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RESEARCH ARTICLE

New insight of itch mediators and proinflammatory cytokines in epidermolysis bullosa

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Abstract

Objectives: Epidermolysis bullosa (EB) is a hereditary disorder characterized by mechanical stress-induced blistering. The presence of extracutaneous complications such as cardiomyopathy and renal disease observed in severe EB subtypes and the fact that pruritus is a common symptom across all EB subtypes indicate that EB is not only a skin fragility disease but also a systemic inflammatory disorder. Our study aims to elucidate the basis of the systemic inflammation seen in EB patients.

Methods: We analyzed serum samples of 20 EB patients by Luminex bead-based cytokine assays and enzyme-linked immunosorbent assays.

Results: The serum levels of sIL-2R, IL-6, HGF, M-CSF, SCGF-β, IL-8, IL-16, IFN-γ, MIF, MIP-1α, and thymic stromal lymphopoietin (TSLP) ($p < .01$, $p < .01$, $p < .01$, $p < .01$, $p < .01$, $p < .05$, $p < .05$, $p < .05$, $p < .05$, $p < .05$, and $p = .01$, respectively) were found to be significantly elevated in EB patients, whereas TNF-β ($p < .01$) was decreased. Th2 cytokines including IL-4, IL-5, and IL-13 were not elevated in EB patients. In contrast, TSLP was significantly increased, and IL-31 and oncostatin M were not statistically significant but tended to be higher in the patients than in healthy controls. Among proinflammatory cytokines such as IL-1β, IL-6, and TNF-α, IL-6 was elevated in EB patients.

Conclusions: The imbalance of several itch mediators and proinflammatory cytokines was identified. Biologics targeting the cytokines found to be elevated in the sera of patients is considered as a beneficial treatment option for EB.

KEYWORDS
cytokines, dystrophic epidermolysis bullosa pruriginosa, IL-1β, IL-31, IL-4, IL-6, thymic stromal lymphopoietin

1 | INTRODUCTION

Epidermolysis bullosa (EB) is a group of inherited disorders characterized by mechanical stress-induced blistering of the skin and mucous membranes caused by defects in proteins of the dermoepidermal junction or within epidermis.[1] The treatment of EB is mainly supportive and unsatisfactory. Classical EB is classified into the four major types depending on the distinguishing ultrastructural site of skin cleavage: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler EB.[1,2] EBS is defined by skin blistering within epidermal basal keratinocytes and is subclassified into localized, intermediate, and severe EBS according to clinical severities.[1] EBS is mostly inherited in an autosomal dominant fashion and is caused by a single mutation in either *KRT5* or *KRT14*, which encode keratin 5 or keratin 14, respectively. DEB is caused by mutations in the *COL7A1* gene encoding type VII collagen and has two patterns of inheritance: autosomal dominant DEB (DDEB) and autosomal recessive DEB (RDEB).[3] Tissue separation occurs in the sublamina densa region. DEB pruriginosa (DEB-Pr) is a rare clinical subtype of DEB with autosomal dominant or recessive inheritance.[3] Characterized by severe pruritus, prurigo nodularis, and lichen simplex chronicus-like lesions, DEB-Pr may resemble other skin diseases, thereby complicating the clinical diagnosis. The mutations causing DEB-Pr do not differ from those observed in other forms of DEB, and the pathomechanisms underlying the clinical manifestations of DEB-Pr are not fully understood.[4]

Patients with a more severe phenotype, such as severe RDEB patients, are at an increased risk of glomerulonephritis, renal amyloidosis, IgA nephropathy, and cardiomyopathy,[5,6] and it has been suggested that proinflammatory cytokines such as interleukin (IL)-1β are responsible for these extracutaneous features.[7,8] In addition, the itch is one of the most common symptoms across all EB subtypes.[9–11] It may occur not only in the blistered or wounded site but also on the whole body. These observations led us to consider the possibility that EB is not only a skin fragility disease but also a systemic inflammatory disorder. Understanding systemic inflammation at the molecular level is necessary to improve clinical management and should be a research priority for EB.

Despite elucidation of the genetic basis and molecular mechanisms of the proteins involved in the pathogenesis of EB,[12–14] itch mediators, and systemic proinflammatory cytokines are poorly understood. To elucidate the imbalance of cytokines seen in patients with EB, we analyzed sera from 20 patients with EBS or DEB.

