Altered Expression of Cytokines in the Skin Bullous Pemphigoid Is Characterized by an Dipeptidyl Peptidase 4 Inhibitor–Associated Bullous Pemphigoid Is Characterized by an Altered Expression of Cytokines in the Skin

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Dipeptidyl peptidase 4 inhibitors (DPP4is), commonly used drugs for treatment of type 2 diabetes, increase the risk for bullous pemphigoid (BP). Currently, the mechanism leading to the loss of immunological tolerance of the cutaneous adhesion molecule BP180 as well as similarities and differences in disease progression between DPP4i-associated BP (DPP4i-BP) and DPP4i-independent regular BP are largely unknown. We analyzed the expression of 32 cytokines and two proteases by Luminex and ELISA assays in samples taken from lesional and nonlesional skin of patients with regular BP or DPP4i-BP and healthy controls. Cytokines mediating B-cell survival and targeting such as BAFF, CCL4, CXCL12, and IL-6 were expressed at a higher level in the lesional regular BP skin than the levels in the lesional DPP4i-BP skin. The DPP4i-BP samples had increased levels of eosiophilic cytokines CCL1, CCL17, CCL26, and IL-5, which correlated with the serum level of anti-BP180 NC16A IgG autoantibodies. The mRNA expression of BAFF, IL6, CCL1, CCL17, CCL26, and IL5 measured by qPCR correlated with the protein levels. Taken together, the cutaneous cytokine profiles were found to provide distinctive molecular fingerprints between regular BP and DPP4i-BP.

INTRODUCTION

Bullous pemphigoid (BP) is the most common autoimmune blistering skin disease (Amber et al., 2018; Hammers and Stanley, 2020; Schmidt and Zillikens, 2013). BP autoantibodies target the cutaneous adhesion molecule BP180 (also known as collagen XVII) (Nishie, 2014; Schmidt and Zillikens, 2013; Tuusa et al., 2021). The main epitope is the juxtamembranous extracellular noncollagenous (NC16A) domain of BP180, and the level of anti-BP180 NC16A IgG antibodies correlates well with the severity of BP (Nishie, 2014; Schmidt and Zillikens, 2013). Disruption of the dermal–epidermal junction in BP requires complement activation as well as inflammatory cytokine–mediated recruitment of monocytes, granulocytes, and lymphocytes (Hiroyasu et al., 2019; Margaroli et al., 2020; Nishie, 2014). In addition, matrix metalloproteases and neutrophil-derived granzymes are believed to contribute to blister formation (Hiroyasu et al., 2019; Russo et al., 2018; Schmidt and Zillikens, 2013).

The loss of immunological tolerance of BP180 is associated with aging, neurological comorbidities, and medication (Bağci et al., 2017; Bastuji-Garin et al., 2011; Försti et al., 2017; Moro et al., 2020). The most prominent risk factor is the previous use of dipeptidyl peptidase 4 (DPP4) inhibitors (gliptins) in the treatment of type 2 diabetes (Dourois et al., 2019; Krิดin and Bergman, 2018; Nishie and Tasanen, 2019; Tasanen et al., 2019; Wu et al., 2021). Currently, it is unclear whether patients with DPP4 inhibitor (DPP4i)-associated BP (DPP4i-BP) have specific immunological or phenotypical properties that are distinct from those of patients with regular BP (rBP) (Nishie and Tasanen, 2019; Tasanen et al., 2019).

