 3.1. Subjects

Patients with active UC (n=69) were recruited with age–sex matched control subjects. Biopsy data sets of ‘paired’ macroscopically inflamed and non-inflamed tissues were available in 54 UC patients. Baseline characteristics of patients and controls are shown (Table 1). Control subjects (n=69) presented with IBS (n=32), rectal polyps (n=4), diverticular disease (n=5), haemorrhoids (n=4), other non-inflammatory presentations (n=5), and normal (n=15).

#### 3.2. Consistency of endoscopic and histological evaluation

Comparison of mucosal concentrations of cytokines from inflamed and non-inflamed mucosa from UC patients (n=54) are shown (Table 2). Mucosal concentration of IL-8 was significantly increased and each of TGFβ1, IFNγ, IL-4 and IL-17 were significantly reduced in inflamed compared with non-inflamed mucosa. There were no differences in concentrations of TNFα.

Comparison of cytokine profiles in inflamed mucosa from UC patients (n=69) and from age–sex matched controls (n=69) is shown (Table 3). Concentration of IL-8 was significantly increased and each of TGFβ1 and IFNγ significantly reduced in inflamed mucosa. There were no differences in concentrations of TNFα, IL-4, IL-17 and IL-10.

There were no significant differences in mucosal concentration of IFNγ, IL-4, IL-8, IL-17, TGFβ1 and TGFβ in non-inflamed mucosa from active UC patients compared with age–sex matched controls adjusted for protein (Table 4).

#### 3.3. Cytokine mucosal profiles

Comparison of mucosal concentrations of cytokines from inflamed and non-inflamed mucosa from UC patients (n=54) were presented with IBS (n=69) is shown (Table 3). Concentration of IL-8 was significantly increased and each of TGFβ1, IFNγ, IL-4 and IL-17 were significantly reduced in inflamed compared with non-inflamed mucosa. There were no differences in concentrations of TNFα.

Comparison of cytokine profiles in inflamed mucosa from UC patients (n=69) and from age–sex matched controls (n=69) is shown (Table 3). Concentration of IL-8 was significantly increased and each of TGFβ1 and IFNγ significantly reduced in inflamed mucosa. There were no differences in concentrations of TNFα, IL-4, IL-17 and IL-10.

There were no significant differences in mucosal concentration of IFNγ, IL-4, IL-8, IL-17, TGFβ1 and TGFβ in non-inflamed mucosa from active UC patients compared with age–sex matched controls adjusted for protein (Table 4).

**3.4. Correlations of cytokine concentration with endoscopic disease activity**

Cytokine concentrations in inflamed mucosa were correlated against endoscopic score (Table 5, Figs. 1 and 2). Mucosal concentration of IL-8 (rα =+0.481, p<0.01) was significantly and positively correlated with endoscopic score. By comparison IFNγ (rα =−0.321, p<0.01) and TGFβ1 (rα =−0.462, p<0.01) were each significantly and negatively correlated. Mucosal concentration of TNFα, IL-4, IL-10 and IL-17 did not correlate with endoscopic activity.
3.5. Correlations of cytokine concentration with histological disease activity

Cytokine concentrations in inflamed mucosa were correlated against histological score (Table 6, Figs. 3 and 4). Mucosal concentration of IL-8 ($r_s = +0.460$, $p < 0.01$) was significantly and positively correlated with histological score. By comparison, IFNγ ($r_s = -0.241$, $p < 0.05$) and TGFβ ($r_s = -0.460$, $p < 0.01$) were each significantly and negatively correlated. Mucosal concentration of TNFα, IL-4, IL-10 and IL-17 did not correlate with histological activity.

3.6. Corticosteroid and 5-ASA subgroup analysis

Subgroup analysis of 18 patients (age 45.3; M=10 [55%], F=8 [45%]) not on 5-ASA or corticosteroid treatment compared to 67 age–sex matched control group, was carried out (Table 7).
Correlated with endoscopic score. Mucosal concentration of IFN\(\gamma\), IL-4, IL-8, IL-17 was significantly reduced in inflamed mucosa; however no IL-10 of 54 (Table 1).

