Role of interleukin-23 in the pathogenesis of Behçet's disease

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Role of interleukin-23 in the pathogenesis of Behçet's disease

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Table of Contents

List of Tables

List of Figures

ABSTRACT

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Behçet's disease (BD) is a multisystemic chronic inflammatory disease with unknown etiology. Studies on the cytokine profiles in BD have been performed extensively with various samples. Biologically active interleukin(IL)-12 is a 70 kDa heterodimeric cytokine mainly produced by antigen-presenting cells and made of disulfide-linked p35 (alpha chain) and p40 (beta chain) subunits. Recently, an additional heterodimeric cytokine IL-23 which is made of shared p40 subunit of IL-12 and p19 subunit of IL-23 was discovered as part of a structure-based bioinformatics search. Therefore, we checked the changes of IL-23 in skin lesions and sera of patients with BD.

In this study, Increased IL-23 p19 mRNA levels were observed in the erythema nodosum-like skin lesions of BD patients and the skin lesions of psoriasis patients in comparison with normal control skins. Immunohistochemical staining of skin sections revealed increased IL-23 expression in dermal and subcutaneous layer of erythema nodosum-like lesion of BD and in dermal layer of psoriatic lesion. No statistically significant changes were observed in serum IL-12 and Il-23 levels between active BD patients and normal controls. No significant changes in the serum IL-12 and Il-23 levels of BD patients after treatment were also detected. Therefore, this study suggests that IL-23 may play a role in the localized pathogenic mechanism in the erythema nodosum-like lesions of active BD patients.

Key words: Behçet's disease, IL-23, IL-12

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I. INTRODUCTION

Behçet's disease (BD) is a multi-systemic chronic inflammatory disease with various combinations of mucocutaneous, ocular, musculoskeletal, vascular, respiratory, gastrointestinal, and neurologic involvements[1-3]. BD has a worldwide distribution, although it shows a higher prevalence along the ancient Silk Road, specifically in the Mediterranean countries, the Middle East, the Far East area, Japan, China and Korea[4]. The skin lesions of BD are erythema nodosum, folliculitis, pyoderma, erythema multiforme and thrombophlebitis[5].

Interleukin (IL)-12, is a heterodimeric cytokine of 70 kDa comprising of covalently linked p40 and p35 subunits[6]. It is known to play a central role in promoting type 1 T helper cell (Th1) responses, which strengthens cell-

mediated immunity[7]. Its ability to stimulate T-helper 1 cells and adaptive immunity has led to the proposal that it also actively contributes to several autoimmune diseases, including rheumatoid arthritis and inflammatory bowel disease[7]. IL-12 was also proposed to play an important pathogenic role in active BD[8]. Recent studies have demonstrated that the production of p40 is independently regulated from that of IL-12 p70. An additional heterodimeric cytokine similar to IL-12 was discovered as a part of structure-based bioinformatics search. This new cytokine named IL-23, comprises of p40 subunit, which is shared with IL-12, and new p19 subunit[9]. In many previous studies on IL-12 in BD, specific information was not available regarding which subunits of IL-12 measured. Sometimes, p40 subunit of IL-12 was measured as a proxy of IL-12 because p40 subunit is an inducible protein. Due to recent discovery of IL-23, p40 subunit can also represent IL-23. Therefore, the results of the previous studies should be re-interpreted and clarified with discovery of IL-23.

Changes in cytokine and chemokine levels in BD were extensively investigated by different research groups with different methods, summarized in Table 1[8,10-21]. The analysis of cytokine mRNA expression within BD lesions showed a high increase in the expression of chemokines and Th1 cytokines and the absence of Th2 cytokines in mucocutaneous BD lesions, suggesting that BD skin lesions are probably related to cell-mediated immunity[8]. Th1-type immune response was suggested with investigation of serum cytokine levels and cytokine production by peripheral blood lymphocytes[10]. However, divergent cytokine production profile of peripheral

blood mononuclear cells from BD patients of increased secretion of IL-4, IL-10, and IL-13 (Th2 cell response), almost normal secretion of IL-2, but minimal or deficient secretion of interferon(IFN)-γ and IL-12 (Th1 cell response) was also reported[18]. IL-23 has never been measured in the study of BD until now. To know the involvement of IL-23 in active Behçet's disease, we performed the followings[22]. Firstly, we compared IL-23 p19 mRNA expression levels in the skin biopsy specimens among active BD group, psoriasis group and normal control group, quantitatively. Secondly, We performed immnunohistochemical staining for IL-23 p19 in the skin biopsy specimens among three groups mentioned above. Thirdly, we investigated serum IL-23 and IL-12 profiles in active BD patients and normal controls.

Table 1. Changes in the levels of cytokines and chemokines in Behçet's disease

Cytokine	Lesion	CD3+ lymphocyte*	PBMC**	Serum	Plasma	Reference
Th1 cytokine						
IL-2		+	±	+		[10,18,20]
IL-12			-	+,-	+	[10,11,17,18,19]
IL-12p40	+					[8,21]
IFN-γ	+	+	-	+,-		[8,10,11,18,19,21]
TNF-α	+			+	±	[12,16,17,20,21]
Th2 cytokine						
IL-4	±	±	+	+		[8,10,11,18,19]
IL-10	+		+	+	±	[8,11,18,19]
IL-13	±		+	+		[8,18,19]
IL-6				+	±	[11,12,17,20]
IL-17				+		[11]
IL-18				+		[11,16]
Chemokine						
IL-8	+			+,		[8,12,14,15]
MCP-1	+			+,±		[8,13,15]
MIP-1α				+		[13,15]
RANTES				+,±		[13,15]