DPP4 is a membrane-bound protease expressed in many tissues and cells, including the skin and T lymphocytes (Fox et al., 1984; Gabrilovac et al., 2017; Mah et al., 2017; Ohnuma et al., 2008; Patel et al., 2021; Willheim et al., 1997). It functions by catalyzing the hydrolysis of N-terminal dipeptides with either proline or alanine at the penultimate position (Lone et al., 2010). Potential DPP4 substrates include several chemokines involved in leukocyte migration and lymphocyte activation (Elmansi et al., 2019; Forssmann et al., 2008; Lambeir et al., 2001; Metzemaekers et al., 2017; Mulvihill and Drucker, 2014; Proost et al., 1998). They might be involved in triggering the immune system to act against BP180 or in downstream signaling in different manners in rBP and DPP4i-BP. It is conceivable that the pathological mechanism of DPP4i-BP may include specific features arising from altered cytokine expression and turnover. This is further supported by the known role of DPP4 as a lymphocyte surface antigen (CD26) and T-cell activator.
(Dang et al., 1990; Hatano et al., 2013; Ishii et al., 2001), which may regulate the binding affinity and specificity of ligands to the receptor, a phenomenon well-characterized for the CXCL12–CXCR4 axis, where CXCR4 receptor–associated DPP4 inactivates the bound CXCL12 ligand (Janssens et al., 2017; Ohnuma et al., 2008).

In this study, we compared the expression of both DPP4 substrate and nonsubstrate cytokines at the protein and mRNA levels between nonlesional and lesional skin biopsies taken from patients with rBP or DPP4i-BP and healthy controls.

**RESULTS**

**Cutaneous expression of several cytokines is increased both in rBP and in DPP4i-BP**

Skin extracts for Luminex analysis were prepared from lesional samples taken from patients with rBP (n = 18) and DPP4i-BP (n = 17) and from skin samples of healthy control subjects (n = 12) (Supplementary Tables S1 and S2). In addition, the 96-well multiplex format allowed us to include nonlesional skin samples from patients with rBP (n = 4) and DPP4i-BP (n = 4). Before initiating a large-scale analysis of clinical samples with restricted availability, the suitability of the Luminex method for measurement of cytokines from skin extracts was confirmed using a four-plex format with IL-6, CXCL16, matrix metalloprotease 1, and IL-1β (Supplementary Table S3).

Ten analytes (CCL1, CCL2, CCL17, CCL26, granzyme B, IL-6, IL-8, matrix metalloprotease 1, TNF-α, and TSLP) were significantly upregulated in the lesional skin of both BP groups compared with that in healthy controls (Supplementary Table S5). The levels of CCL17, CCL26, and TSLP were also increased in the nonlesional DPP4i-BP skin. The levels of two analytes, IL-2 and IL-33, were slightly but significantly decreased in rBP and DPP4i-BP skin compared with that in healthy control skin (Supplementary Table S5).

**BAFF, CCL4, CXCL12, and IL-6 are expressed in rBP skin at higher levels than in DPP4i-BP skin and/or healthy control skin**

BAFF and CCL4 were expressed at 1.8-fold (adjusted P = 0.007) and 3.1-fold (adjusted P = 0.012) greater levels in the lesional skin of rBP than in the skin of healthy controls, whereas no statistically significant increase was detected in the lesional DPP4i-BP skin (Figure 1a, b, e, and f and Supplementary Table S5). IL-6 was very strongly upregulated (322-fold) in the lesional rBP skin compared with that in the healthy controls. This was much more than in the DPP4i-BP skin where IL-6 was still markedly elevated (72-fold), although the difference between rBP and DPP4i-BP was not statistically significant. Interestingly, IL-6 was also elevated in the nonlesional skin of both patients with rBP and DPP4i-BP (7.6- and 45-fold) compared with that in the skin of the healthy control group (Figure 1c and d and Supplementary Figure S1 and Supplementary Table S5).

BAFF and IL-6 are known as lymphocyte survival factors in germinal centers of lymph nodes and skin-associated lymphoid tissue (Fetter et al., 2020). Therefore, we investigated whether another lymphocyte chemokine, CXCL12 (or SDF-1α), a well-characterized DPP4 substrate playing a crucial role in lymphocyte attraction and maintenance in germinal centers, also shows differential expression between rBP and DPP4i-BP skin. We found that CXCL12 was significantly downregulated in the lesional skin of DPP4i-BP but not in that of rBP compared with that in healthy control skin (~2.0-fold) (Figure 2).