Only 2 (11.1%) patients were receiving maintenance therapy (azathioprine). Extent of colitis (Proctitis 12 [66.7%], sigmoiditis 5 [27.8%], left sided 1 [5.5%], extensive 0 [0%]), endoscopic severity (1:13 [72.2%]; 2:3 [16.7%]; 3:2 [11.1%]) and histological severity (0:3 [16.7%]; 1:7 [38.9%]; 2:6 [33.3%]; 3:2 [11.1%]; 4:0 [0%]) were similar to the full group and histological severity (grade 4, [Table 9]) compared to controls.

Concentration of IL-8 was significantly increased and TGF\(\beta\) significantly reduced in inflamed mucosa; however no difference in IFN\(\gamma\) was noted. Cytokine concentration was correlated with endoscopic score. Mucosal concentration of IL-8 \((r_s=+0.333, p<0.01)\) was significantly and positively correlated and TGF\(\beta\) \((r_s=−0.373, p<0.01)\) was significantly and negatively correlated. By comparison, IFN\(\gamma\), TNF\(\alpha\), IL-4, IL-10 and IL-17 did not correlate with disease activity.

### 3.7. High endoscopic and histological severity subgroup analysis

Subgroup analysis of 12 patients (age 38; M=9 [75%], F=3 [25%]) with high endoscopic score (grade 3, [Table 8]) and 10 patients (age 36.5; M=6 [60%], F=4 [40%]) with high histological score (grade 4, [Table 9]) compared to controls was carried out. Mucosal IL-8 concentration was significantly increased in the severe subgroup measured endoscopically and histologically \((p<0.001)\); conversely, mucosal TGF\(\beta\) concentration was significantly reduced in the severe subgroup measured endoscopically \((p<0.001)\) and histologically \((p<0.01)\). Mucosal IFN\(\gamma\) concentration was also significantly reduced in the severe subgroup measured endoscopically, with a non-significant trend towards reduced concentration in the severe subgroup measured histologically \((p=0.1)\). By comparison TNF\(\alpha\), IL-4, IL-10 and IL-17 did not correlate with disease activity.

### 4. Discussion

The results of this study suggest that colonic mucosa in active distal UC is associated with contrasting mucosal cytokine production in inflamed compared to non-inflamed mucosa proximal to the inflammatory demarcation line in the same

### Table 2

Comparison of mucosal cytokine profiles in UC patients, ‘paired’ inflamed and non-inflamed mucosa (median±inter-quartile range).

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Paired (n=54)</th>
<th>Non-inflamed (n=54)</th>
<th>Inflamed/non-inflamed</th>
<th>p-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN(\gamma)</td>
<td>1.01 (0.83–1.25)</td>
<td>1.17 (0.97–1.44)</td>
<td>0.87 (0.73–0.98)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-4</td>
<td>1.49 (0.70–2.47)</td>
<td>1.86 (0.70–3.21)</td>
<td>0.91 (0.67–1.1)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.66 (0.40–0.86)</td>
<td>0.47 (0.34–0.59)</td>
<td>1.38 (0.95–1.86)</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-17</td>
<td>0.53 (0.27–2.69)</td>
<td>0.70 (0.39–3.64)</td>
<td>0.74 (0.53–1.05)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TNF(\alpha)</td>
<td>1.50 (1.19–1.90)</td>
<td>1.71 (1.09–1.98)</td>
<td>0.91 (0.70–1.20)</td>
<td>0.572</td>
</tr>
<tr>
<td>IL-10</td>
<td>4.73 (2.43–5.90)</td>
<td>5.95 (3.61–7.06)</td>
<td>0.81 (0.57–1.08)</td>
<td>0.01</td>
</tr>
<tr>
<td>TGF(\beta)</td>
<td>44.4 (0.0–87.0)</td>
<td>75.3 (23.5–145.7)</td>
<td>0.30 (0.01–0.82)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* Wilcoxon signed rank pair analysis.

### Table 3

Comparison of mucosal cytokine profiles in UC patients (inflamed mucosa) compared with age–sex matched controls (median±inter-quartile range).