2 | METHODS

2.1 | Patients and controls

This study was approved by the human research ethics committees of Niigata University Medical and Dental Hospital (approval number: 2021-0285), Hokkaido University Hospital (approval number: 011-0089, 13-043), Nara Medical University (approval number: 2790). Written informed consent was obtained from the enrolled subjects. For individuals younger than 20 years of age, informed consent was signed by their parents. Serum samples of 20 EB patients were collected from multiple hospitals and institutions in Japan.[15] The diagnosis of EB was made by certified dermatologists according to the international criteria.[1,16] We also assembled the following clinical information on the patients: gender, age, EB subtype, and affected body surface area (BSA) (%).[15] The affected area was defined as including erythema, erosions, ulcers, and scars.[15] Serum samples from 16 healthy volunteers without any dermatological disorders were also collected as controls.

2.2 | Luminex bead-based cytokine assays

A total of 48 human cytokines were measured by Bio-Plex Pro Human Cytokine Screening Panel, 48-Plex (Bio-Rad Laboratories) using 12.5 μl of serum per assay following the manufacturer's instruction manual. The assay plate was read on the Bio-Plex 200 System (Bio-Rad Laboratories). Standards and samples were measured in duplicates and analyzed using Bio-Plex Manager Software 6.1 (Bio-Rad Laboratories) to calculate concentrations in pg/ml.

2.3 | Enzyme-linked immunosorbent assay

IL-31, thymic stromal lymphopoietin (TSLP), thymus and activation-regulated chemokine (TARC), and Oncostatin M (OSM), which are not included in the Bio-Plex Pro Human Cytokine Screening Panel, 48-Plex, were quantitatively analyzed using the following enzyme-linked immunosorbent assay (ELISA) kits, according to the manufacturer's instructions: IL-31 Human ELISA kit (Abcam), TSLP Human ELISA kit (Abcam), Human CCL17/TARC Quantikine ELISA kit (R&D Systems), and Human Oncostatin M/OSM ELISA kit (Abcam). The absorbance was measured at 450 nm with the iMark Microplate Reader (Bio-Rad Laboratories). The protein levels were calculated from standard curves generated by Microplate Manager 6 Software (Bio-Rad Laboratories).

2.4 | Statistical analyses

Statistical analysis was performed using GraphPad Prism 9 software. Since the data were not normally or equally distributed, the Kruskal-Wallis 1-way analysis of variance on ranks was performed to compare the concentrations of each cytokine among healthy control, DEB, and EBS groups followed by Dunn's method to detect significant differences between each group. The p-value $< .05$ was considered statistically significant. The significant difference between healthy controls and EB was detected by Mann-Whitney rank-sum test.

3 | RESULTS

3.1 | The profiles of the EB patients

Sera from the following patients were obtained: 14 DEB (9 RDEB, 1 DDEB, 2 DEB-Pr, and 2 DEB with unknown inheritance) and 6 EBS patients (Table 1). The mean affected BSA (%) ± standard error of the mean of the DEB and EBS patients was 51.0% ± 8.4 and 9.0% ± 6.5, respectively.

3.2 | Luminex bead-based cytokine assays reveal several serum cytokines are significantly increased

Cytokine levels of FGF basic, Eotaxin, G-CSF, GM-CSF, IFN-γ, IL-1β, IL-1ra, IL-1α, sIL-2R, IL-3, IL-12 (p40), IL-16, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, GRO-α, HGF, IFN-α2, LIF, MCP-3, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, IP-10, MCP-1, MIG, β-NGF, SCF, SCGF-β, SDF-1α, MIP-1α, MIP-1β, PDGF-BB, RANTES, TNF-α, VEGF, CTACK, MIF, TRAIL, IL-18, M-CSF, and TNF-β were analyzed using Luminex bead-based cytokine assays in serum samples for 20 EB patients and 16 healthy controls (Table 2). In EB patients, sIL-2R, IL-6, HGF, M-CSF, SCGF-β, IFN-γ, IL-16, MIF, IL-8, and MIP-1α were found significantly increased compared to healthy controls, whereas TNF-β was significantly decreased.

TABLE 1 Profiles of the serum samples

Cases	Gender	Age (years)	EB subtypes	BSA (%)
1	Female	15	RDEB	90
2	Male	13	EBS	40
3	Female	14	EBS	1
4	Male	6	RDEB	15
5	Male	83	RDEB	18
6	Male	35	RDEB	90
7	Male	0	DEB	40
8	Male	11	RDEB	90
9	Male	1	EBS	1
10	Female	20	DDEB	70
11	Male	11	DEB	80
12	Male	24	EBS	0
13	Female	56	RDEB	50
14	Male	22	RDEB	50
15	Female	16	DEB-Pr	10
16	Female	16	RDEB inversa	30
17	Female	10	RDEB	5
18	Male	38	EBS	0
19	Female	9	EBS	10
20	Female	17	DEB-Pr	70

Abbreviations: BSA, body surface area; DDEB, dominant dystrophic epidermolysis bullosa; DEB, dystrophic epidermolysis bullosa; DEB-Pr, dystrophic epidermolysis bullosa pruriginosa; EBS, epidermolysis bullosa simplex; RDEB, recessive dystrophic epidermolysis bullosa.