**The expression of IL-5, CCL1, CCL17, and CCL26 is increased in both nonlesional and lesional DPP4i-BP skin**

In lesional DPP4i-BP skin, IL-5 was significantly increased compared with that in the healthy control skin (6.8-fold but not in rBP skin (Figure 1m and n and Supplementary Table S5). Although the average concentrations of CCL1, CCL17, and CCL26 were elevated (7–42-fold) in both BP groups compared with that in the healthy control group, the samples with very high values were mostly in the DPP4i-BP group (Figure 1g, i, and k and Supplementary Figure S1 and Supplementary Table S5). IL-5, CCL1, CCL17, and CCL26 were increased (2.7–7.2-fold) in the nonlesional skin of DPP4i-BP compared with that of the healthy controls (Figure 1h, j, and l and Supplementary Table S5). These cytokines have been implicated in eosinophil chemotraction, but it is of note that the well-characterized eosinophil chemokine eotaxin (CCL11) was unchanged (Supplementary Table S5). When cytokine levels were analyzed in relation to disease severity and duration, highly increased levels were found exclusively in patients with DPP4i-BP with severe symptoms, whereas in rBP, the few high values were distributed evenly among patients with mild, moderate, or severe symptoms (Supplementary Figures S2 and S3). In BP blister fluid, the amount of CCL5 (RANTES) chemokine has been shown to correlate with IL-5 and eosinophil cationic protein (D’Auria et al., 1998). When we analyzed the expression of CCL5 in our skin samples by ELISA, its amount remained unchanged (Supplementary Table S5).

**The mRNA expression of BAFF, IL6, CCL1, CCL17, CCL26, and IL5 correlate with their protein expression**

Observed differences in cytokine concentrations between rBP or DPP4i-BP suggest that at least in most cases, they were not caused by a straightforward inhibition of DPP4-mediated cytokine turnover. Therefore, we examined the mRNA levels of BAFF, CCL4, CXCL12, IL-6, CCL1, CCL17, CCL26, and IL-5, which may regulate the binding affinity and specificity of ligands to the receptor, a phenomenon well-characterized for the CXCL12–CXCR4 axis, where CXCR4 receptor–associated DPP4 inactivates the bound CXCL12 ligand (Janssens et al., 2017; Ohnuma et al., 2008).
Figure 1. Cytokine expression in nonlesional and lesional skin differentiates regular BP and DPP4i-BP. A total of 32 cytokines and other proteins were measured from skin extracts by Luminex assay and normalized to total protein (Supplementary Table S4). Healthy control skin samples (n = 12) were compared with (a, c, e, g, i, k, m) nonlesional skin samples (n = 4) and (b, d, f, h, j, l, n) lesional skin samples (rBP, n = 18; DPP4i-BP, n = 17). Boxplots are shown for (a, b), BAFF (c, d), IL-6 (e, f), CCL4 (g, h), CCL1 (i, j), CCL17 (k, l), CCL-26, and (m, n) IL-5 whose expressions were different between rBP and/or DPP4i-BP and/or healthy controls. Statistical testing was performed with the Kruskal–Wallis test, and pair-wise posthoc test comparisons were made using the Dunn–Bonferroni test. Statistically significant differences are marked by asterisks: *P < 0.05, **P < 0.01, ***P < 0.001. BP, bullous pemphigoid; Ctrl, control; DPP4i, dipeptidyl peptidase 4 inhibitor; DPP4i-BP, dipeptidyl peptidase 4 inhibitor–associated bullous pemphigoid; rBP, regular bullous pemphigoid.
(i.e., those cytokines that had notable differences in their expression in either BP group) by qPCR using total RNA isolated from the remaining half of the skin biopsy of patients with rBP ($n = 6$, nonlesional and lesional) and DPP4i-BP ($n = 6$, nonlesional and lesional) and healthy controls ($n = 6$).

