BRIEF COMMUNICATION

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Elevated IL-6 levels in the intestinal mucosa of patients with gastrointestinal Behçet's disease

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Behçet's disease (BD) is a multisystem inflammatory disorder. Its cause is unknown, but it is believed to involve a combination of genetic and environmental factors. Gastrointestinal BD (GIBD) is rare but potentially life threatening due to the risk of intestinal perforation and haemorrhage.[1,2] Its incidence varies geographically, with high frequencies among Japanese and Korean patients with BD.[1] GIBD presents clinically as abdominal pain, bleeding, and diarrhea, similar to inflammatory bowel disease (IBD).[3] The clinical presentation of GIBD can be quantified using the disease activity index for intestinal Behcet's disease (DAIBD), an 8 item measure that quantifies the symptoms of GIBD on a 0–325 scale. The DAIBD has been shown to correlate with GIBD disease activity and clinical course.[3] Current treatment strategies for GIBD rely on immunomodulatory approaches, particularly 5 aminosalicylic acid, corticosteroids, thiopurines, and anti tumor necrosis factor-α (anti-TNF-α) biologics. One focus of new treatment approaches has been on neutralization of the cytokine interleukin-6 (IL-6), considered an important mediator of IBD.[4,5] IL-6 binds to the membrane-bound IL-6 receptor (IL-6Ra), and the IL-6/IL-6Ra complex then associates with glycoprotein-130 (gp130) to initiate intracellular signaling. This may promote anti-inflammatory and homeostatic responses in certain cell types, such as intestinal epithelial cells.[6] IL-6 can also bind to the soluble IL-6Ra (sIL-6Ra) isoform however, allowing the IL-6/sIL-6Ra complex to associate with gp130 in non-IL-6Ra-expressing cells. This may lead to pro-inflammatory responses in certain immune cell populations.[4] Systemic levels of IL-6 above 7 pg/mL have been reported to correlate with BD disease activity, especially GIBD.[7] Whether and to what extent IL-6 is implicated in the pathogenesis of GIBD is unresolved. There is a lack of data on mucosal IL-6 expression in GIBD in particular, and so the involvement of local immune responses in the most affected organ system remains unclear.

In this study, the levels of IL-6, IL-6Ra, and gp130 in paired intestinal mucosal biopsies of inflamed and uninflamed tissues from patients with GIBD were measured to explore this possible connection between IL-6 and GIBD. Male and female patients with confirmed GIBD based on established diagnostic criteria were enrolled in the study.[8] Specifically, GIBD is defined when a patient with BD has intestinal symptoms and characteristic oval-shaped deep ulcers with discrete borders identifiable by endoscopic examination. All patients were treated at Severance Hospital, Yonsei University (Seoul, Republic of Korea), and provided informed consent for participation in the study. The study protocol (No. 4-2024-0133) was approved by the Severance Hospital. Paired mucosal biopsies from both inflamed (ulcerated) and uninflamed intestinal mucosal tissues were obtained from each patient during routine colonoscopy procedures from October 2016 to December 2020. Inflamed tissue samples were obtained from the site of the ulcer, and non inflamed tissue samples were obtained from the cecum or ileocecal valve. For patients with multiple ulcers, the largest ulcer was chosen for biopsy.

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The biopsies were placed in 0.5 mL tubes containing 1.4 mm ceramic beads (tubes for soft tissue homogenizing CK14; Bertin Technologies, Montigny-le-Bretonneux, France) with phosphate-buffered saline (Sigma-Aldrich, St. Louis, MO, USA) and protease inhibitors (Roche, Basel, Switzerland). The biopsies were then mechanically homogenized at 6,800 rpm (2 cycles of 15 seconds with 30 seconds pause, 4°C) using a Precellys Evolution Touch (Bertin Technologies). Beads and tissue debris were removed from the homogenates by centrifugation (10,000×g, 10 minutes, 4°C), and the total protein concentrations in the supernatants were determined by absorbance measurement at 280 nm using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA). The concentrations of IL-6, IL-6Ra (membrane bound and soluble), and gp130 were then measured with ready-to-use cartridges containing pre-loaded calibrators (SPCKB-PS-003028, SPCKB-PS-000505, SPCKB-PS-000494) in an automated flow-based sandwich immunoassay platform (Ella system; Bio Techne, Minneapolis, MN, USA). Cytokine levels were reported as pg/mg of total protein. The results were analyzed using GraphPad Prism (version 10). *P*-values <0.05 were considered statistically significant.

