Analysis of IL-10 and IL-35 in DPP-4 inhibitor-related bullous pemphigoid

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Short Report

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Abstract

The association between immunoregulatory cytokines, such as IL-10 or IL-35, and DPP-4 inhibitor-related bullous pemphigoid (BP) has not been evaluated. Sera were collected from 39 Japanese patients with BP (24 males and 15 females; 77.0 ± 11.1 years old) including 6 patients with non-DPP-4 inhibitor-related BP before treatment in our hospital. Ten healthy Japanese individuals (4 males and 6 females; 42.2 ± 9.37 years old) were enrolled as healthy controls. No significant difference was observed in serum IL-10 levels (BP patients: 7.63 ± 5.03 pg/ml; healthy individuals: 6.88 ± 0.52 pg/ml; DPP-4 inhibitor-related BP: 6.77 ± 0.24 pg/ml; non-DPP-4 inhibitor-related BP: 6.84 ± 0.20 pg/ml; BP vs healthy: $P = 0.368$; DPP-4 inhibitor-related BP vs non-DPP-4 inhibitor-related BP: $P = 0.553$), nor in serum IL-35 levels (BP patients: 2.62 ± 0.20 pg/ml; healthy individuals: 2.60 ± 0.17 pg/ml; DPP-4 inhibitor-related BP: 2.63 ± 0.17 pg/ml; non-DPP-4 inhibitor-related BP: 2.63 ± 0.21 pg/ml; BP vs healthy: $P = 0.727$; DPP-4 inhibitor-related BP vs non-DPP-4 inhibitor-related BP: $P = 0.949$). Bullous Pemphigoid Disease Area Index (BPDAI) before treatment was not related with serum IL-10 levels (r = 0.159; Fig. 1A), nor with serum IL-35 levels (r = 0.227; Fig. 1B). The number of serum eosinophils was significantly higher in patients with non-DPP-4 inhibitor-related BP (911.3 ± 948.8) than in patients with DPP-4 inhibitor-related BP (476.1 ± 234.0; $P = 0.038$). DPP-4 is also known as a CD26 molecule expressed on the surface of T lymphocytes. The mean rate of infiltrating $CD26^+$ cells was significantly increased in 6 patients with DPP-4 inhibitor-related BP (32.9 ± 7.1) than in 6 patients with non-DPP-4 inhibitor-related BP (15.7 ± 4.4; $P = 0.002$; Fig. 2). It was reported that the co-engagement of CD3 and CD26 induces the preferential production of IL-10 from human CD4 + T cells, which might reflect the clinical characteristics of faint inflammatory bulla in DPP-4 inhibitor-related BP.

Introduction

Bullous Pemphigoid (BP) is characterized histologically by subepidermal blister formation induced by the dermal-epidermal junction (DEJ) component. Interleukin (IL)-10 and IL-35 are thought to play an important role in immunoregulatory and autoimmune disease processes [1–3]. Although IL-35 may act as an efficient therapeutic cytokine for various autoimmune diseases [4], the association has not been investigated between IL-35 and BP. Recently, the association has been well discussed between dipeptidyl peptidase-4 (DPP-4) inhibitor and BP [5]. Although DPP-4 is known as a CD26 molecule expressed on the surface of T lymphocytes and other cell types [6], the association has not been evaluated between immunoregulatory cytokines, such as IL-10 or IL-35. This study conducted a comprehensive evaluation of serum IL-10 and IL-35 levels in patients with BP including DPP-4 inhibitor-related BP. This study also evaluated the association between $CD26^+$ cells in the dermis around bulla and DPP-4 inhibitor-related BP using immunohistochemical staining.

Methods

Sera were collected from 39 Japanese patients with BP (24 males and 15 females; 77.0 ± 11.1 years old) including 6 patients with non-DPP-4 inhibitor-related BP before treatment in our hospital. Ten healthy Japanese individuals (4 males and 6 females; 42.2 ± 9.37 years old) were enrolled as healthy controls. BP

was diagnosed by clinical and histopathological features and detection of anti-DEJ antibodies using immunofluorescence staining and/or anti-BP180NC16a antibody. The disease severity of BP was evaluated using the Bullous Pemphigoid Disease Area Index (BPDAI) defined by the International Pemphigoid Committee using skin score [7]. Serum anti-BP180NC16a antibodies were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (MBL, Nagoya, Japan).