3.3 | No significant difference in Th2 cytokines in EB patients compared to healthy controls

Type 2 helper T (Th2) lymphocyte differentiation is induced by the stimulation of naïve T cells with cytokines such as IL-4.[17] Cytokines produced by Th2 cells, which are called Th2 cytokines, are known to be involved in the pathogenesis of pruritus and allergic diseases by increasing antibody production from plasma cells and activating cells such as eosinophils.[18] None of the major Th2 cytokines, IL-4, IL-5, or IL-13, were elevated in EB patients including patients with DEB-pr shown as yellow dots (Figure 1A-C). Eotaxin, a potent inducer of eosinophils, was also not elevated.[19]

3.4 | TSLP is significantly elevated and IL-31 and oncostatin M are tended to increase in sera of EB patients

In addition to the 48 cytokines analyzed by Luminex bead-based cytokine assays, serum levels of 4 itch mediators (IL-31, TSLP, TARC, and OSM)[20] were analyzed using ELISA in serum samples for 20 EB patients and 4 healthy controls (Table 3). TSLP was significantly increased in DEB patients compared with healthy controls (Figure 1D), and IL-31 and OSM were not statistically significant but tended to be higher than in healthy controls only in DEB patients, but not in EBS patients (Figure 1E,F). In contrast, there were no differences in TARC (Figure 1G).

3.5 | IL-6, a major proinflammatory cytokine, is elevated in DEB, whereas IL-1β and TNF-α are not

The main proinflammatory cytokines are known to be IL-1β, IL-6, and tumor necrosis factor (TNF)-α.[21] These proinflammatory cytokines are up-regulated during viral infections, such as the influenza virus, and down-regulated when the virus is eliminated.[22] Among these proinflammatory cytokines, serum IL-6 levels were significantly higher in DEB patients than in healthy controls (Figure 1H). On the other hand, there was no significant difference in IL-6 in EBS patients compared to the healthy subject group. IL-1β and TNF-α were not elevated in either DEB or EBS patients compared to healthy controls (Figure 1I,J).

3.6 | Among the cytokines produced by Th1 cells, only IFN-γ is elevated

Type 1 helper T (Th1) lymphocytes are stimulated by interferon-γ (IFN-γ) or IL-12 to differentiate from naïve T cells in the thymus.[17]

TABLE 2 Serum cytokine concentrations in patients with epidermolysis bullosa and healthy controls (Luminex bead-based cytokine assays)

Cytokines	Healthy controls ($n = 16$)[a] Median (interquartile range) (pg/ml)	Epidermolysis bullosa cases ($n = 20$)[a,b] Median (interquartile range) (pg/ml)	p Value[c]
sIL-2R	33.07 (28.84-42.18)	84.58 (42.84-123.15) ↑	.001**
IL-6	0 (0-1.26)	21.08 (0.4-50.78) ↑	.001**
HGF	234.68 (175.91-320.6)	392.8 (277.27-497.39) ↑	.002**
M-CSF	7.39 (5.44-13.94)	15.36 (10.99-20.88) ↑	.002**
TNF-β	536.76 (390.18-610.54)	397.24 (374.77-430.23) ↓	.006**
SCGF-β	121,907.41 (107,854.99-144,291.53)	167,813.48 (138,759.5-204,515.01) ↑	.007**
IFN-γ	2.53 (2.03-3.06)	4.2 (2.47-5.02) ↑	.014*
IL-16	27.94 (21.79-32.31)	55.17 (26.9-92.08) ↑	.015*
MIF	287.43 (155.29-410.28)	531.92 (299.21-1553.54) ↑	.016*
IL-8	3.26 (0.69-8.47)	10.28 (4.46-669.13) ↑	.020*
MIP-1α	1.36 (0.92-3.42)	3.45 (1.59-191.07) ↑	.049*
CTACK	659.81 (477.25-870.52)	517.05 (318.67-729.65)	.062
IL-4	2.71 (2.09-3.15)	1.76 (1.42-4.42)	.072
IL-17A	5.34 (4.49-6.9)	3.72 (2.66-8.12)	.077
RANTES	9593.73 (8818.03-11,756.34)	11,194.92 (9365.67-17,706.65)	.077
MCP-1	27.42 (16.08-45.91)	16.44 (12.11-30.64)	.095
GM-CSF	0 (0-2.12)	1.19 (0-4.38)	.140
IL-1β	0.89 (0.74-1.25)	1.14 (0.77-4.39)	.149
GRO-α	522.38 (466.73-685.27)	596.96 (503.97-809.44)	.189
LIF	11.35 (9.02-15.21)	33.57 (2.01-76.97)	.211
IL-12 (p40)	20.13 (6.55-29.9)	28.21 (5.77-48.48)	.236
β-NGF	0 (0-14.01)	1.97 (0-21.96)	.276
G-CSF	43.72 (17.64-62.27)	51.71 (30.49-1373.63)	.276
IL-9	273.65 (262.33-290.48)	267.29 (253.38-284.21)	.290
IL-15	0 (0-191.75)	0 (0-384)	.290
IL-18	33.49 (20-60)	45.14 (27.2-65.6)	.305
IL-12 (p70)	0 (0-3.98)	0 (0-0)	.320
IL-1Rα	82.73 (53.79-201.87)	286.3 (0-479.41)	.352
IP-10	215.54 (162.7-281.57)	237.72 (169.21-380.84)	.440
SDF-1α	871.1 (776.01-1007.9)	971.05 (778.65-1339.54)	.440
MIP-1β	209.73 (199.01-225.2)	213.21 (193.41-629.02)	.459
IL-1α	4.02 (1.56-8.86)	0.28 (0-27.94)	.479
VEGF	0 (0-71.57)	0 (0-242.81)	.560
MCP-3	0 (0-0.65)	0 (0-1.13)	.582
TNF-α	36.11 (30.8-39.8)	36.06 (30.75-87.26)	.589
PDGF-bb	2338.65 (1302.88-3294.21)	2618.15 (1607.78-4135.28)	.604
IFN-α2	0 (0-2.35)	0 (0-6.45)	.648
TRAIL	46.21 (33.03-55.44)	43.1 (36.94-56.27)	.648
MIG	65.19 (50.12-147.08)	77.97 (63.81-138.35)	.671
SCF	61.55 (48.11-76.23)	62.31 (43.39-74.84)	.694
IL-7	0 (0-6.35)	0 (0-5.74)	.718
FGF basic	31.27 (28.56-33.4)	25.47 (22.84-75.08)	.741
IL-3	0 (0-0)	0 (0-0.17)	.741
IL-5	0 (0-0)	0 (0-0)	.765