A total of 20 patients (all adult Koreans) with confirmed GIBD were included in the study. The mean age (mean±standard deviation [SD]) of the patients was 48±11 years, and the ratio of males to females was 50:50. The mean disease duration (mean±SD) at sampling was 6.9±8.7 years. The mean DAIBD score at sampling (mean±SD) was 52.5±32.7, and mean C reactive protein (CRP) level (mean±SD) was 23.1±40.4 mg/L. There were 5 patients with intestinal involvement only and 15 cases with extraintestinal manifestations. In all, 10 patients were anti-TNF-α-exposed (bioexposed) and 10 were biologic-naive (ratio of bioexposed to bionaive: 50:50). Nine of the 10 bioexposed patients had a history of adalimumab use, and one had a history of infliximab use. Most patients (n=17/20, 85%) had ulcers in the region of the ileocecal valve. The median number of intestinal ulcers was 1 (range, 1–10) and the median size of the biopsied intestinal ulcers was 20 mm (range, 5–80 mm). Other documented patient characteristics are described in Table 1. Data on human leukocyte antigen profiles were not obtained.

The concentrations of IL-6, IL-6Ra, and gp130 were measured in paired intestinal mucosal biopsies from inflamed and uninflamed tissue. Proteins were measurable in samples from 95% of patients (n=19/20); both samples from one patient and the uninflamed sample from another patient could not be processed due to paper in the collection tube that could not

Table 1. Patient Characteristics

Characteristic	Total
No of patients, No. (%)	20 (100)
Weight (kg), mean±SD	61.3±14.3
Height (cm), mean±SD	166.8±10.7
BMI (kg/m^2), mean±SD	21.8±3.5
Time since diagnosis (yr), mean±SD	6.9±8.7
Age at diagnosis (yr), mean±SD	41±12
Age at sampling (yr), mean±SD	48±11
Sex, No. (%)	
Male	10 (50)
Female	10 (50)
Manifestations of BD[a], No. (%)	
GIBD only	5 (25)
GIBD plus additional manifestation(s)	15 (75)
Oral ulcers present	12 (60)
Joint conditions present	8 (40)
Genital ulcers present	5 (25)
Dermatological conditions present	7 (35)
DAIBD score[b], mean±SD	52.5±32.7
Biologic treatment status, No. (%)	
Bionaive	10 (50)
Bioexposed	10 (50)
Medication(s) at sampling, No. (%)	
5-ASA	18 (90)
Immunomodulator	13 (65)
Infliximab or adalimumab	10 (50)
Previous corticosteroid medication, No. (%)	
Prednisolone	14 (70)
No. of intestinal ulcers, median (range)	1 (1–10)
Size of biopsied intestinal ulcers (mm)[c], median (range)	20 (5–80)
Location of intestinal ulcer[d], No. (%)	
Anastomosis site	3 (15)
Ileocecal valve	17 (90)
Transverse colon	4 (20)
Ascending colon	3 (15)
Descending colon	1 (5)
Hepatic flexure	3 (15)
Cecum	1 (5)
CRP level (mg/L), mean±SD	23.1±40.4

[a]Total >20 as 15 patients had >1 manifestation.
[b]DAIBD consists of 8 items: general well-being, fever, extraintestinal manifestations, abdominal pain, abdominal mass, abdominal tenderness, intestinal complication, and number of liquid stools. Scores range from 0 to 325.
[c]Only largest ulcer biopsied for patients with multiple ulcers.
[d]Total >20 as 6 patients had >1 intestinal ulcers.
SD, standard deviation; BMI, body mass index; BD, Behçet's disease; GIBD, gastrointestinal BD; DAIBD, disease activity index for intestinal BD; 5-ASA, 5-aminosalicylic acid; CRP, C-reactive protein.