+, increased in BD or active BD; ±, no change; -, decreased in BD or active BD

*CD3+ lymphocytes stimulated by PHA, ** peripheral blood mononuclear cell (PBMC) stimulated by anti-CD3 and anti-CD40

II. MATERIALS

For quantitative reverse transcription polymerase chain reaction analysis of skin lesions, we studied 10 patients with BD fulfilling the criteria of the International Study Group for BD[23]. One patient was man, and nine patients were women, ages 38.7 ± 6.5 years (mean ± SD). All patients had clinically active disease at time of the study (more than two clinical manifestations of BD) and presented with erythema nodosum-like skin lesions. The patients were not receiving any specific treatment for at least four weeks when they presented with active disease. Biopsy specimens were obtained from erythema nodosum-like skin lesions with a 4-mm skin biopsy punch after administration of local anesthesia (lidocaine and epinephrine). Specimens were immediately snap frozen in liquid nitrogen and stored at -80°C. Biopsy specimens were obtained from erythema nodosum-like skin lesions 2 to 5 days after their appearance. Biopsy specimens of normal skin were obtained from 5 healthy volunteers. Additionally, biopsy specimens of psoriatic skin lesions were obtained from 5 psoriasis patients.

For immunohistochemical staining of skin lesions, we studied 8 patients with BD fulfilling the criteria of the International Study Group for BD[23]. Two patient were male, and six patients were female. All patients had clinically active disease at time of the study (more than two clinical manifestations of BD) and presented with erythema nodosum-like skin lesions without treatment for at least for four weeks. Additional sections were cut from eight paraffin blocks form biopsy specimens obtained from erythema nodosum-like skin lesions. Sections were also cut from paraffin blocks from seven psoriasis patients and six normal controls.

To determine cytokine levels in BD sera, we studied 24 patients with BD fulfilling the criteria of the International Study Group for BD[23]. Three patients were male, and twenty one patients were female. All patients had clinically active disease at time of the study (more than two clinical manifestations of BD). The patients were not receiving any specific treatment for at least four weeks when they presented with active disease. In a subgroup of ten patients, the sera were taken again seven to fourteen days after the initiation of colchine therapy during follow-up visits. We also studied 9 healthy volunteers who served as normal controls.

All patients and controls gave written informed consent for the tests and biopsies. The institutional review board of Yonsei University College of Medicine approved the protocol.

III. METHODS

1. Quantitative reverse transcription polymerase chain reaction

Total cellular RNA was prepared from the frozen specimens using rotor-stator PowerGen 700 (Fisher Scientific, Pittsburgh, PA, U.S.A.) and the RNeasy Mini Kit according to the manufacturer's protocol (Qiagen, Valencia, CA, U.S.A.). The RNA was treated directly in the column with 27 units of RNase-Free DNase I (Qiagen) for 15 min at room temperature during the extraction procedure. The RNA was eluted by diethylpyrocarbonate-treated sterile water, and the RNA concentration was determined by measuring the optical absorbance at 260 nm. Then, rTth DNA polymerase was used to reverse transcribe and amplify 5 ng of total RNA in a single tube assay using the Perkin Elmer TaqMan EZ RT-PCR kit (Perkin Elmer Applied

Biosystems, Foster City, CA, U.S.A.) with gene-specific sense and antisense primers and a probe fluorescently labeled at the 5' end with 6-carboxy-fluorescein (6-FAM)[24,25]. IL-23 p19 Assay-on-demand primers and fluorescently labeled probe set were purchased from Perkin Elmer-ABI, in which primer pairs were designed to avoid amplification of contaminating genomic DNA. The gene for human acidic ribosomal protein (hARP), a constitutively expressed housekeeping gene, primers and fluorescently labeled probe were generated using Primer Express software version 1.5 (Perkin Elmer-ABI) and were synthesized by Perkin Elmer-ABI. Duplicate samples were reverse transcribed and amplified consecutively: 2 min at 50 ℃, 30 min at 60 ℃ and then, 40 rounds of amplification for 15 s at 95 ℃ and 1 min at 60 ℃ using the ABI Prism 7700 sequence detection system as described by the manufacturer (Perkin Elmer-ABI)[24]. Sequence-specific amplification was detected as an increased fluorescent signal of 6-FAM during the amplification cycle. Quantification of gene-specific message levels was based on a comparison of the fluorescence intensity in the unknown total RNA sample with the fluorescence intensity from a standard curve of control mRNA levels. Amplification of hARP was performed on all samples tested to control for variations in RNA amounts[26]. All genes were subsequently normalized against hARP mRNA levels. Levels of gene-specific messages were graphed as normalized message units as determined from the standard curve. A no-template control was included in each amplification reaction to control for contaminating templates. For valid sample analysis the fluorescence intensity in the no-template control was required to be zero.

2. Immunohistochemical staining and scoring

가. Immunohistochemical staining

Sections were cut from 21 paraffin blocks from 8 BD patients, 7 psoriasis patients, and 6 normal controls for immunohistochemistry. Commercially available purified mouse monoclonal anti-human IL-23 (p19) IgG (clone HTL2736, BioLegend, San Diego, CA, U.S.A.), Catalyzed Signal Amplification (CSA) System (DakoCytomation, Carpinteria, CA, U.S.A.) and CSA Ancillary System (DakoCytomation) were used in immunohistochemical staining. The detailed procedures are as follows. Prior to staining, tissue slides were de-paraffinized with xylene and gradient alcohols to remove embedding media and then re-hydrated. Tissue sections were immersed with Target Retrieval Solution (DakoCytomation) diluted in 1:10 from 10X concentrate and micro-waved for 1 minute with high power and 9 minutes with medium power, then cooled at room temperature for 20 minutes. Tissue sections were incubated with avidin solution for 10 minutes. Avidin solution was then rinsed off with Tris-buffered saline with Tween 20 (TBST) (50 mM Tris-HCl, 300 mM NaCl, 0.1% Tween 20, 0.01% preservative, pH 7.6) and the slides were placed in a bath of TBST for three minutes. Next, tissue sections were incubated with biotin solution for 10 minutes. Biotin solution was then rinsed off and the slides were placed in a fresh bath of TBST for three minutes. Peroxidase block on specimens were performed with 3% hydrogen peroxide in water, followed by protein block using serum-free protein in phosphate buffered saline (PBS) with 0.015 M sodium azide. Then, specimens were covered with application of monoclonal mouse anti-human IL-23 p19 antibody [20 μg/ml (1:50)] or negative

