Clinics in Surgery

Research Article
Published: 13 May, 2022

IL-36, 37 and 38 in Ulcerative Colitis

Christopher M Issa[1], Brett D Hambly[2], Jose Garrido-Mesa[3], Geoff Watson[3], Kun Tao[1] and Shisan Bao[1]**

[1]Department of Pathology, Tongren Hospital, Shanghai Jiaotong University School of Medicine, China

[2]Department of Pathology, Royal Prince Alfred Hospital, Central Clinical School, University of Sydney, Australia

[3]CIBER-EHD, Department of Pharmacology, ibs. GRANADA, Centre for Biomedical Research (CIBM), University of Granada, Avenida del Conocimiento, Spain

Abstract

Ulcerative colitis, a chronic disorder of the gastrointestinal tract, results in inflammation. The precise underlying mechanism is still unclear; however it is believed that immunological, genetic and environmental factors contribute to development of colonic inflammation. The Interleukin-1 (IL-1) family and IL-1 ligands are associated with acute and chronic inflammation. Recently, it has been demonstrated that IL-36α, β, γ, IL-37 and IL-38 play an important role in the initiation of inflammation in a number of organs, but the possible role of IL-36α, β, γ, and IL-38 in human colitis remains, except for IL-37, to be explored. Our data show that substantial upregulated production of IL-36α, -β, -γ, IL-37 and IL 38 in the inflamed colonic mucosa from UC occurs, compared to that from the control colon, using quantitative immunohistochemistry. Furthermore, chronic disease in these patients showed severity in both clinical presentation and histopathology score, suggesting that IL 36α, β, γ, IL-37 and IL-38 associate the severity of UC. These data may provide useful information for potential therapeutic intervention for UC patients.

Keywords: Ulcerative colitis; IL-36; IL-37; IL-38; Inflammation

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Correspondence:
Kun Tao, Department of Pathology, Tongren Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China,
E-mail: taokun20119@163.com
Shisan Bao, Department of Pathology, Tongren Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China,
E-mail: profbao@hotmail.com

Received Date: *23 Mar 2022*
Accepted Date: *05 May 2022*
Published Date: *13 May 2022*

Citation:
Issa CM, Hambly BD, Garrido-Mesa J, Watson G, Tao K, Bao S. IL-36, 37 and 38 in Ulcerative Colitis. Clin Surg. 2022; 7: 3506.

Background

Ulcerative Colitis (UC) is a chronic inflammatory disease confined to the large intestine [1] of a relapsing – remitting nature. UC is characterized by mucosal ulceration, that usually starts in the rectum and can ascend proximally affecting the whole colon [1]. Despite decades of extensive research, the precise underlying mechanisms mediating UC still remain unclear. It has been suggested that the aetiology of UC involves a convergence of a genetic predisposition and immunological and environmental factors, in particular a deregulated immune response to otherwise normal microbiota [2]. In the absence of a clearly identified cause, the current available therapies are aimed at ameliorating symptoms and/or complications but in general do not cure the disease [3]. Cytokines play an essential role in the maintenance of normal gut homeostasis, substantiated through the interplay of the innate immune system and its regulatory components [4]. Cytokines from the IL-1 superfamily possess a variety of immunoregulatory properties in response to infection and inflammation, including host defence against infection, injury and stress [4], but their main biological role is generally pro-inflammatory. The different IL-1 family cytokines can be categorized into subfamilies based on their length and precursors [5]. Although great progress has been made in our understanding of the biology of some of the IL-1 family members and their role in different diseases, there is still much to be learnt about the newly discovered IL-36α, IL-36β, IL-36γ, IL-37 and IL-38. IL-36α transgenic mice led to acanthuses and hyperkeratosis, which can be ameliorated with IL-36RA antibody [6], implying that IL-36α contributes to the dysregulation of host immunological homeostasis. IL-36β stimulates synoviocyte proliferation *via* IL-6, IL-8 and TNF *in vitro* [6,7]. Furthermore, substantially higher neutrophil influx, hyper-responsiveness and pro-inflammatory cytokine production was observed in the lungs of IL-36γ knockout mice following post-house dust challenge [5]. Taken together, these findings suggest a possible role of the IL-36 family in the development of immunologically mediated disease. On the other hand, IL-37 and IL-38 appear to exhibit anti-inflammatory properties. IL-36α mRNA is increased in the treatment naïve UC [8]. Recently, McNamee et al. [9] reported attenuated DSS-induced experimental colitis in human IL-37 transgenic mice, and this phenotype was attributed to a downregulation of TFN production [6]. Finally, IL-38 has been reported to reduce Candida-induced Th-17 responses *via* binding to IL-36R *in vitro* [5,6]. Colonic, but not circulating IL-38 is upregulated in IBD patients and DSS induced colitis animal model [10]. However, the whole panel of the IL-36 family, IL-37 and

IL-38 in the samples from UC of same cohorts, as for comparison, was unclear. We demonstrate here that higher levels of the recently discovered IL-36α, β, γ, IL-37 and IL-38 in colonic specimens from UC patients compared to healthy controls, shedding new light on the role of these cytokines in intestinal inflammatory diseases.