(Continues)

TABLE 2 (Continued)

Cytokines	Healthy controls ($n = 16$)[a] Median (interquartile range) (pg/ml)	Epidermolysis bullosa cases ($n = 20$)[a,b] Median (interquartile range) (pg/ml)	p Value[c]
Eotaxin	28.28 (25.64-79.59)	34.72 (28.43-45.75)	.814
IL-10	0.32 (0-1.85)	0.48 (0-1.92)	.863
IL-2	0 (0-2.35)	0 (0-1.72)	.888
IL-13	0.77 (0.52-4.76)	1.09 (0.42-3.9)	.962

Abbreviations: CTACK, cutaneous T cell-attracting chemokine; FGF basic, fibroblast growth factor basic; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GRO-α, growth regulated oncogene-alpha; IL, interleukin; INF-γ, interferon-gamma; IP-10, interferon gamma-induced protein 10; LIF, leukemia inhibitor factors; MCP-1, monocyte chemoattractant protein-1; MCP-3, monocyte chemotactic protein-3; M-CSF, macrophage colony-stimulating factor; MIF, macrophage migration inhibitory factor; MIG, monokine induced by interferon-gamma; MIP-1α, macrophage inflammatory protein-1 alpha; MIP-1β, macrophage inflammatory protein-1 beta; PDGF-bb, platelet-derived growth factor bb; RANTES, regulated upon activation, normal T cell expressed and presumably secreted; SCF, stem cell factor; SCGF-β, stem cell growth factor-beta; SDF-1α, stromal cell-derived factor-1 alpha; sIL-2R, soluble IL-2Receptor; TNF-β, tumor necrosis factor-beta; TRAIL, TNF related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor; β-NGF, beta-nerve growth factor.

[a]Values less than the detection limit are described as 0.

[b]Cytokines that are significantly elevated or decreased compared to healthy controls are indicated by ↑ and ↓, respectively.

[c]Significant differences between two groups are detected by Mann-Whitney U-test. p Value < .05 is considered to be statistically significant.
$^{*}p < .05$, $^{**}p < .01$.

Cytokines produced by Th1 cells are known to be involved in the removal of viral and intracellular antigens, the development of autoimmune diseases and cellular immunity responsible for antitumor immunity by activating immune cells such as macrophages and cytotoxic T cells. Among the cytokines produced by Th1 cells including GM-CSF, IFN-γ, IL-2, and TNF-α, only IFN-γ was elevated in the sera of EB patients compared with healthy controls.[18] Serum levels of IFN-γ were significantly higher in DEB compared to healthy controls, but not significantly different in EBS compared to healthy controls (Figure 1K). IL-12 introducing Th1 cells differentiation was not significantly different between EB patients and healthy controls.