Serum IL-10 and IL-35 levels were measured by ELISA according to the manufacturer's protocol [IL-10 (Bender MedSystems, Vienna, Austria) and IL-35 (Cloud-Clone, Wuhan, China)]. The number of $CD26^+$ cells in the dermis around bulla in 6 patients with DPP-4 inhibitor-related BP and 6 patients with non-DPP-4 inhibitor-related BP was counted on sections immunohistochemically stained with monoclonal anti-CD26 antibody (ab215711; Abcam plc, Cambridge, UK) in three random grids per section at ·200 magnification. The mean frequency of infiltrating $CD26^+$ cells ($CD26^+$ mononuclear cells/all mononuclear cells) was decided based on these three values.

Statistical analysis was performed using Student's *t*-test to compare patients with BP to healthy controls. *P* values < 0.05 were considered statistically significant. The Pearson product-moment correlation coefficient was used to examine the relationship between two continuous variables. Data were shown as the mean ± standard deviation. The experimental protocol was established according to the Declaration of Helsinki and approved by the Ethics Committee of Shiga University of Medical Science (reference no. R2017-227).

Results

Serum IL-10 and IL-35 levels were measured in 39 patients with BP and 10 healthy controls. No significant difference in serum IL-10 levels in patients with BP (7.63 ± 5.03 pg/ml) compared to healthy individuals (6.88 ± 0.52 pg/ml; $P = 0.368$) was observed. Serum IL-35 levels in patients with BP (2.62 ± 0.20 pg/ml) also showed no significant difference compared to healthy controls (2.60 ± 0.17 pg/ml; $P = 0.727$).

We further assessed whether the severity of BP was correlated with serum IL-10 or IL-35 levels. The use of BPDAI to assess the disease severity of BP is widely accepted. No significant relationship was observed between serum IL-10 levels and BPDAI before treatment (r = 0.159; Fig. 1A). Although serum IL-35 levels decreased in BP patients, no significant relationship was also detected between serum IL-35 levels and BPDAI before treatment (r = 0.227; Fig. 1B).

We compared the clinical features, such as anti-BP180NC16a antibody titer, the number of serum eosinophils, and BPDAI, between 6 patients with DPP-4 inhibitor-related BP and 33 patients with non-DPP-4 inhibitor-related BP (Table 1). Regarding the types of the DPP-4 inhibitor, four were taking sitagliptin and two were taking vidagliptin among 6 patients with DPP-4 inhibitor-related BP. Although anti-BP180NC16a antibody titers were lower in patients with DPP-4 inhibitor-related BP, no significant difference was

observed. The number of serum eosinophils was significantly higher in patients with non-DPP-4 inhibitor-related BP (911.3 ± 948.8) than in patients with DPP-4 inhibitor-related BP (476.1 ± 234.0; $P = 0.038$).

Table 1
Clinical and laboratory differences between DPP-4 inhibitor- and non-DPP-4 inhibitor-related BP

	DPP-4 inhibitor-related BP (n = 6)	Non-DPP-4 inhibitor-related BP (n = 33)	*P*-value
Anti-BP180NC16a antibody titer (U/mL)	67.31 ± 37.40	76.18 ± 54.59	0.706
No. eosinophils (/mm^3)	476.1 ± 234.0	911.3 ± 948.8	0.038
BPDAI (erosions/blisters)	14.50 ± 10.98	16.66 ± 13.71	0.717
BPDAI (urticaria/erythema/other)	15.00 ± 9.75	17.54 ± 10.52	0.585
BPDAI (mucosa)	0	0.63 ± 2.24	0.113
BP: Bullous Pemphigoid; BPDAI: Bullous Pemphigoid Disease Area Index			

Serum IL-10 and IL-35 levels were compared with regard to DPP-4. Although serum IL-10 levels were lower in patients with DPP-4 inhibitor-related BP (6.77 ± 0.24 pg/ml) than in patients with non-DPP-4 inhibitor-related BP (6.84 ± 0.20 pg/ml; $P = 0.553$), no significant difference was observed. Serum IL-35 levels in patients with DPP-4 inhibitor-related BP (2.63 ± 0.17 pg/ml) also showed no significant difference compared to patients with non-DPP-4 inhibitor-related BP (2.63 ± 0.21 pg/ml; $P = 0.949$).