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Matched</th>
<th>p-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN(\gamma)</td>
<td>Inactive (n=69)</td>
<td>Control (n=69)</td>
</tr>
<tr>
<td>IL-4</td>
<td>1.46 (0.53–2.43)</td>
<td>1.16 (0.79–2.45)</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.67 (0.42–0.95)</td>
<td>0.44 (0.30–0.57)</td>
</tr>
<tr>
<td>IL-17</td>
<td>0.59 (0.27–2.73)</td>
<td>0.51 (0.31–2.83)</td>
</tr>
<tr>
<td>TNF(\alpha)</td>
<td>1.45 (1.10–1.85)</td>
<td>1.55 (1.22–2.09)</td>
</tr>
<tr>
<td>IL-10</td>
<td>4.78 (2.44–5.90)</td>
<td>5.72 (1.51–6.92)</td>
</tr>
<tr>
<td>TGF(\beta)</td>
<td>57.7 (38.7–120)</td>
<td>107 (78–208)</td>
</tr>
</tbody>
</table>

* Independent samples Mann–Whitney U test.

### Table 4

Comparison of mucosal cytokine profiles in UC patients (non-inflamed) compared with age–sex matched controls (median±inter-quartile range).

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Matched</th>
<th>p-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN(\gamma)</td>
<td>Inactive (n=69)</td>
<td>Control (n=69)</td>
</tr>
<tr>
<td>IL-4</td>
<td>1.93 (0.64–3.17)</td>
<td>1.16 (0.79–2.45)</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.50 (0.35–0.64)</td>
<td>0.44 (0.30–0.57)</td>
</tr>
<tr>
<td>IL-17</td>
<td>0.77 (0.40–3.52)</td>
<td>0.51 (0.31–2.83)</td>
</tr>
<tr>
<td>TNF(\alpha)</td>
<td>1.66 (1.08–1.97)</td>
<td>1.55 (1.22–2.09)</td>
</tr>
<tr>
<td>IL-10</td>
<td>5.95 (3.84–7.05)</td>
<td>5.72 (1.51–6.92)</td>
</tr>
<tr>
<td>TGF(\beta)</td>
<td>75.3 (23.8–146)</td>
<td>107 (78–208)</td>
</tr>
</tbody>
</table>

* Independent Samples Mann–Whitney U Test.

### Table 5

Correlations of cytokine mucosal profiles (inflamed mucosa) and endoscopic score in UC patients.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Spearman correlation (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN(\gamma)</td>
<td>(r_s=−0.321^*)</td>
</tr>
<tr>
<td>IL-4</td>
<td>(r_s=−0.022)</td>
</tr>
<tr>
<td>IL-8</td>
<td>(r_s=0.481^*)</td>
</tr>
<tr>
<td>IL-17</td>
<td>(r_s=−0.048)</td>
</tr>
<tr>
<td>TNF(\alpha)</td>
<td>(r_s=−0.112)</td>
</tr>
<tr>
<td>IL-10</td>
<td>(r_s=−0.003)</td>
</tr>
<tr>
<td>TGF(\beta)</td>
<td>(r_s=−0.462^*)</td>
</tr>
</tbody>
</table>

* Spearman's correlation, \(p<0.01\).
patient, and to external matched controls; the pattern indicates elevated concentrations of IL-8 and reduced concentrations of TGF$\beta$ in inflamed mucosa. Furthermore IL-8 concentration is significantly positively correlated with endoscopically and histologically determined disease activity, in contrast TGF$\beta$ is significantly inversely correlated with the same. Finally, these contrasting findings are consistent in the subgroup analyses.

This is the first time that the inverse relationship between TGF$\beta$ concentration and disease activity has been documented; in addition, it is the first time that IL-8 concentration has been demonstrated to correlate positively with validated endoscopic and histological scoring systems in the same model. These findings are consistent in the subgroup not on 5-ASA or corticosteroids, which demonstrates that they are not related to therapeutics.

The results also suggest a significantly lower concentration of IFN$\gamma$, IL-4, IL-17, and IL-10 (non-significant trend), but not TNF$\alpha$, in inflamed compared to non-inflamed mucosa from UC patients. TNF$\alpha$ and the other measured cytokines did not demonstrate a consistent correlation with disease activity. Finally, there did not appear to be significant differences between non-inflamed mucosa from UC patients and controls.