Materials and Methods

Colonic samples from chronic ulcerative colitis patients (n=12) and controls (n=3), embedded in paraffin blocks, were obtained from the Department of Anatomical Pathology, Royal Prince Alfred Hospital, The University of Sydney. This study has been approved by *The Human Ethics Committee, The University of Sydney*. Demographic information of these colon tissues is presented in Table 1. All the samples from the patients were collected using colonoscopic biopsy for pathological confirmation of clinically active disease; whereas the three controls were collected from patients undergoing a hemicolectomy for either adenomatous polyps, appendiceal mucinous tumour or colonic carcinoma of the hepatic flexure. All the tissues were fixed in 10% formalin and wax embedded. The area of the colon selected for examination was both macroscopically and microscopically normal in morphology. Paraffin embedded blocks was sectioned to 5 μm in thickness and primary stained with Haematoxylin and Eosin (H&E). Immunohistochemistry was performed as described [11]. Primary antibodies for cytokines IL-36α, IL-36β, IL-36γ, IL-37 and IL-38 were obtained from (Abcam, Sydney, Australia) and were incubated at room temperature for 60 min at 1:1000 dilution, followed by a Dako anti-rabbit secondary for 30 min (Dako, Sydney, Australia). Slides were subjected to several high salt buffer washes (TBST), before being incubated with DAB + chromagen (3'3-diaminobenzidine) for 5 min. Sections were rinsed and counterstained with Harris haematoxylin. These specific cytokine expression stains in the intestine were photographed and quantified, using *Image pro Plus 9.1, Media Cybernetics*, as described [11]. Briefly, Staining is expressed as image units. A pathologist who was blinded to the treatment determined the threshold for positive staining. The resulting values were used to obtain an average staining for each group [12].

Statistics

All data are presented as mean ± SEM. Statistical analysis was performed using two-way and one-way ANOVA, whichever was appropriate. Differences with $p<0.05$ were regarded as statistically significant.

Results

In this study colonic tissue samples were collected from UC patients (n=12) (Table 1). Mean age was 40 ± 13 years, with 83.3% of patients were male. Furthermore 75% of patients had severe disease, 8.3% moderate disease and 16.7% mild disease, respectively. Immunohistochemistry was used to evaluate IL-36α, IL-36β, IL-36γ, IL-37 and IL-38 cytokines. The basal level of IL-36α on the colonic mucosal surface from control samples was almost undetectable (Figure 1c). Higher levels of IL-36α were detected in the intestinal sections from UC patients (Figure 1b), particularly within the highly immune cell infiltrated region, where levels >100-fold higher than in the control samples ($p<0.001$) (Figure 1a). In addition, a correlation between IL-36α production and inflammation was observed both in immunohistochemical (Figure 1b, 1c) and histopathological scoring (Figure 1p, 1q) (Table 1). Similarly, constitutive IL-36β expression was observed in the healthy human colon, which was substantially higher in colon samples from UC patients ($p<0.001$) (Figure 1e, 1f), consistent with the higher levels of IL-36α. This was also the case for IL-36γ, which was also found to be increased ($p<0.001$) (Figure 1h, 1i), being more than 40-fold higher in UC samples than in controls (Figure 1g) ($p<0.001$). IL-37 and IL-38 were also increased in inflamed samples (Figure 1k, l1). IL-37 levels detected in UC patient samples were over 60-fold higher than those observed in control tissue (Figure 1j) ($p<0.01$), and a more than 100 fold increase was found in IL-38 concentration ($p<0.001$) (Figure 1m-1o). We observed substantial upregulation on IL-36α, IL-36β, IL-36γ, IL-37 and IL-38 production in the inflamed colonic mucosa from UC patients. Upregulated cytokine production was observed in those areas strongly affected by mucosal destruction and crypt architect distortion, which also showed heavy leucocyte infiltration, all these being characteristic features of UC (Table 1).