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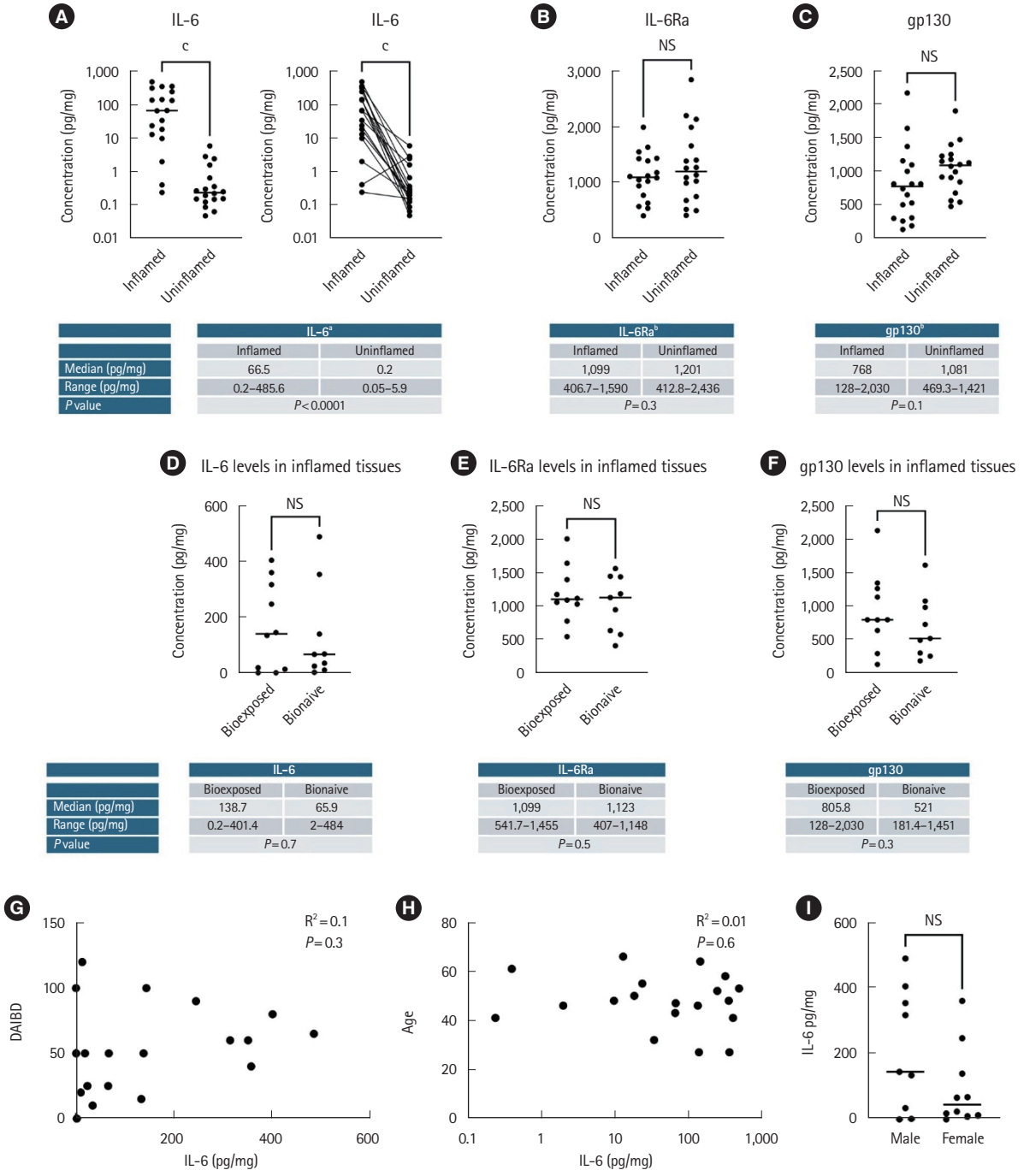