4 | DISCUSSION

It is becoming clear that itch is the result of dysregulation between keratinocytes, immune cells, and sensory nerves, caused by endogenous or exogenous irritants.[10,20,23,24] In addition to histamine, a number of non-histaminergic itch mediators and receptors have recently been identified.[20,25] In EB, the itch is a common symptom with a significant impact on quality of life and is often accompanied by pain and skin infections in the patients.[10,11,26] In a study of the prevalence of itch in 40 adults with either of three EB types (EBS, JEB, or DEB), 85% reported itch, with a prevalence similar to that of atopic dermatitis (AD).[10,11,27] JEB (100%) and RDEB (100%) had the highest prevalence, followed by DDEB (87%) and EBS (74%).[11] In a study of itch using the visual analog scale (VAS) in 13 patients with severe or intermediate RDEB, the mean VAS score was 7.54 ± 2.07, which is considered severe itch.[28] To manage EB successfully, it is important to address pruritus.[29] The mechanisms involved in pruritus in EB are still unclear. It is likely that skin inflammation secondary to barrier disruption, wound healing cascades, and dysregulated activation of epidermal sensory nerve endings are all involved in its pathogenesis at the molecular and cellular level.[10]

In this study, Th2 cytokines such as IL-4, IL-5, and IL-13 were investigated and, in agreement with previous reports, Th2 cytokines in the sera of EB patients were not significantly different from those of healthy individuals (Table 4).[30,31] On the other hand, TSLP in DEB was significantly higher than in healthy controls. TSLP is an epithelial cell-derived immunostimulatory factor that activates immune cells such as dendritic cells, T cells, and mast cells. Recently, epithelial cell-derived TSLP has attracted much attention as cytokines that induce allergic immune responses.[32] TSLP is released by primary epithelial cells in response to certain microbial products, physical injury, or inflammatory cytokines.[33] Although the results of this study did not show a trend toward higher TSLP levels in patients with higher BSA indicated by the red dots in Figure 1D, it may be that TSLP is more likely to be produced by epidermal keratinocytes in patients with EB who often have chronic wounds prone to infection and cutaneous dysbiosis.[34,35] In addition, there was a tendency for IL-31, known as a pruritogen, to be elevated in DEB patients compared to healthy controls. IL-31, a cytokine belonging to the IL-6 family, has been reported to be involved in the development of chronic dermatitis through the induction of severe pruritus in transgenic mice with lymphocyte-specific overexpression of IL-31.[36,37] The expression of the IL-31 gene is upregulated in itchy human skin diseases such as AD, allergic contact dermatitis, psoriasis, and prurigo nodularis.[38] In addition, serum levels of IL-31 are elevated in AD, and IL-31 levels correlate with disease severity, suggesting that IL-31 plays a pivotal role in the induction and maintenance of pruritus and chronic skin inflammation in AD.[37] Schulz et al.[39] have shown that the specific IL-31 gene haplotype associated with the regulation of IL-31 gene expression is more common in non-atopic eczema subjects. On the other hand, the association between IL-31 haplotypes and DEB-Pr has not been demonstrated, and the pathophysiology of IL-31 in EB remains unclear.[40] Recently, nemolizumab, the humanized antihuman IL-31 receptor A (IL-31RA) monoclonal antibody, has been reported to improve pruritus in AD and prurigo nodularis, and IL-31 may also