Discussion

The IL-36 family of cytokines are generally thought to exhibit pro-inflammatory properties, and in this study were found to be markedly elevated in the inflamed colonic wall of patients suffering from ulcerative colitis. On the other hand, evidence suggests that IL-37 and IL-38 primarily exhibit anti-inflammatory properties. Paradoxically, in this study IL-37 and IL-38 were also found to be substantially elevated in these patients. IL-36α a pro-inflammatory cytokine binds and signals *via* IL-1RL2/IL-36R, activating NF-kappa-B and/or MAPK pro-inflammatory responses [13]. IL-36α is highly expressed in blood and salivary glands from primary Sjogren's syndrome patients [14], *via* enhancing production of IL-17, IL-22 and IL-23p19. Furthermore IL-36 pathway involves in epithelial barriers eliciting local inflammatory responses [13]. Thus, such reports are consistent with our data, showing upregulated IL-36α in the highly inflamed mucosa from UC patients, reflecting both in local inflammation and breakdown of epithelial barrier. Similarly, IL-36β has been found substantially increased in articular chondrocytes and/or synovial fibroblasts cultured from rheumatoid arthritis patients in response to IL-36β stimulation *in vitro*. Increased IL-36β was also associated to the production of other pro-inflammatory cytokines, suggesting that IL-36β acts in both paracrine and autocrine fashions in the micro-environment during inflammation. In addition, IL-36γ has recently been described to stimulate the

Table 1: Characteristics of ulcerative colitis *vs.* controls.

Characteristic	Controls (n=3)	Ulcerative colitis (n=12)	p-value
Age, (mean ± SD)	29 ± 1.5	40 ± 13	
Gender			
Male	2 (66.7%)	10 (83.3%)	
Female	1 (33.3%)	2 (16.6%)	
Specimen position			
Ascending colon	2 (66.7%)	2 (16.7%)	
Descending colon	1 (33.3%)	4 (33.3%)	
Transverse colon		2 (16.7%)	
Rectum		4 (33.3%)	
Microscopic Inflammation grade			
Mild disease		2 (16.7%)	
Moderate disease		1 (8.3%)	
Severe disease		9 (75%)	
Normal	3		