To investigate the association between DPP-4 inhibitor and BP pathologically, we counted the number of $CD26^+$ cells in the dermis around bulla on sections immunohistochemically. The mean rate of infiltrating $CD26^+$ cells was significantly increased in 6 patients with DPP-4 inhibitor-related BP (32.9 ± 7.1) than in 6 patients with non-DPP-4 inhibitor-related BP (15.7 ± 4.4; $P = 0.002$; Fig. 2).

Discussion

The production of pathogenic autoantibodies is key to the development of autoimmune bullous disease. Many immunological steps, including impaired immunotolerance, are needed for autoantibody production. Immunotolerance to self-antigens is essential to protect the host against chronic inflammatory diseases and tissue damage. We previously reported that B10 cells were associated with long-term remission after intravenous immunoglobulin treatment for pemphigus [8]. We also reported that peripheral blood levels of B10 cells in patients with pemphigus but not patients with BP decreased compared to healthy individuals [9]. Because serum IL-10 levels were not evaluated in these two studies, this study measured serum IL-10 levels. As for serum IL-10 levels in patients with BP, no significant increase was observed in a recent study [10], but there was a reported increase [11]. In this study, we found no significant difference in serum IL-10 levels in patients with BP compared to healthy individuals.

Moreover, Fig. 1A shows no significant relationship between serum IL-10 levels and BPDAI. These observations indicated that serum IL-10 levels are not usually increased in patients with BP and corresponded to a previous observation that B10 cells in peripheral blood are decreased in pemphigus but not in BP [9].

IL-35 is another immunoregulatory cytokine. Although serum IL-35 levels have been evaluated in various autoimmune diseases, such as rheumatoid arthritis and systemic sclerosis [4], it has not been investigated in BP. No significant difference in serum IL-35 levels was observed between patients with BP and healthy individuals. Figure 1B shows no significant relationship between serum IL-35 levels and BPDAI. Although further investigations are needed regarding IL-35, this immunoregulatory cytokine may not be a candidate of a therapeutic target for BP, unlike other autoimmune diseases.

Because the role of CD26 in DPP-4 inhibitor-related BP has not been evaluated, this study evaluated infiltrating $CD26^+$ cells on bullous lesions. Figure 2 showed that the mean frequency of infiltrating $CD26^+$ cells on bullous lesions significantly increased in DPP-4 inhibitor-related BP than non-DPP-4 inhibitor-related BP. It is speculated that this increase in $CD26^+$ cells might be associated with the clinical characteristic of a non-inflammatory phenotype with less erythema in DPP-4 inhibitor-related BP than that seen in patients with non-DPP-4 inhibitor-related BP [12], because the co-engagement of CD3 and CD26 induces the preferential production of IL-10 in human $CD4^+$ T cells [6].

Declarations

Financial support: None

Conflict of interest: None

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Figures

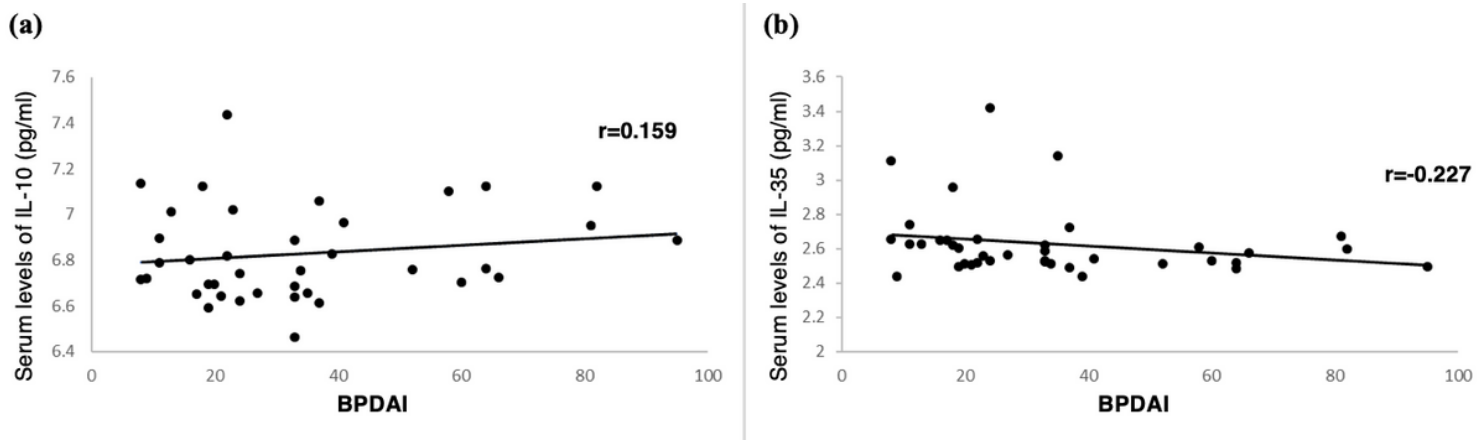

Figure 1

(A and B) Relationship of IL-10 or IL-35 with BPDAI. No significant relationship was observed between serum IL-10 levels and BPDAI (A). Although serum IL-35 levels decreased in BP patients, no significant relationship was detected between serum IL-35 levels and BPDAI (B).