Histological evaluation of endoscopically determined non-inflamed mucosa (in patients with active colitis) confirmed the absence of a microscopic inflammatory infiltrate in 74% of samples, and demonstrated a minor inflammatory infiltrate in all but 1 (2%) of the remainder. This finding and the failure to detect a difference between cytokine profile in endoscopically determined non-inflamed mucosa and matched controls, justified endoscopic assessment as a tool to delineate inflamed from non-inflamed mucosa.

Previous investigators have examined cytokine profiles in UC, both in serum and mucosa, examining a wide array of

<table>
<thead>
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<th>Table 6 Correlations of cytokine mucosal profiles (inflamed mucosa) and histological score in UC patients.</th>
</tr>
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<tbody>
<tr>
<td><strong>Spearman correlation (n=69)</strong></td>
</tr>
<tr>
<td>IFN$\gamma$</td>
</tr>
<tr>
<td>IL-4</td>
</tr>
<tr>
<td>IL-8</td>
</tr>
<tr>
<td>IL-17</td>
</tr>
<tr>
<td>TNF$\alpha$</td>
</tr>
<tr>
<td>IL-10</td>
</tr>
<tr>
<td>TGF$\beta$</td>
</tr>
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<td></td>
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<td></td>
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</table>

The results also support a significantly lower concentration of IFN$\gamma$, IL-4, IL-17, and IL-10 (non-significant trend), but not TNF$\alpha$, in inflamed compared to non-inflamed mucosa from UC patients. TNF$\alpha$ and the other measured cytokines did not demonstrate a consistent correlation with disease activity. Finally, there did not appear to be significant differences between non-inflamed mucosa from UC patients and controls.

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Previous investigators have examined cytokine profiles in UC, both in serum and mucosa, examining a wide array of
cukines. The results are frequently contradictory which may reflect differences in study design, sample size or scientific methodology. The current study is the largest cohort study of mucosal cytokine expression in UC; the scientific methodology included quantitative cytokine measurement, not mRNA production (a surrogate marker of cytokine production).

Our results are consistent with an association between IL-8 mucosal concentrations and mucosal inflammation in UC. This cytokine, commonly associated with the innate immune response, has a recognised chemoattractant effect on neutrophils. Raised IL-8 concentrations have been demonstrated in previously published studies of active UC and are considered to be an independent prognostic indicator of risk of future relapse in quiescent colitis.

By comparison, TGFβ, which is a pleiotropic cytokine and an inhibitor of Th1, Th2 differentiation, NK cell activity and is involved in tissue repair, is reduced in inflamed mucosa in our study; this is a novel finding. Experimental studies using cyclosporine (a potent anti-inflammatory drug used in patients with fulminant steroid resistant UC) demonstrate cyclosporine induced TGFβ-mediated inhibition of epithelial apoptosis, reversed by addition of anti-TGFβ antibodies. Previous studies with transgenic mice expressing mutant TGFβ receptors demonstrate increased susceptibility to spontaneous colitis, DSS (dextran sodium sulphate) colitis and with delay in healing when TGFβ signalling is interrupted. This suggests TGFβ is central in the maintenance of intestinal homeostasis and repair.

Significantly lower concentrations of the cytokines IL-4, IL-17 and IFNγ (with a trend towards a lower level of IL-10) may represent a down-regulation of the respective immune cell types. IL-17 upregulation, reflecting Th17 cell activation, stimulates cytokines TNFα and IL-8 as part of the adaptive immune response and IFNγ reflects Th1 cell activation (as commonly seen in Crohn’s disease). IL-4, reflecting Th2 cell activation, is elevated in inflamed UC mucosa in some studies while in others it is normal or downregulated.

The failure to note a difference in TNFα levels, a ubiquitous cytokine, is disappointing and conflicts with published experimental and clinical studies demonstrating elevated TNFα in active UC and clinical efficacy of anti-TNFα antibodies respectively. However, subgroup analysis in published studies of mucosal cytokines used to classify disease activity has demonstrated that TGFβ is a marker of disease activity and an indicator of remission.

Table 8: Comparison of mucosal cytokine profiles in UC patients with age–sex matched controls: subgroup analysis of endoscopically determined severely inflamed mucosa (median ±inter-quartile range).