The expression of IL6 was 15- and 51-fold upregulated in the lesional skin of patients with rBP and DPP4i-BP, respectively, compared with that in the skin of the healthy controls. CCL1 was 34-fold upregulated in the lesional skin of patients with DPP4i-BP, and CCL26 was 9- and 13-fold upregulated in nonlesional and lesional skin of patients with DPP4i-BP, respectively, compared with that in the healthy controls. CCL17 and IL5 had a trend to be upregulated in the skin of patients with DPP4i-BP, albeit without statistical significance (Supplementary Table S6 and Supplementary Figure S4). The correlation analysis indicated that protein and mRNA expression of BAFF, IL6, CCL1, CCL17, CCL26, and IL5 had a positive correlation (Figure 3).

Levels of serum anti-BP180 NC16A autoantibodies correlate positively with the expression of CCL1, CCL17, and CCL26 in DPP4i-BP skin

When the association between anti-BP180 NC16A IgG autoantibody levels in serum samples and the cytokine concentrations in lesional skin was examined in each patient, we found that the autoantibody values of patients with DPP4i-BP correlated positively with the amounts of CCL1, CCL17, and CCL26, which are increased in lesional DPP4i-BP skin (Supplementary Figure S5b, e, and h). IL-5 behaved similarly but without statistical significance (Supplementary
FIGURE S5c). In the lesional rBP skin, a positive correlation was found between the anti-BP180 NC16A autoantibody levels and TNF-α and IL-21 (Supplementary Figure S5d and g) and with CCL1, TSLP, and granzyme B (in both BP groups) (Supplementary Figure S5a, b, j, k, m, and n). The relationship between the cytokine concentrations and NC16A autoantibodies was further analyzed by grouping patients with BP according to positive (>9 U/ml, n = 30) and negative (<9 U/ml, n = 5) anti-BP180-NC16A ELISA values instead of DPP4i use (Supplementary Figure S6 and Supplementary Table S9). The concentration of CCL1, CCL2, CCL4, granzyme B, IL-6, IL-21, TSLP, and TNF-α were increased in patients with positive BP180-NC16A ELISA (Supplementary Figure S6 and Supplementary Table S9).

DISCUSSION

The use of DPP4is is a major risk factor for the development of BP, but the molecular signatures and pathomechanisms that cause the association between DPP4is and BP are currently poorly understood (Nishie and Tasanen, 2019). In this study, we have analyzed the expression of cytokines in skin samples obtained from patients with BP to address the similarities and differences between rBP and DPP4i-BP, which have not previously been investigated systematically. In addition, to the best of our knowledge, a large number of relevant cytokines have not been previously measured in skin extracts from patients with BP. Preceding studies have used samples of sera, peripheral inflammatory cells, or blister fluid (Kowalski et al., 2019) (Supplementary Table S7). Our Luminex and ELISA measurements revealed notable differences in the cutaneous cytokine spectrum, which predict that the signals between skin-resident and -homing cells such as B lymphocytes and eosinophils are at least partially different between rBP and DPP4i-BP.

We found that BAFF was upregulated at the protein level in lesional rBP skin but not in that of DPP4i-BP. This is in line with observations that BAFF is upregulated in the sera of patients with BP (Asashima et al., 2006) and in circulating naive and memory B lymphocytes in BP (Qian et al., 2014). BP180-specific autoantibodies have been shown to induce IL-6 and IL-8 in cultured keratinocytes (Schmidt et al., 2000). We found that these cytokines were strongly upregulated both in rBP and in DPP4i-BP. The very high levels of IL-6 in lesional rBP skin were more than four-fold higher than in DPP4i-BP skin, although this difference was not statistically significant. This difference in IL-6 levels is in line with a previous study that showed that sitagliptin downregulates the expression of IL-6 in PBMCs and inhibits lymphocyte proliferation and T helper 1/T helper 17 differentiation (Pinheiro et al., 2017). Similar to BAFF and IL-6, the expression of CXCL12 was also lower in DPP4i-BP than in rBP. An interesting topic for future investigation is to clarify whether in rBP, the induced BAFF and IL-6 together with CXCL12 act as survival and homing factors for follicular helper T cells as well as B lymphocytes in skin-associated lymphoid tissue driving local autoantibody production (Bombardieri et al., 2011; Eto et al., 2011; Fetter et al., 2020). These structures have been described in Schnitzler syndrome, secondary syphilis, and lupus erythematosus profundus (Kamido et al., 2021; Kogame et al., 2021). Our preliminary histopathological analysis did not show typical skin-associated lymphoid tissue structures or germinal centers in BP skin but instead found increased infiltration of lymphocytes typical for dermatitis (Lindgren et al., unpublished data). Interestingly, IL-21, which has previously been shown to be upregulated in BP sera (Li et al., 2013), was also elevated in rBP skin but in a nonsignificant manner (Supplementary Table S5). CCL4, which was also upregulated in rBP but not in DPP4i-BP, has previously been implicated in atopic dermatitis (Kaburagi et al., 2001) and is produced by activated B cells in germinal centers (Krzysiek et al., 1999). Of these cytokines, which are expressed at higher levels in rBP skin than in DPP4i-BP skin, CXCL12 and CCL4 but not BAFF or IL-6 are DPP4 substrates (Guan et al., 2004). This challenges the assumption that inhibition of DPP4 would increase the substrate levels.