Fig. 1. Analysis of GIBD mucosal biopsies. (A-C) IL-6 but not IL-6Ra or gp130 levels are elevated in inflamed tissue from patients with GIBD. Measured concentrations of (A) IL-6, (B) IL-6Ra, (C) gp130 in paired mucosal biopsies from inflamed and uninflamed tissue. Median, range, and statistical significance are shown in the tables below the charts. Statistical significance was calculated using Note that the data in panel A are plotted on a log scale and are shown as both a dot plot and paired-sample dot plot. (D-F) IL-6, IL-6Ra, and gp130 levels are not influenced by bioexposure status in inflamed tissue. Measured concentrations of (D) IL-6, (E) IL-6Ra, (F) gp130 in inflamed tissue biopsies from bioexposed and bionaive patients with GIBD. Median, range, and statistical significance are shown in the tables below the charts. Statistical significance was calculated using unpaired *t*-tests. (G) IL-6 levels in inflamed tissue do not correlate with DAIBD. Statistical significance was calculated using the Spearman's rank correlation coefficient test. (H) IL-6 levels in inflamed tissues do not correlate with age ($R^2=0.01$, $P=0.6$ Spearman's rank correlation coefficient test). (I) IL-6 levels in inflamed tissues of male and female patients (median IL-6: 143.9 pg/mg vs. 44.8 pg/mg, respectively) were not significantly different ($P=0.3$, Mann Whitney test).

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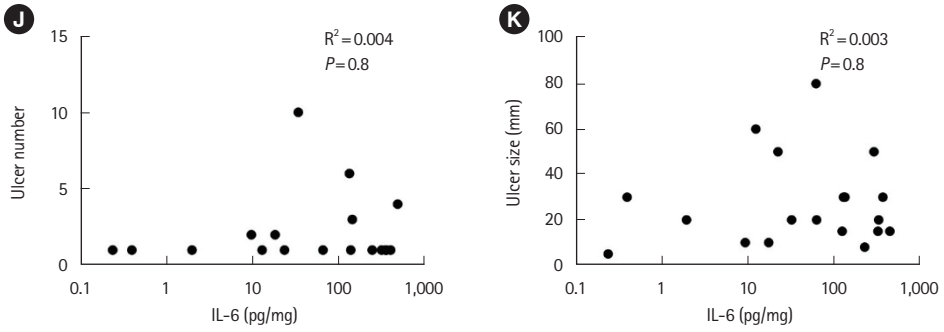

Fig. 1. Continued. (J, K) IL-6 levels do not correlate with ulcer number or size. Statistical significance was calculated using the Spearman's rank correlation coefficient test. Ulcer size refers to the largest ulcer if multiple ulcers were identified. [a]Wilcoxon matched-pairs signed-rank test or [b]paired *t*-test; [c]$P < 0.0001$. IL-6, interleukin 6; IL-6Ra, interleukin-6 receptor a; gp130, glycoprotein-130; NS, not significant; GIBD, gastrointestinal Behçet's disease; DAIBD, disease activity index for intestinal Behcet's disease.

be separated from the biopsy.

Median IL-6 level was significantly elevated in samples from inflamed tissue compared with uninflamed tissue (66.5 pg/mg vs. 0.2 pg/mg; $P < 0.0001$), with 94% of the paired samples (n = 17/18) exhibiting this trend (Fig. 1A). The median levels of IL-6Ra and gp130 were both slightly lower in inflamed versus uninflamed tissue (IL-6Ra: 1,099 pg/mg vs. 1,201 pg/mg, $P = 0.3$; gp130: 768 pg/mg vs. 1,081 pg/mg, $P = 0.1$), but the differences were not statistically significant (Fig. 1B and C). The levels of IL-6, IL-6Ra, and gp130 in inflamed samples showed no significant differences according to bioexposure status (Fig. 1D-F).