control IgGκ. Link antibody was attached using biotinylated rabbit anti-mouse immunoglobulins in Tris-HCl buffer containing stabilizing protein and 0.015 M sodium azide. Streptavidin-biotin complex (40 ㎕ Streptavidin in PBS buffer containing an anti-microbial agent, 40 ㎕ biotin conjugated to horseradish peroxidase in PBS buffer containing an anti-microbial agent and 1 ㎖ PBS buffer containing stabilizing protein and an anti-microbial agent were mixed at least 30 minutes before use) was applied to specimens. Amplification reagent (biotinyl tyramide and hydrogen peroxide in PBS containing stabilizing protein and an anti-microbial agent) was then added. Specimens were covered with streptavidin conjugated to horseradish peroxidase in PBS containing stabilizing protein and an anti-microbial agent. Specimens were covered with the prepared substrate-chromogen solution (CSA System, DakoCytomation). Finally specimens were dehydrated with gradient alcohol and xylene and mounted with permanent mounting medium.

나. Scoring of staining intensity

Two independent investigators independently reviewed all of the sections without knowledge of which antibody they were scoring. Discrepancies in estimations were reconciled by a concurrent review using a multi-headed microscope. The expression of the IL-23 p19 subunit was blindly assessed in two sections per paraffin block, scoring separately in the basal and suprabasal epidermis, upper and lower dermis and subcutaneous fat tissue. The quantity of staining was evaluated using dermis using the following arbitrary units: 0, no staining; 1, weak staining; 2, moderate

staining; 3, strong staining. The score was adjusted for each part of the skin, adding scores on two sections of each part of the skin, so that score 6 referred to the maximum within all specimens. Epidermal expression was defined as the average of basal and suprabasal scores, whereas dermal expression was the average upper and lower dermal scores. Subcutaneous expression was the sum of scores on subcutaneous layers of two sections.

3. Cytokine levels in sera

Serum levels IL-12 (Quantikine®, R&D Systems, Minneapolis, MN, U.S.A.) and IL-23 (Bender MedSystems GmbH, Vienna, Austria) were measured using an enzyme-linked immunosorbent assay (ELISA) according to the procedures suggested by the manufacturers. Briefly, IL-12 assay employed the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-12 had been pre-coated onto a microplate. Standards and samples were pipetted into the wells and any IL-12 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-12 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-12 bound in the initial step. The color development is stopped and the intensity of the color is measured. For IL-23 assay, an anti-IL-23 monoclonal antibody was absorbed onto micro-wells. IL-23 present in the sample or standard were bound to antibodies absorbed to the micro-wells. Then, a biotin-conjugated IL-23 antibody was added and bound to IL-23 captured by the

first antibody. Following incubation, unbound biotin conjugated anti-IL-23 was removed during a washing step. Streptavidin-HRP was added and bound to the biotin conjugated anti-IL-23. Following incubation, unbound Streptavidin-HRP was removed during a washing step, and substrate solution reactive with HRP was added to the wells. A colored product was formed in proportion to the amount of IL-23 present in the sample. The reaction was terminated by addition of acid and absorbance was measured. Standard curves were used to quantify serum levels of cytokines. IL-12 ELISA kit was known to have no significant cross-reactivity or interference was observed with recombinant human IL-12 p40, recombinant mouse IL-12, recombinant human IL-23, or recombinant mouse IL-23. IL-23 ELISA kit was known to have no cross-reactivity with IL-12 p40 or IL-12 p70. The lowest levels of sensitivity were 5.0 pg/ml of IL-12 and 20 pg/ml of IL-23.

4. Statistical analysis

Quantitative measures of changes in IL-23 mRNA expression were evaluated statistically. Differences among BD group, psoriasis group and normal controls were compared with ANOVA test. Difference between BD group and normal controls was also compared with two-tailed Student *t*-test.

Semiquantitative measures of Il-23 p19 immunohiscochmical stainings were evaluated statistically. Differences in arbitrary scores among BD group, psoriasis group and normal controls were compared with ANOVA test.

Quantitative measures of changes in cytokine levels were also evaluated statistically. Differences between BD group and normal controls were also

compared with two-tailed Student *t*-test. Differences between serum cytokines levels at initial presentation and follow-up presentation after 10 ± 4 days with oral colchicine treatment were compared with two-tailed paired Student *t*-test.

IV. RESULTS

1. IL-23 p19 mRNA expression in skin lesions

Increased IL-23 p19 mRNA levels were observed in the erythema nodosum-like skin lesions of BD patients (5.977 ± 4.21) and the skin lesions of psoriasis patients (7.58 ± 5.26) in comparison with normal control skins (0.892 ± 1.00) (Fig. 1). The differences were statistically significant with ANOVA ($p = 0.042$). The differences with post hoc multiple comparisons using LSD were significant ($p = 0.034$ and 0.017 respectively).