In contrast to rBP skin, the eosinophilic cytokines IL-5, CCL1, CCL7, and CCL26 (Bishop and Lloyd, 2003; Furue et al., 2019; Kouro and Takatsu, 2009; de Lavareille et al., 2002; Miyagaki et al., 2009) were strongly upregulated in the DPP4i-BP skin, and their concentrations correlated with the level of anti-BP180 NC16A IgG autoantibodies in sera. CCL1 and CCL26 were also increased at the mRNA level in the lesional BP skin. CCL1 was increased at the mRNA level only in DPP4i-BP, and CCL26 was also increased at the mRNA level in the nonlesional skin of DPP4i-BP. All the four showed a correlation between the protein and mRNA levels. In contrast, the level of CCL11 (eotaxin) was not increased at the protein level, although its DPP4i-induced upregulation is known to enhance eosinophil infiltration in a rodent model (Forssmann et al., 2008). The induction of eosinophilic cytokines, especially in DPP4i-BP, was unexpected because low numbers of lesional-infiltrated eosinophils in the skin have been described in Japanese, Israeli, Spanish, and Hungarian DPP4i-BP studies (Chijiwa et al., 2018; Izumi et al., 2016; Kinyo et al., 2021; Kridin and Bergman, 2018; Nieto-Benito et al., 2021). No significant differences in the number of infiltrated eosinophils have been reported in Greek, Croatian, French, and Finnish studies of patients with DPP4i-BP (Bukvić-Mokos et al., 2020; Lindgren et al., 2019; Patsatsi et al., 2018; Plaquevent et al., 2019; Ständer et al., 2021). In this study, the number and proportion of eosinophils in blood samples were slightly lower in DPP4i-BP than in rBP, but the difference was not statistically significant (Supplementary Table S2). IL-5 and CCL11 but not CCL1, CCL17, or CCL26 are DPP4 substrates. Even though eosinophil attraction is a shared property of these cytokines, it should be clarified why eosinophil infiltrations are not increased in DPP4i-BP skin. Further research is needed to determine whether there is a causal relationship between DPP4i use and cytokine levels and to identify the cellular origin of increased cytokines. Keratinocytes may not be the source of DPP4i-induced upregulation because their expression of IL-5 and CCL11 was not increased in DPP4i-BP skin.

Similar to IL-5 and CCL11, CXCL12 is also a highly eosinophilic chemokine (Nagase et al., 2000), whose N-terminal proteolysis is inhibited by DPP4is. The significant decrease of CXCL12 in the DPP4i-BP skin may result from
feedback regulation, possibly through increased CXCR7-mediated turnover (Boldajipour et al., 2008), because the majority of patients with DPP4i-BP in this study have used gliptins from several months or even years (5–96 months) before the onset of BP and the collection of skin biopsies.