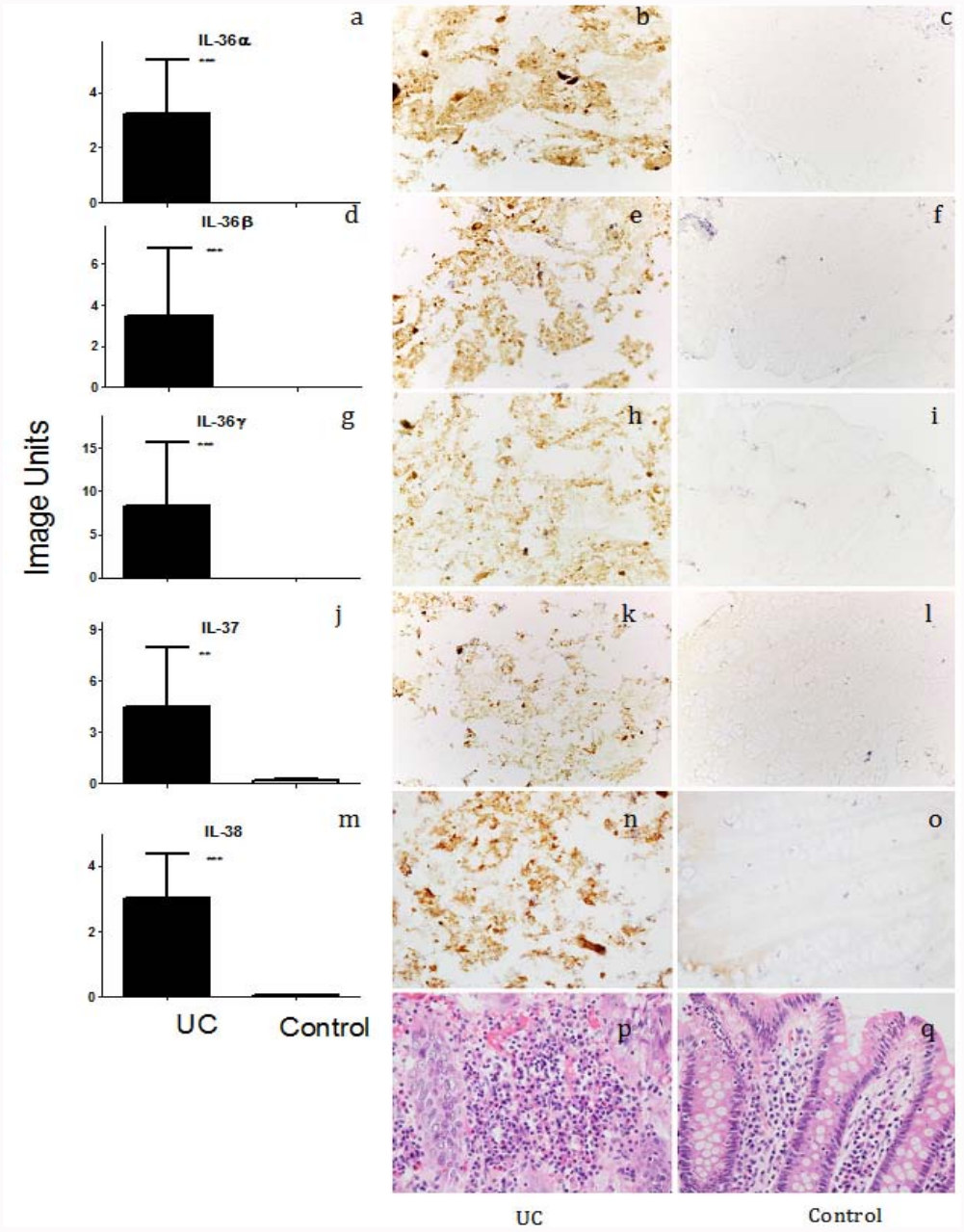

Figure 1: Presence of IL-36α, IL-36β, IL-36γ, IL-37 and IL-38 in active UC patients (b, e, h, k, n) versus asymptomatic control (c, f, i, l, o). H&E stain of active colitis (p) *vs.* control (q).

production of different pro-inflammatory mediators in human colonic sub-epithelial myofibroblasts, such as IL-6, CXCL1, CXCL2, and CXCL8, *via* the activation of MAPKs and NF-κB [15]. It has been reported UC is a risk factor for the development of colorectal cancer (World J Gastroenterol. 2008 July 7th; 14(25):3937–3947). We have demonstrated that (*BMC Cancer* 2020;20:92) that subsets of IL-36 have a differential role during the development of colorectal cancer, showing that IL-36α or IL-36γ are reliable biomarkers in predicting the prognosis of CRC during both the later or early stages of the disease, respectively. These findings, together with our observations described in this manuscript, support a role for the IL-36 subfamily of cytokines mediating both the initiation and progression of disease pathogenesis in UC, and possibly involving the development of colorectal cancer. Interestingly, on the other hand it has been demonstrated IL-37 acts as an anti-inflammatory mediator in response to inflammatory stimuli [6]. This is consistent with the observation that human IL-37-expressing transgenic mice are less susceptible to develop experimental colitis, independent of the IL-10 pathway [6]. Paradoxically, our data show increased IL-37 staining in samples of patients with UC which was positively associated with disease activity. It is unclear whether upregulated IL-37 in UC represents an attempt by the body to mount an anti-inflammatory response to the inflammation present to suppress colitis or whether the increase in IL-37 has been orchestrated by the inflammatory micro-environment present within the inflamed bowel wall. It has been reported that IL-37 is highly produced in the mucosa of active Crohn's disease patients, compare to that from UC and the control [16]. Interestingly, IL-37 production is higher in the mucosa from UC than the control in the same study [16]. Thus, such a finding is consistent with our data, showing increased IL-37 in the inflamed

colon from UC patients. Elucidating the precise role of IL-37 in the highly inflamed mucosa during remission and relapse may shed light on this mechanism and may be valuable in the development of potential therapeutic agents in the treatment of this devastating condition. A previous study demonstrated that IL-38 inhibits IL-1R, IL-18R, and IL-36R pathways [17] on Th17 cells, down-regulating IL-17 and IL-22 secretion. Our data show substantial upregulation of IL-38 in the inflamed colonic mucosa from UC patients when compared to controls. Our observation invites speculation that increased colonic IL-38 in UC patients may function as an anti-inflammatory activity, which may help dampen the local inflammatory micro-environment, *via* suppressing the IL-17 pathway. However, there may be some unidentified mechanism which compromised the anti-inflammatory response in the UC mucosal, perhaps *via* IL-36 common receptor (ref). Such data is consistent with our previous finding, showing that the expression of colonic IL-38 is associated with the differentiation of colorectal cancer (ref). There were some limitations in our current study. First, the patient's sample number was relatively small. In addition, there was no comparison between active and non-active colon tissues from UC patients. The drug treatment history of these patients was unclear. All of these concerns are currently being investigated.

In conclusion, we found substantial up-regulation of IL-36α, β, γ, IL-37 and IL-38 in the inflamed colonic mucosa from UC patients, which was consistent with the severity of both clinical presentation and histopathology score. Despite the current knowledge pointing to a local role for these newly discovered cytokines, it is unclear whether there is correlation between circulating and local cytokine production, which could be useful in clinical diagnosis and/or specific therapeutic drug target(s). The precise underlying mechanism of these cytokines in the development of colitis in humans is currently being investigated.

Acknowledgment

This work was partially supported by Bosch Institute Small equipment grant, the University of Sydney.

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