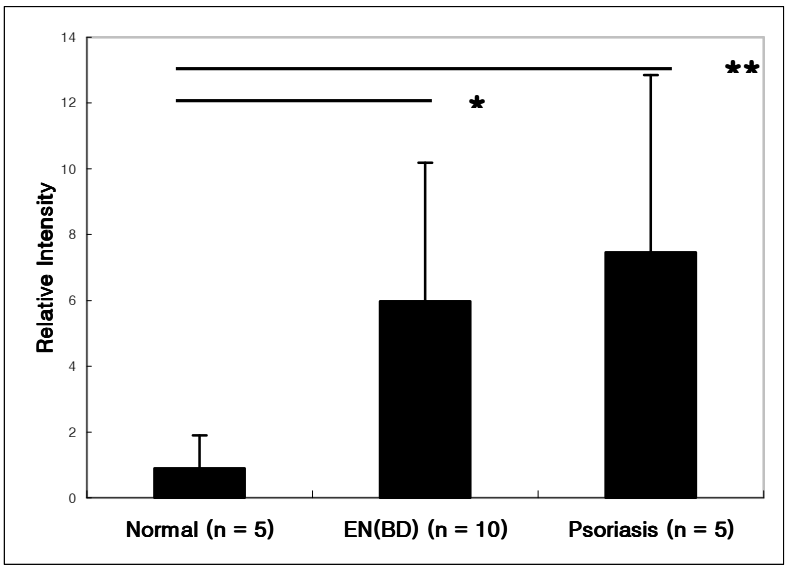

Figure 1. Increased IL-23 p19 mRNA levels were observed in the erythema nodosum-like skin lesions of BD patients (5.977 ± 4.21) (*****p=0.034) and psoriatic lesions of psoriasis patients (7.58 ± 5.26) in comparison with normal controls (0.892 ± 1.00) (******p=0.017).

2. IL-23 p19 immunohistochemical stains on paraffin sections of skin lesions

To confirm the expression and localization of IL-23, we stained normal (n = 6), erythema nodosum-like lesional (BD) (n = 8), and psoriatic lesional (n = 7) skin paraffin embedded sections with the IL-23 p19-specific monoclonal Ab. The expression of the p19 subunit in the epidermis of skin was diffuse and strong in all three groups (Fig. 2A, B, C). In the dermis, perivascular cells abundantly expressed this molecule in erythema nodosum-like lesional (BD), and psoriatic lesional skin (Fig. 2E, F). In contrast, in normal human skin the IL-23 p19 expression in the dermis was present in only a limited number of cells, mainly around the capillaries (Fig. 2D). In the subcutaneous tissue, Septal infiltrating cells abundantly expressed this molecule in erythema nodosum-like lesion (BD) (Fig. 2H). In contrast, in normal and psoriatic human skin the IL-23 p19 expression in the subcutaneous tissue was much weaker (Fig. 2G, I).

The expression of IL-23 p19 in normal, erythema nodosum-like lesion (BD), and psoriatic lesion was scored in arbitrary units and was summarized in Fig. 3. The

expression of IL-23 p19 in epidermis did not differ significantly among three groups. However, the expression of IL-23 p19 in dermis was stronger in erythema nodosum-like lesion (BD) and psoriatic lesion compared with normal controls. The expression of IL-23 p19 in subcutaneous tissue was stronger in erythema nodosum-like lesion (BD) compared with normal controls and psoriatic lesion.