The severity of skin symptoms, classified by Bullous pemphigoid Disease Area Index scores, has been reported to correlate with the level of anti-BP180 IgG autoantibodies similarly in rBP and DPP4i-BP (Chijiwa et al., 2018; Patsatsi et al., 2018), but also lower levels of anti-BP180 autoantibodies and higher Bullous pemphigoid Disease Area Index of patients with DPP4i-BP than of patients with rBP have been found (Ständer et al., 2021). Previously, a positive correlation between the level of CCL17, IL-21, and TWEAK in sera and the level of anti-BP180 NC16A auto-IgG has been described in patients with BP. However, in these studies, the use of DPP4is was not reported (Li et al., 2013; Liu et al., 2017; Suzuki et al., 2021). We found that besides CCL17, the increased levels of CCL26 and IL-5 in the lesional DPP4i-BP group also correlated with the serum anti-BP180 NC16A auto-IgG levels. A positive correlation of lesional skin cytokines with serum autoantibody levels was also observed with IL-21 and TNF-α in the rBP skin and with TSLP, granzyme B, and CCL1 in both rBP and DPP4i-BP skin. Increased eosinophilic cytokines seem to be associated specifically with DPP4i usage because the NC16A auto-IgG status alone did not discriminate between eosinophilic and other cytokines. Interestingly, the highest cytokine values were concentrated among patients with the most severe DPP4i-BP, but similar skewness was not detected in the rBP group (Supplementary Figures S2 and S6 and Supplementary Table S9). It remains to be investigated whether the correlations between cytokines and autoantibodies as well as severity among DPP4i users have any mechanistic significance. Moreover, the possible relationship between cytokine expression and DPP4i-BP–linked anti-BP180 antibodies targeting non–NC16A epitopes (Lindgren et al., 2019; Mai et al., 2019; Salemme et al., 2022) will be an interesting topic for future research.

The main strength of our study is that we have measured and compared the expression of 32 cytokines and two proteases in the skin of patients with rBP and DPP4i-BP, whereas previous studies have targeted serum and blister fluid levels without separation of patients on the basis of their use of DPP4is and, with a few exceptions, focused only on one or a few cytokines at a time (Supplementary Table S7) (Kowalski et al., 2019). Several observations of elevated cytokines in serum or blister fluid were reproduced from skin extracts such as IL-5, IL-6, IL-8, CCL1, CCL17, CCL26, or BAFF, now with resolution between rBP and DPP4i-BP, whereas CCL4 and CXCL12 were implicated as previously unreported BP effectors.

The limitations of our work include first the large biological variation among patients with BP in the clinical stage and disease severity as well as the heterogeneity of individual skin lesions. The very high concentrations of certain cytokines such as CCL1 and IL-6 in a few lesional skin samples likely reflects real elevations, but as a result of the limited number of samples, large variation between patients and/or lesions, and skewed distributions, these differences did not reach statistical significance between rBP and DPP4i-BP. Second, the results concerning CXCL12 should be interpreted with caution because the ELISA assay is not able to separate intact CXCL12 (SDF-1) (aa 1–68) from DPP4-processed (aa 3–68) form and may also cross-react to some extent with the intact (aa 1–72) and processed (3–72) isoforms of CXCL12 (SDF-1β), which differ in stability and biological function (Elmansri et al., 2019; Sun et al., 2010). Third, it is not known whether described cytokine spectra are universally associated with DPP4i use or restricted to populations in which eosinophil counts and the anti–BP180-NC16A titers do not differ between patients with rBP and those with DPP4i-BP.

Taken together, this work shows that skin cytokine expression differs between patients with rBP and those with DPP4i-BP (Table 1). The cytokines possibly involved in the local lymphocyte function in rBP skin (BAFF, CCL4, CXCL12, IL-6) and the eosinophilic cytokines in DPP4i-BP skin (IL-5, CCL1, CCL17, CCL26) were generally more pronounced in lesional than in nonlesional skin, which suggests that these changes probably represent disease process after the loss of immunological tolerance toward self-antigens rather than preceding events.