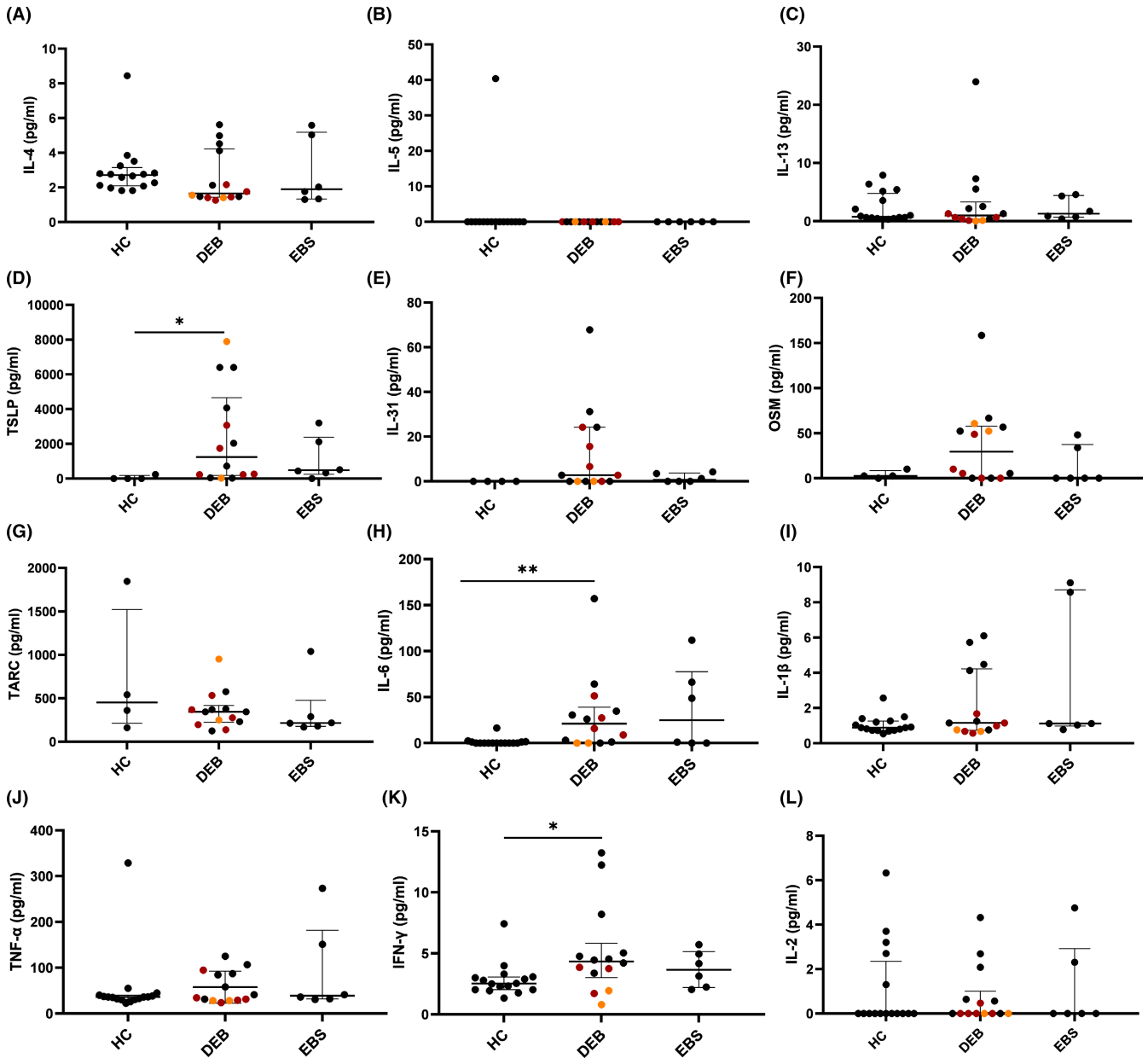

FIGURE 1 Serum levels of IL-4 (A), IL-5 (B), IL-13 (C), TSLP (D), IL-31 (E), OSM (F), TARC (G), IL-6 (H), IL-1β (I), TNF-α (J), IFN-γ (K), and IL-2 (L) in patients with dystrophic epidermolysis bullosa (DEB), epidermolysis bullosa simplex (EBS) compared with those of healthy control subjects (HC). Data are presented as scattergrams with medians and interquartile range. $^{*}p < .05$, and $^{**}p < .01$. Red dots: patients with skin lesions covering more than 70% of body surface area. Yellow dots: patients with DEB pruriginosa

provide a new target for antipruritic and antiinflammatory activity in the treatment for EB.[41–43] OSM was also not statistically significant, but tended to be higher in DEB patients than in healthy controls. OSM, a cytokine belonging to the IL-6 family, which is released from T cells is a potent stimulator of keratinocyte migration and increases the thickness of the epidermis, and OSM transcripts are enhanced in the skin of patients with AD.[44] In contrast, TARC was not increased in EB patients compared to healthy controls. TARC, also known as CC chemokine ligand 17, is one of the CC chemokines that stimulate CC chemokine receptor 4 (CCR4) expressed on Th2 lymphocytes.[45,46] CCR4 mobilizes Th2 lymphocytes to local sites of inflammation, triggering a Th2-type immune response.[47] Serum TARC levels correlate with the severity of AD, as Th2 lymphocytes are increased in patients with AD.[48]

DEB-Pr is notoriously resistant to be the treatment of pruritus.[4,49,50] Recently, six previous reports have documented successful treatment of DEB-Pr with dupilumab, a monoclonal antibody targeting the IL-4 receptor alpha (IL-4Rα) that provides dual blockade of IL-4 and IL-13, which play an important role in the pathogenesis of AD.[4,26,51–53] In addition, Darbord et al.[54] reported mast cell infiltration of the lesional skin, high serum total IgE levels and increased Th2 subsets in circulating T cells in patients with DEB-Pr. These findings suggest that DEB-Pr is driven by the Th2 immune response.[4] In this study, two patients with DEB-Pr, indicated in yellow dots,

TABLE 3 Serum cytokine concentrations in patients with epidermolysis bullosa and healthy controls (enzyme-linked immunosorbent assays)