N **EN (BD)** **P**

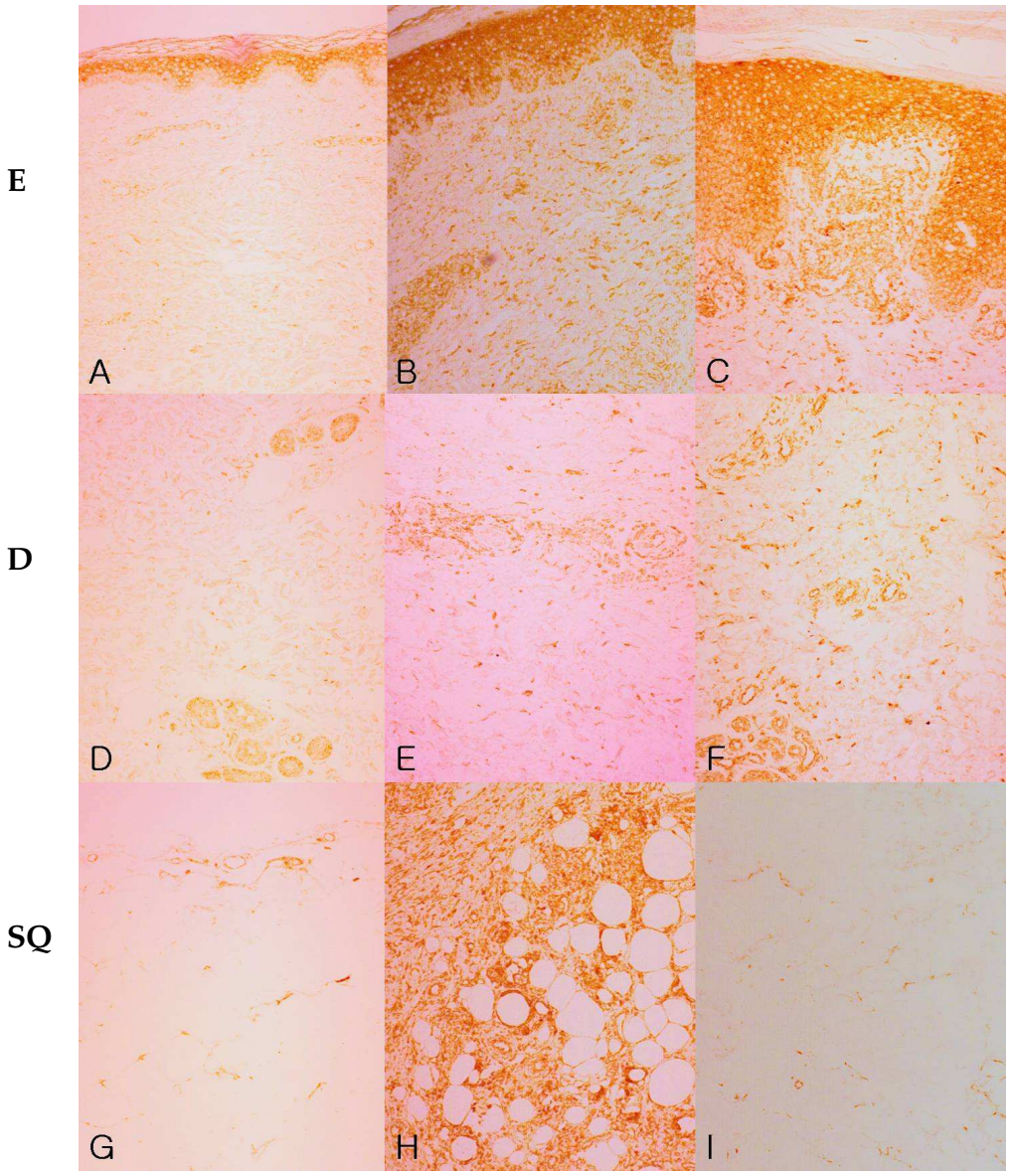

Figure 2. Immunohistochemical staining of IL-23 p19 in normal, erythema nodosum-like lesional (BD) and psoriatic lesional skin. The expression of the IL-23 p19 in the epidermis of skin was diffuse and strong in all three groups (A, B, C). In the dermis, perivascular cells abundantly expressed this molecule in erythema

nodosum-like lesional (BD), and psoriatic lesional skin (E, F). In contrast, in normal human skin the IL-23 p19 expression in the dermis was present in only a limited number of cells, mainly around the capillaries (D). In the subcutaneous tissue, septal infiltrating cells abundantly expressed this molecule in erythema nodosum-like lesion (BD) (H). In contrast, the IL-23 p19 expression in the subcutaneous tissue was much weaker in normal (G) and psoriatic skin (I) (original magnification, ×100). Abbreviations: N, normal; EN, erythema nodosum-like lesion (BD); P, psoriasis; E, epidermis; D, dermis; SQ, subcutaneous fat.

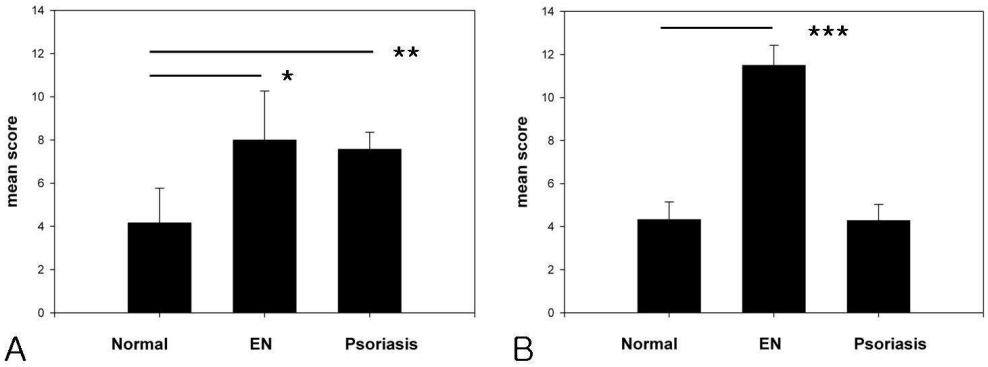

Figure 3. High level of IL-23 p19 immunostains in erythema nodosum-like lesion of BD and psoriatic lesion in dermis (A.). High level of IL-23 immunostains in erythema nodosum-like lesion of BD in subcutaneous layer (B). The levels in the lesions were significantly different from normal skins. (* $p = 0.001$, ** $p = 0.002$, *** $p < 0.001$) .

3. Cytokine levels in sera

Serum IL-12 and IL-23 levels of the twenty four patients with active BD were compared with the levels of nine healthy control sera. No statistically significant difference between serum IL-12 levels of the twenty four patients with BD (13.14 ± 19.46 pg/ml) and the levels of nine healthy control sera (10.07 ± 12.57 pg/ml) were observed (Fig. 4). No statistically significant difference between serum IL-23 levels of the twenty four patients with BD (224.68 ± 322.32 pg/ml) and the levels of nine healthy control sera (432.62 ± 663.42 pg/ml) were observed (Fig. 5).

Serum IL-12 and IL-23 levels of the subgroup of ten patients with active BD were compared with the levels of serum IL-12 and IL-23 seven to fourteen days after the initiation of treatment. There was no statistically significant difference between serum IL-12 levels at initial presentation (11.37 ± 10.78) and follow-up visit (14.83 ± 19.73) after 10 ± 4 days with oral colchicine treatment with two-tailed paired *t*-test ($p = 0.56$) (Fig. 6). There was no statistically significant difference between serum IL-23 levels at initial presentation (232.56 ± 321.89) and follow-up visit (184.03 ± 280.97) after 10 ± 4 days with oral colchicine treatment with two-tailed paired *t*-test ($p = 0.085$) (Fig. 7).