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Matched</th>
<th>Control (69)</th>
<th>p-Value</th>
<th>Correlation with endoscopic score</th>
</tr>
</thead>
<tbody>
<tr>
<td>INFγ</td>
<td>0.89 (0.72–1.00)</td>
<td>1.17 (0.96–1.38)</td>
<td>&lt;0.005</td>
<td>r_s = −0.141</td>
</tr>
<tr>
<td>IL-4</td>
<td>1.20 (0.53–2.16)</td>
<td>1.16 (0.79–2.45)</td>
<td>=0.528</td>
<td>r_s = 0.186</td>
</tr>
<tr>
<td>IL-8</td>
<td>1.17 (0.72–1.60)</td>
<td>0.44 (0.30–0.57)</td>
<td>&lt;0.001</td>
<td>r_s = 0.333 *</td>
</tr>
<tr>
<td>IL-17</td>
<td>0.53 (0.23–2.68)</td>
<td>0.51 (0.31–2.83)</td>
<td>=0.599</td>
<td>r_s = −0.130</td>
</tr>
<tr>
<td>TNFα</td>
<td>1.38 (0.90–1.58)</td>
<td>1.55 (1.22–2.09)</td>
<td>=0.123</td>
<td>r_s = 0.071</td>
</tr>
<tr>
<td>IL-10</td>
<td>5.48 (4.50–5.85)</td>
<td>5.72 (1.51–6.92)</td>
<td>=0.799</td>
<td>r_s = 0.373 *</td>
</tr>
<tr>
<td>TGFβ</td>
<td>35.66 (13.26–71.71)</td>
<td>107 (78–208)</td>
<td>&lt;0.001</td>
<td>r_s = 0.141</td>
</tr>
</tbody>
</table>

* Spearman’s correlation, p < 0.01.
  ** Mann–Whitney U test.
  *** control group for TGFβ analysis 54/69 results due to undetectable values in control data set.
studies suggests significant differences between TNFα mRNA in severely inflamed compared with mild-moderately inflamed mucosa in UC which may reflect a switch to a TNFα phenotype during the inflammatory process.\(^{41}\) It is possible that TNFα-mediated inflammation becomes apparent in severe disease (explaining the response to anti-TNFα biologic agents) that was not well represented in our patient cohort (17% of study acute colitis patients).

In this study, we have used patients as their own controls, and with non-related age–sex matched controls, and have demonstrated reproducibility in the major results. Comparisons of cytokine production between individuals may be confounded by genetic polymorphism in cytokine production,\(^{42}\) drug therapy, environmental factors, amongst other factors. Our methodology attempted to control for such factors by providing internal comparisons using non-inflamed tissue adjacent to colitis samples in patients.

The clinical significance of these results is that IL-8 and TGFβ production seem to be intimately and consistently related to the severity of the inflammatory response measured in vivo, whereas TNFα, IL-4, IFNγ, IL-17α and IL-10 do not consistently demonstrate close association. Both elevated IL-8 and reduced TGFβ may be fundamentally important in the loss of intestinal homeostasis which develops in UC; the clinical significance of the other measured cytokines may become more apparent in more severe disease states.

In conclusion, the results of this study suggest a major role for IL-8 mediated neutrophil infiltration, seen histologically, and failure of TGFβ mediated maintenance of intestinal homeostasis and tissue healing. This is consistent with the hypothesis that the primary driver of the inflammatory response in distal UC (at least in mild to moderate disease) is the innate immune response. Furthermore, inflammation in these patients appears to be limited to the area of colitis, supporting the use of topical in addition to systemic treatment. Finally, the cytokine profile suggests that IL-8 and TGFβ may be considered as potential targets for therapeutic intervention; the role of the other measured cytokines is less clear, and may represent a consequence rather than a direct cause of the pathophysiology of UC.

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### Competing interests

None.

### Contributors

Study design: DSP, TMT, MAW, JFB, JKS. Patient recruitment: DSP, TMT. Data collection: DSP, TMT, KS, MAW, HNS. Data analysis and interpretation: DSP, TMT, KS, MAW, JFB, JKS. Manuscript drafting: DSP, TMT, JFB, JKS.

### Data sharing

Data sharing not available at this time due to unpublished source data. However, data sharing may be available at later date.

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### References