Cytokines	Healthy controls ($n = 4$)[a] Median (interquartile range) (pg/ml)	Epidermolysis bullosa cases ($n = 20$)[a,b] Median (interquartile range) (pg/ml)	p Value[c]
TSLP	4.18 (0.4-179.17)	620.61 (226.06-3168.11) ↑	.010*
IL-31	0 (0-0)	2.03 (0-13.37)	.097
OSM	2.53 (0.67-8.28)	7.825 (0-52.32)	.682
TARC	451.91 (211.97-1520.94)	283.52 (201.31-376.62)	.388

Abbreviations: IL-31, interleukin 31; OSM, oncostatin M; TARC, thymus and activation regulated chemokine; TSLP, thymic stromal lymphopoietin.

[a]Values less than the detection limit are described as 0.

[b]Cytokine that is significantly elevated compared to healthy controls is indicated by ↑.

[c]Significant differences between two groups are detected by Mann-Whitney *U*-test. p Value < .05 is considered to be statistically significant.

*$p < .05$.

showed no elevation of Th2 cytokines such as IL-4, IL-5, IL-13, and IL-31, while TSLP was markedly elevated in only one patient with DEB-Pr (Figure 1A-E). The role of Th2 cytokines in the pathogenesis of DEB-Pr needs to be further investigated.

We also analyzed serum proinflammatory cytokines, including IL-1β, IL-6, and TNF-α, in patients with EB. In the present study, serum IL-6 levels were significantly elevated in patients with DEB (Figure 1H). IL-6, mainly produced by activated macrophages, lymphocytes, and fibroblasts, is well known to be a proinflammatory cytokine with pleiotropic functions and induce the production of acute-phase proteins, inducing systemic fever and C-reactive protein and immunoglobulin production.[55,56] Although the etiological relevance of IL-6 in EB patients with fever is unknown, there are potentially sufficient prospective data to understand the nature of its action in inflammatory skin diseases, such as generalized pustular psoriasis, palmoplantar pustulosis, and acute urticaria.[55,57–59] Kawakami et al.[55] reported that all the sera from their five unrelated patients with EB had markedly increased IL-6 levels. In addition, Odorisio et al.[60] reported a case of RDEB in which the expression of IL-6 was upregulated in a twin with a more severe phenotype. Consistent with these reports, in two previous studies, serum IL-6 levels were significantly elevated in patients with EB and correlated with the severity of the disease (Table 4).[30,31] These facts suggest that IL-6 can play a pathophysiological role in triggering serological inflammation in patients with EB.

Previous studies revealed that IL-1β level was elevated in sera of EB patients compared with healthy controls (Table 4).[30,31] In contrast, no significant difference in IL1-β was observed among the groups in this study. IL-1β secreted from keratinocytes and the other cells activate the c-Jun N-terminal-kinase (JNK)/mitogen-activated protein kinase (MAPK) stress pathway, resulting in higher migratory potential and hyperproliferative activity in keratinocytes.[61] The JNK/MAPK stress pathway is known to be upregulated in severe EBS keratinocytes,[62] and IL-1β signaling has been found to be constitutively active in keratinocytes from EBS patients.[7] Other investigators also demonstrated the significantly upregulated mRNA level of IL-1β in the *Krt5* knockout mouse skin.[63] In line with these findings, a clinical trial using diacerein, which has been reported to block the release of active IL-1β by inhibiting plasma membrane-bound IL-1 converting enzyme,[64] was conducted; and topical 1% diacerein was shown to be an effective and safe treatment for patients suffering from EBS.[65] In addition, caspase-1 overexpressing transgenic mice, in which keratinocytes highly release IL-1α and IL-1β, showed severe dermatitis as well as systemic complications, such as abnormal weight loss, cardiovascular disease, and extensive amyloid deposition with organ dysfunction, similar to those observed in patients with severe EB subtypes.[8] Taken together, IL-1β may be involved in the skin as well as systemic manifestations in EB. In this study, some EBS patients showed a marked increase in serum IL-1β (Figure 1I), which may indicate that elevation of IL-1β is specific to a subgroup of EBS. In addition, the present study did not show any tendency for severe DEB patients to have particularly high levels of IL-1β, and further research is needed on the role of IL-1β in EB patients.

There was no significant difference in serum TNF-α levels between patients with EB and healthy subjects (Figure 1J), as reported by Esposito et al.[31] On the other hand, Annicchiarico et al.[30] reported that TNF-α in patients with DEB was significantly lower than in healthy controls, and, therefore, the consensus has not yet been reached. Gubinelli et al.[66] reported the improvement of DEB in a patient treated with etanercept, a TNF-α inhibitor, for concomitant psoriatic arthritis. In this case, a remarkable improvement of intractable pruritus and blisters, associated with a reduction of the appearance of new lesions, was progressively observed during the first 3 months of therapy. Etanercept was continued and, after 3 years, persistent good control of DEB with the sporadic occurrence of new lesions was observed. Although to the best of our knowledge, only one patient with EB has been treated with a TNF-α inhibitor, it is hoped that more such cases will be accumulated in the future to provide new insights.