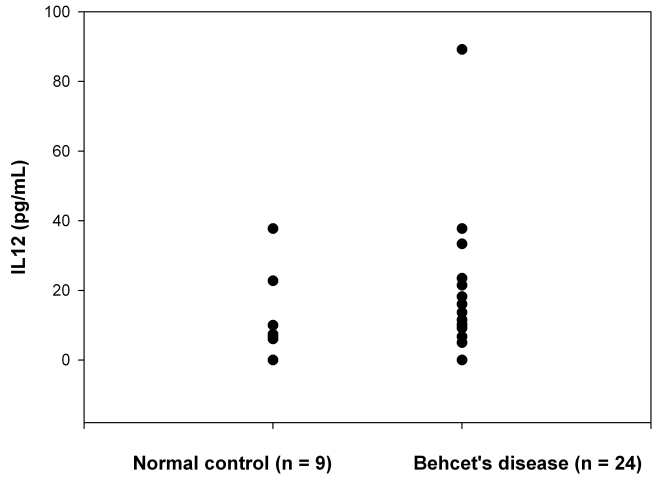

Figure 4. Seum IL-12 levels in Behçet's disease. No statistically significant difference between serum IL-12 levels of the twenty four patients with Behçet's disease (13.14 ± 19.46 pg/ml) and the levels of nine healthy control sera (10.07 ± 12.57 pg/ml).

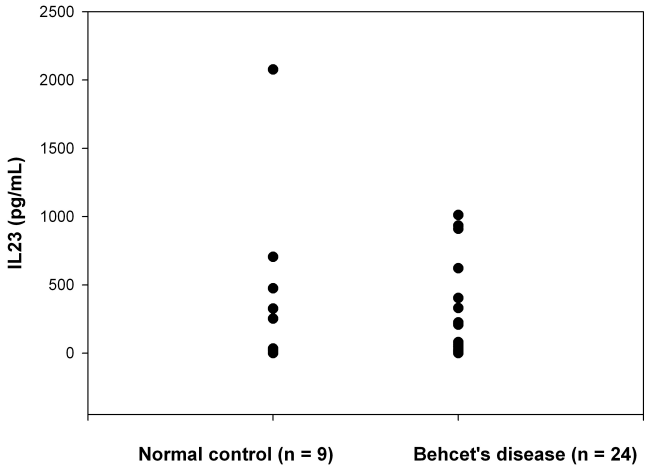

Figure 5. Seum IL-23 levels in Behçet's disease. No statistically significant difference between serum IL-23 levels of the twenty four patients with Behçet's disease (224.68 ± 322.32 pg/ml) and the levels of nine healthy control sera (432.62 ± 663.42 pg/ml).

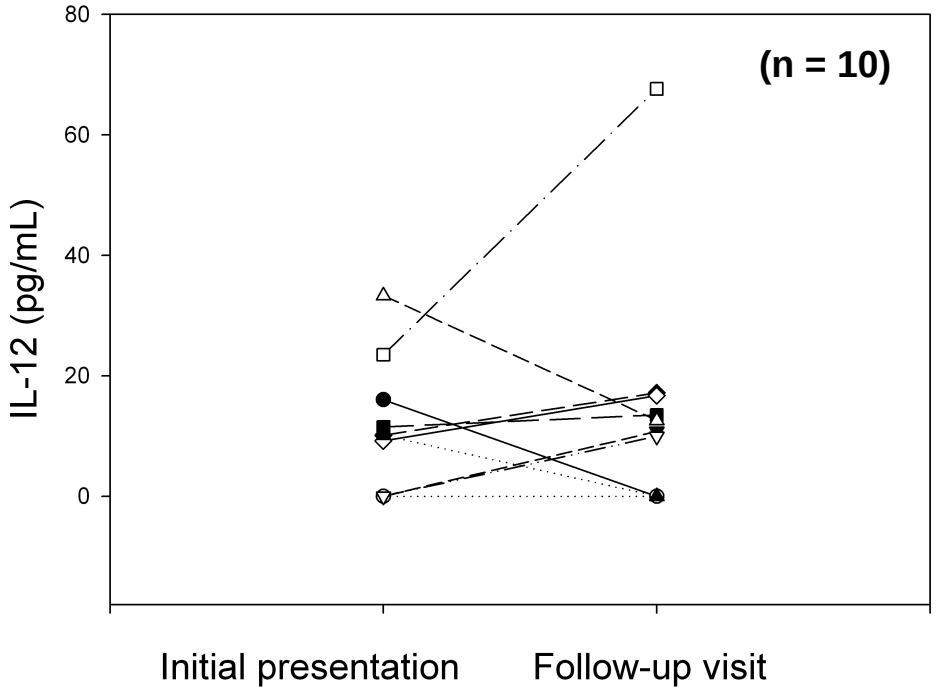

Figure 6. Changes in seum IL-12 levels in Behçet's disease after treatment. There was no statistically significant difference between serum IL-12 levels at initial presentation (11.37 ± 10.78) and follow-up visit (14.83 ± 19.73) after 10 ± 4 days with oral colchicine treatment with two-tailed paired *t*-test.