Although IL-6 levels were significantly elevated in inflamed tissues, they did not correlate with DAIBD scores ($R^2 = 0.1$, $P = 0.3$) (Fig. 1G). IL-6 levels in inflamed tissues also did not correlate with age ($R^2 = 0.01$, $P = 0.6$) (Fig. 1H). Median IL-6 levels in samples from inflamed tissues of male and female patients were not significantly different (143.9 pg/mg vs. 44.8 pg/mg, respectively, $P = 0.3$) (Fig. 1I). IL-6 levels did not correlate with either ulcer number or ulcer size ($R^2 = 0.004$ and $R^2 = 0.003$, respectively, $P = 0.8$ for both) (Fig. 1J and K). To determine whether a correlation between IL-6 levels and endoscopic results existed, the patients were stratified according to an endoscopic scoring system.[9] In all, 4 patients were e1 (< 20 mm sized solitary ulcer), 10 patients were e2 (≥ 20 mm sized solitary ulcer), and 6 patients were e3 (multiple ulcers regardless of size). No statistically significant difference in IL-6 levels was observed between the 3 groups in the biopsies of inflamed tissues, however (e1, 1,106 ± 1,178; e2, 903 ± 1,608; e3, 567 ± 773; one-way analysis of variance test).

While IL-6 elevation in systemic BD is well documented, its role in GIBD–a distinct and undercharacterized clinical entity–remains much less clear. In this study, the intestinal tissue levels of IL-6, IL-6Ra, and gp130 were measured in paired mucosal biopsies of inflamed and uninflamed tissues from patients with GIBD for the first time. Intestinal tissue levels of IL-6 were significantly elevated in mucosal biopsies where inflammation was present (Fig. 1A) and therefore appeared to be associated with intestinal disease activity in these patients. This is consistent with a previous study which showed that IL-6 levels > 7 pg/mL were associated with an 8.23 times higher risk of gastrointestinal involvement for patients with BD.[7] There is a growing consensus that high IL-6 levels are associated with poor prognostic outcomes and correlate with disease severity.[10]

The measured IL-6 levels did not correlate with the DAIBD estimate of GIBD disease activity (Fig. 1G) or endoscopic indices. Nonetheless, the correlation between IL-6 and DAIBD ($R^2 = 0.1$) was 10 times higher than the correlations between IL-6 and age, ulcer number, or ulcer size (Fig. 1G, H, J, K). Studies with a larger number of samples will be needed to determine whether a stronger correlation between IL-6 levels and DAIBD-measured disease activity can be detected. The mean DAIBD score (mean ± SD) at sampling was 52.5 ± 32.7, which corresponds to mild-to-moderate disease activity.[3] A stronger correlation might therefore also be observed if more patients with severe disease were assessed. The DAIBD score, which consists of 8 subjective items, showed good performance in terms of capturing the physician's overall evaluation but, in the authors' experience, tends to have a somewhat lower correlation with endoscopic indices. IL-6 levels might therefore correlate more closely with specific symptoms rather than overall disease activity. In this context, it would be informative to compare IL-6 levels with other inflammatory indices such as the fecal calprotectin level or neutrophil-lymphocyte ratio to further investigate the relationship between IL-6 levels and

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GIBD severity and activity. However, a correlation could not be demonstrated between either IL-6 levels and serum CRP (R^2 = 0.273, P = 0.258) or IL-6 levels and erythrocyte sedimentation rate (R^2 = 0.218, P = 0.368).