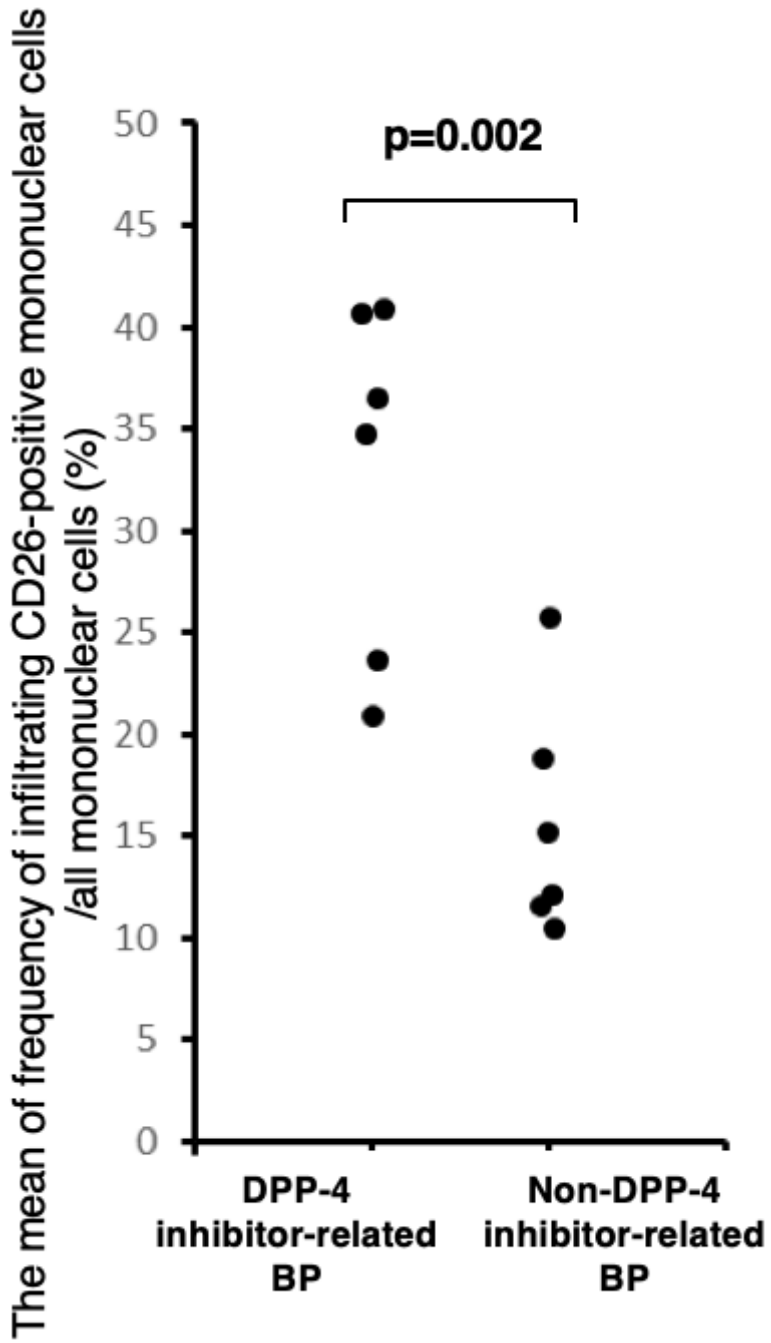

Figure 2

Mean frequency of infiltrating $CD26^+$ cells ($CD26^+$ mononuclear cells/all mononuclear cells) in 6 patients with DPP-4 inhibitor-related BP and 6 patients with non-DPP-4 inhibitor-related BP. $CD26^+$ cells were significantly increased in patients with DPP-4 inhibitor-related BP than non-DPP-4 inhibitor-related BP.