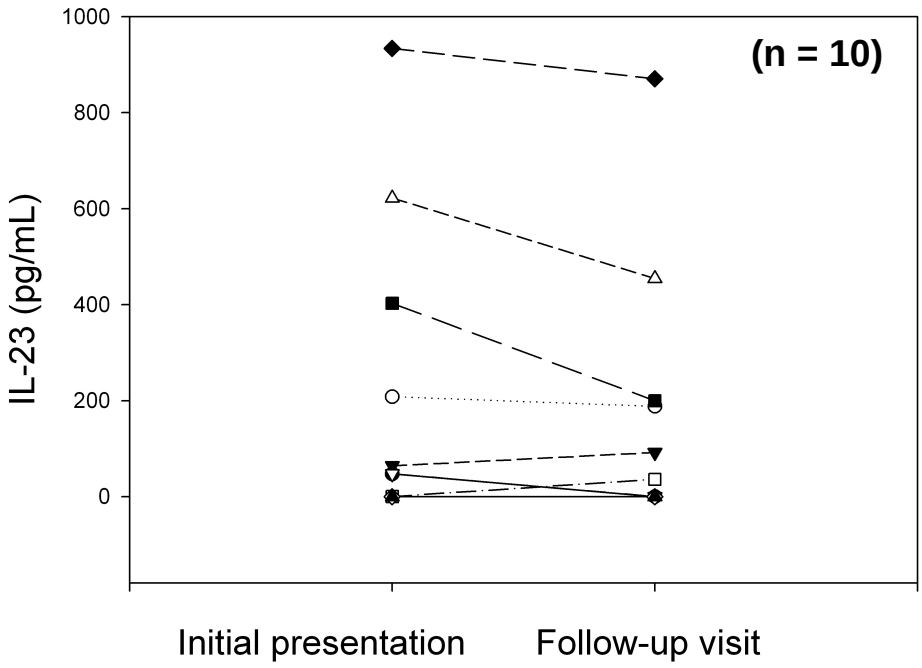

Figure 7. Changes in serum IL-23 levels in Behçet's disease after treatment. There was no statistically significant difference between serum IL-23 levels at initial presentation (232.56 ± 321.89) and follow-up visit (184.03 ± 280.97) after 10 ± 4 days with oral colchicine treatment with two-tailed paired *t*-test (p = 0.085).

V. DISCUSSION

We observed that IL-23 p19mRNA expression and immunoreactivity were increased in BD lesions. In literature, increased expression of IL-12 p40 mRNA in the skin lesions (oral ulcers, genital ulcers and pseudofolliculitis lesions) of active BD patients[8], and increased expression of interferon IFN-γ, TNF-α and IL-12 p40 mRNA in intestinal lesions of BD were reported, indicating Th1 skewed responses[21]. Because IL-23 is composed of p40 and p19 subunits, we predict that IL-23 may play a role in the BD lesions.

By using IL-12/23 p40, IL-12 p35 and IL-23 p19 knockout mice, it was shown that IL-23, but not IL-12 is important in the pathogenesis of multiple sclerosis[28,29]. Increased expression of IL-23 p19 and p40 in psoriatic lesional skin of patients with psoriasis vulgaris was reported[30]. Therefore, IL-23 is suggested to involve in the pathogenesis of psoriasis. Recent advances in immunology have shown that IL-12 is important for the development of IFN-γ producing T helper (Th)1 cells that are essential for host defense and tumor suppression, but a T cell population driven by IL-23 is characterized by the production of the IL-17-related cytokines IL-17A and IL-17F[31,32]. Mononuclear cells isolated from mouse sphenocytes which were cultured with IL-2 in presence of IL-23 secreted increased levels of IL-17 in culture supernatants using ELISA[31]. Gene expression profile of rIL-23-stimulated draining lymph node CD4+ T cells isolated from proteolipid protein peptide immunized mice showed preferentially increased mRNA levels of IL-17, IL-17F, TNF, IL-6, integrin-

α3 and IL-23R whereas rIL-12-stimulated cells expressed increased mRNA levels of IFN-γ, IL-12Rβ2 and granzyme G[32]. By knockout mouse study, IL-12–deficient p35-/- mice developed more IL-17–producing CD4+ T cells, as well as elevated mRNA expression of proinflammatory cytokines such as tumor necrosis factor, IL-1, IL-6, and IL-17 in affected tissues of diseased mice, indicating that IL-23 is an essential promoter of end stage joint autoimmune inflammation, whereas IL-12 paradoxically mediates protection from autoimmune inflammation[33]. Recent studies have suggested that IL-23-IL17 axis may play a dominant role in provoking chronic autoimmune inflammation in central nervous system and joints[28,32,33]. Even though we need more supporting evidences, it is possible that IL-23 may be important in the development and maintenance of BD. To prove the hypothesis that IL-23 is involved in the pathogenesis of BD, it is necessary to check IL-17 levels in BD lesions.

Since, no association between IL-12 p40 gene (IL12B) polymorphism and BD were found in Japanese BD patients[27], IL-12 p40 may not involve as a predisposing factor in the pathogenesis of BD. The significance of IL-12 in BD needs to be further verified by detecting p35 changes in BD lesions.

In immunohistochemical staining for erythema nodosum-like skin lesion using IL-23 p19-specific mouse monoclonal antibody, staining was stronger in the dermis and subcutaneous layer compared with normal controls. In the epidermal layer, staining was diffuse and strong in all three groups. In the previous immunochemical staining study using polyclonal rabbit anti-human IL-23 p19, diffuse positive staining pattern for epidermis similar to our result was observed [34]. On the contrary to our result, they could observe marked stronger staining in the epidermal layer of

the psoriatic lesional skin and relatively stronger reaction in the nonlesional skin compared with normal controls with statistical significance[34]. This discrepancy between previous report and our result on epidermis is thought to be caused by the difference in primary antibodies employed in the procedures