In this study, there was no significant difference in serum IL-2 levels in the EB patient group compared to healthy controls (Figure 1L). Annicchiarico et al.[30] reported a significant increase in serum IL-2 in DEB patients compared with EBS; in contrast, Esposito et al.[31] reported increased IL-2 serum concentration in EB patients, regardless of disease severity (Table 4). IL-2 is the most potent of the mitogenic cytokines and induces the proliferation of CD4+ T cell

TABLE 4 Summary of previous studies analyzing cytokines in patients with EB

	G. Annicchiarico (2015)	S. Esposito (2016)	Our study
IL-1β	DEB > HC EBS > HC DEB-EBS: no difference	EB > HC RDEB > HC RDEB-other EB types: no difference	EB-HC: no difference
IL-2	DEB-HC, EBS-HC: no differences DEB > EBS	EB > HC RDEB > HC RDEB-other EB types: no difference	EB-HC: no difference
IL-4	DEB-HC, EBS-HC, DEB-EBS: no differences	EB-HC: no difference	EB-HC: no difference
IL-6	DEB > HC DEB > EBS EBS-HC: no difference	EB > HC RDEB > HC RDEB-other EB types: no difference	EB > HC DEB > HC DEB-EBS: no difference
IL-8	DEB-HC, EBS-HC, DEB-EBS: no differences	EB-HC: no difference	EB > HC DEB-HC, EBS-HC, DEB-EBS: no differences
IL-10	DEB-HC, EBS-HC, DEB-EBS: no differences	EB > HC RDEB-other EB types, RDEB-HC, other EB types-HC: no differences	EB-HC: no difference
IL-12	DEB-HC, EBS-HC, DEB-EBS: no differences	EB > HC RDEB-other EB types, RDEB-HC, other EB types-HC: no differences	EB-HC: no difference
TNF-α	DEB < HC	EB-HC: no difference	EB-HC: no difference
TNF-β	DEB > HC	EB > HC RDEB > HC	EB < HC DEB-HC, EBS-HC, DEB-EBS: no differences
IFN-γ	DEB > HC	EB > HC RDEB-other EB types, RDEB-HC, other EB types-HC: no differences	EB > HC DEB > HC

Note: Statistically significant differences ($p < .05$) are indicated by > or <.

Abbreviations: DEB, dystrophic epidermolysis bullosa; EB, epidermolysis bullosa; EBS, epidermolysis bullosa simplex; HC, healthy controls; RDEB, recessive dystrophic epidermolysis bullosa.

subsets, CD8+ T cell subsets, B cells, and natural killer cells[67] and is essential for the development, clonal expansion, and survival of regulatory T cells.[68] It is also implicated in the symptom of itch, as some EB patients with severe pruritus responded to topical tacrolimus, which inhibits IL-2 production.[69] Further data are needed to clarify the pathogenic role of IL-2 in EB patients.

Epidermolysis bullosa often causes systemic complications, such as abnormal weight loss, cardiovascular disease, and extensive amyloid deposits with organ dysfunction. In addition, it is often associated with intense, intractable pruritus, which has been suggested to involve systemic inflammation. In the present study, as in previous reports, several cytokine imbalances were found. In recent years, cytokine-targeted biologics have been widely used in various dermatological diseases such as AD, psoriasis, and prurigo nodularis with high therapeutic efficacy. Further investigation is needed to determine whether cytokines such as TSLP, IL-31, and IL-6, which were found to be elevated in EB patients in this study, could be targets for biologic therapy for EB patients. The accumulation of these findings is expected to open up new avenues for the treatment of EB which often leads to life-threatening complications and reduced quality of life due to intense pruritus.

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DECLARATION SECTION

Approval of the research protocol: This study was approved by the human research ethics committees of Niigata University Medical and Dental Hospital (approval number: 2021-0285), Hokkaido University Hospital (approval number: 011-0089, 13-043), and Nara Medical University (approval number: 2790).

Informed Consent: All patients were provided written informed consent and ethics committees approved the protocol.

Registry and the Registration No. of the study/trial: N/A.

Animal Studies: N/A.

CONFLICT OF INTEREST

The authors declare no conflict of interest. Dr. Riichiro Abe is a member of the Journal of Cutaneous Immunology and Allergy Editorial Board. Management of the peer review process, and all editorial decision-making, for this article was undertaken by Editor in Chief.

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