Despite the elevation in IL-6 levels, the levels of IL-6Ra and gp130 (the proteins necessary for IL-6 signal transduction) were not significantly different in samples of inflamed and uninflamed tissue from patients with GIBD. IL-6Ra and gp130 exist in both membrane-bound and soluble forms; IL-6 can signal to gp130-expressing cells via both cell membrane-bound (classical) and soluble (trans) IL-6Ra. The bioanalytical method employed in this study involved homogenization of mucosal biopsy tissue. This process disrupts cellular membranes, causing mixing of (intra-)cellular and extracellular proteinaceous material. It was therefore not possible to establish whether cell surface expression levels of these proteins were altered under inflammatory conditions, or if a change in the relative amounts of membrane-bound and soluble protein occurred. The results from this study suggest the potential for augmented IL-6 signaling in the inflamed GIBD intestinal mucosa. Investigation of IL-6Ra and gp130 cell surface expression in a subsequent study using a method such as immunohistochemistry may help elucidate whether the inflamed intestinal environment in GIBD is more conducive to enhanced classical or trans IL-6 signaling. There were additionally no significant differences in the levels of IL-6, IL-6Ra, and gp130 in inflamed samples according to bioexposure status. This might suggest that drugs involving the IL-6 pathway may be effective in both biologic-exposed and non-exposed subjects.

This study had several limitations. There was a relatively low number of patients and only a single site. The samples were of different ages and were collected over an extended time period. Only the largest ulcer was assayed (n = 6/20 patients had multiple ulcers), and measurements were taken at only a single timepoint. Confounding factors were not considered, and the pathology report did not include details on acute or chronic inflammation, presence of eosinophils, any signs of vasculitis, or microscopic colitis. The yield from the mechanical homogenization and bead-based system used for tissue processing was not known, and the Ella system for cytokine measurement, while advanced, may have introduced some variability to the results. Differences in tissue handling and processing could affect the accuracy and reproducibility of the cytokine measurements. The absence of significant differences between bioexposed and bionaive patients may be due to the lack of consideration of treatment duration, drug levels, or treatment response. The lack of correlation between IL-6 and serum CRP should be treated with caution, as these calculations were made without paired measurements of systemic IL-6 levels.

IL-6 signaling is currently a candidate for targeted biologic therapy of patients with IBD. Tocilizumab, a monoclonal IL-6R antibody, has shown efficacy in systemic BD, particularly in vascular and neurological manifestations.[4] To date, however, there is no published clinical evidence specifically evaluating its use in GIBD. The results here provide some insight into the local immune responses that occur in the most affected organ system of GIBD and may assist in understanding the role of IL-6 in patients with GIBD. The data suggest that further evaluation of the role of IL-6 in GIBD is warranted.

ADDITIONAL INFORMATION

Funding Source
This project was funded by Ferring Pharmaceuticals.

Conflict of Interest
Ravi A and Bruzelius B are employees of Ferring Pharmaceuticals. Pinton P is an employee of Ferring Pharmaceuticals, a member of the board of Directors of PharmaBiome, and owns stocks in Takeda Pharmaceutical Co., Ltd. Park J and Cheon JH are editorial board members of the journal but were not involved in the peer reviewer selection, evaluation, or decision process of this article. Park IS declares no conflicts of interest. No other potential conflicts of interest relevant to this article were reported.

Data Availability Statement
All data relevant to the study are in the article or available from the corresponding author upon reasonable request.

Author Contributions
Conceptualization; Data curation: Cheon JH, Ravi A, Pinton P. Formal analysis: all authors. Investigation: Park J, Ravi A, Bruzelius K, Park IS. Methodology: all authors. Project administration; Supervision: Cheon JH, Pinton P. Visualization: Park J, Ravi A. Writing - original draft: Park J, Ravi A, Bruzelius K. Writing - review & editing: all authors. Approval of final manuscript: all authors.

Additional Contributions
Sample homogenization and analysis was performed by ICON Bioanalytical Laboratories (Assen, the Netherlands).

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