The previous studies on the serum cytokine profile in BD suggested that polarization toward Th1 cytokines including IL-12, IFN-γ and TNF-α even though the converse had been also found[10-12,16-20]. Elevated serum IL-12 levels in active BD patients or correlation of IL-12 plasma levels with severity of the disease suggested pathogenic role of a Th1-type immune response in active BD[10,18]. However, Aridogan et al. reported increased levels of IL-4, IL-10 and IL-13 (Th2 cell response) but decreased levels of IL-12 and IFN-γ (Th1 cell response) in active BD patients[19]. In our study, we could not observe statistically significant changes of IL-12 or IL-23 levels between BD patients and normal controls. Frassanito et al[10] reported that serum IL-12 levels in BD were were 76.7 ± 24.7 pg/ml and that those in normal controls were 30.1 ± 10.9 pg/ml. Hamzaoui et al[11]. reported that serum IL-12 levels in BD were were 32.5 ± 4.2 pg/ml and that those in normal controls were 7.6 ± 2.8 pg/ml. In Aridogan's report[19], serum IL-12 levels were 25.20 ± 14.96 pg/ml in active BD patients, 45.97 ± 15.65 pg/ml in inactive BD patients and 12.31 ± 3.54 pg/ml in normal controls. Serum IL-12 levels of patients with Behcet's disease (13.14 ± 19.46 pg/ml) in our study were significantly lower than those reported by others (*t*-test, $p < 0.05$, respectively). Serum IL-12 levels of normal controls (10.07 ± 12.57 pg/ml) in our study were only significantly lower than those reported by Frassanito et al(*t*-test, $p < 0.05$). However, the differences were not significant when serum IL-12 levels of normal controls in our study and those

reported by Hamazaoui et al. or Aridogan et al. were compared. Therefore, we could predict that the differences in serum IL-12 levels might be due to differences in ethnic groups, detection antibodies or serum preparations. We also compared serum IL-12 or IL-23 levels pairwise at initial presentations and follow-up visits after oral colchine treatment in a subgroup. We could not observe any statistically significant differences in IL-12 levels. Even though there was a trend of slight decrease in IL-23 levels after oral colchine therapy, the difference was not statistically significant when compared pairwise. Hamzaoui et al. reported a striking increase of IL-17 in the sera of active BD patients when compared with patients in remission or healthy controls[11]. Although IL-23 was reported to be one of the essential factors required for the expansion of a pathogenic CD4+ T cell population which produces IL-17, IL-17F, IL-6, and tumor necrosis factor[31,32], we could not observe any difference in seum IL-17 levels between BD patients and normal controls (data not shown). However it is possible that IL-17 may relate to localized pathogenesis in BD instead of systemic involvement.

In summary, we observed elevated expression of IL-23 p19 in erythema nodosum-like lesions of active BD patients using quantitative real-time PCR and immunohistochemical staining with IL-23 p19-specific mouse monoclonal antibody. However, we could not observe statistically significant changes of Il-12 or IL-23 serum levels between BD patients and normal controls. The overall data in our study suggests that IL-23 may play a role in the localized pathogenic mechanism in the erythema nodosum-like lesions of active BD patients.

VI. CONCLUSION

In this study, we performed quantitative reverse transcription-polymerase chain reactions and immunohistochemical stains for IL-23 p19 on normal skins, erythema nodosum-like BD skin lesions and psoriatic skin lesions. ELISA for IL-12 and Il-23 were also performed in the sera of active BD patients and normal controls. The results are summarized as follows:

1. Increased IL-23 p19 mRNA levels were observed in the erythema nodosum-like skin lesions of BD patients and the skin lesions of psoriasis patients in comparison with normal control skins.
2. The expression of the p19 subunit in the epidermis of skin was diffuse and strong in all three groups. However, expression of IL-23 in dermis was significantly stronger in erythema nodosum-like lesions of BD and psoriatic lesions compared with normal control skins. The expression of IL-23 in subcutaneous layer was significantly stronger in erythema nodosum-like BD skin lesions.
3. Serum IL-12 and IL-23 levels of the twenty four patients with active BD were compared with the levels of nine healthy control sera. No statistically significant difference between serum IL-12 and Il-23 levels of the twenty four patients with BD and the levels of nine healthy control sera were observed.
4. Serum IL-12 and IL-23 levels of the subgroup of ten patients with active BD were compared with the levels of serum IL-12 and IL-23 seven to fourteen days

after the initiation of treatment. There was no statistically significant difference between serum IL-12 and Il-23 levels at initial presentation and follow-up visit.

Therefore, this study suggests that IL-23 may play a role in the localized pathogenic mechanism in the erythema nodosum-like lesions of active BD patients.

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국 문 요 약

베체트병의 병인에 있어서 IL-23의 역할

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장재용

베체트병은 아직 병인이 밝혀지지 않고 신체 여러 장기를 침범하는 만성 염증성 질환이다. 베체트병에서 싸이토카인의 변화는 여러 가지 표본에서 조사되었다. 생물학적으로 활동적인 인터루킨(IL)-12는 p35 (알파체인) 와 p40 (베타체인) 소단위로 이루어진 70 kDa의이질이량체적 싸이토카인으로 주로 항원전달세포에서 생산된다. 최근 IL-12의 p40 소단위를 공유하고 IL-23의 IL-19 소단위로 이루어진 추가적인 이질이량체적 싸이토카인인 IL-23이 구조에 근거한 생물정보학적 탐구에 의해 발견되었다. 따라서 저자들은 베체트병 환자의 혈청과 조직에서 IL-23의 변화를 알아보았다.

본 연구에서는 정상인과 비교시 베체트병 환자의 결절홍반양 피부병변과 건선환자의 피부병변에서 IL-23 p19 mRNA가 증가가 관찰되었다. 피부조직의 면역조직화학염색을 시행하였을 때 베체트병환자의 결절홍반양 병변의 진피와 피하지방층 및 건선환자의 건선 병변의 진피에서 IL-23의 발현의 증가를 보였다. 정상인과 베체트병 환자군 간에 혈청 IL-12나 IL-23 수준을 비교하였을 때 통계적으로 유의한 차이가

발견되지 않았다.베체트병 환자에서 치료 후에도 이들 수준의 차이는 발견되지 않았다. 그러므로 본 연구는 IL-23이 활동성 베체트병 환자의 결절홍반성 병변에서 국소적 병리 기전에 관여할 수도 있다는 점을 시사한다.

핵심 되는 말: 베체트병, IL-12, IL-23