### MATERIALS AND METHODS

#### Patients and samples

The collection of human sera and skin samples of patients with BP and healthy subject controls was approved by the Ethical Committee of the Northern Ostrobothnia Hospital District (Approval number 20/2015). The study was performed according to the principles of the Declaration of Helsinki, and all samples were taken after written informed consent. Patients with BP were diagnosed as described previously (Fürsti et al., 2014) in the Department of Dermatology, Oulu University Hospital (Oulu, Finland). Patients were designated as gliptin treated (DPP4i-BP) or nongliptin treated on the basis of their gliptin treatment status at the time of BP diagnosis. BP samples were taken from nonblistering erythematous skin and healthy-looking skin. Control samples were taken from healthy-looking, sun-protected skin areas of patients who were treated for skin tumors. The age and sex of patients and control subjects are indicated in Supplementary Table S1. The anti–BP180-NC16A autoantibody

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**Table 1. Summary of the Differences between Cytokine Expression in the rBP and DPP4i-BP Skin Compared with that of Healthy Control Skin**

| Lymphocyte targeting signals | rBP | DPP4i-BP |
|-----------------------------|-----|----------|
| BAFF                        | ↑   | ➥        |
| IL-6                        | ↑   | ↑        |
| CCL4                        | ↑   | -        |
| CXCL12                      | -   | -        |

**Eosinophil targeting signals**

| CCL1                        | ↑   | ↑        |
| CCL17                       | ↑   | ↑        |
| CCL26                       | ↑   | ↑        |
| IL-5                        | -   | ↑        |

Abbreviations: DPP4i-BP, dipeptidyl peptidase 4 inhibitor–associated bullous pemphigoid; rBP, regular bullous pemphigoid. The arrows indicate the roughly estimated magnitude of increase or decrease of expression of each cytokine.
levels, disease severity (Eming et al., 2015), and duration as well as absolute and relative blood eosinophil counts are shown in the Supplementary Table S2. A total of 5-mm punch skin biopsies were split into two parts (for Luminex/ELISA and RNA isolation), immediately frozen with liquid nitrogen, and stored at −80 °C.

Preparation of skin extracts
Frozen skin samples were pulverized and dispersed in Tissue extraction reagent buffer (Thermo Fisher Scientific, Waltham, MA) supplied with the cOmplete Protease Inhibitor Cocktail (KGaA, Merck, Darmstadt, Germany; 10 μl/g tissue, at least 250 μl). The homogenization was completed by a 5-minute mechanical disruption in Tissuelyser device (Qiagen, Düsseldorf, Germany) with a 50-Hz frequency. Samples were incubated on ice for 15 minutes and centrifuged (20,000g, 20 minutes, +4 °C). The supernatants were aliquoted, frozen in liquid nitrogen, and stored at −80 °C. Protein concentrations were determined using a DC protein assay kit (Bio-Rad Laboratories, Hercules, CA).

Luminex assays
A customized human magnetic Luminex assay 4-plex (R&D Systems, Minneapolis, MN) (Supplementary Table S3) was used for analytical testing and optimization. Two customized magnetic Luminex 16-plex assay kits (R&D Systems) (Supplementary Table S4) with technical replicates were used for measuring cytokines and enzymes from skin extracts according to the manufacturer’s instructions. Two-fold constant dilutions were used. Spike and recovery and dilution series were done for three samples (one from each group). Measurements were performed with a MAGPIX reader (Luminex, Austin, TX).

ELISA
Quantikine ELISA human CXCL12/SDF1α and CCL5 (RANTES) immunoassays (R&D Systems) were used to measure CXCL12 and CCL5 from skin extracts according to the manufacturer’s instructions, with 2.5-fold dilutions. Anti-BP180 NC16A autoantibodies in serum samples were measured using a DC protein assay kit (Bio-Rad Laboratories, Hercules, CA).

RNA isolation and qPCR
Frozen skin samples were pulverized, and total RNA was isolated with the RNeasy Plus universal kit (Qiagen). The quality of RNA was verified with the 2100 Bioanalyzer and RNA Nano 6000 kit (Agilent Technologies, Santa Clara, CA) at the Biocenter Oulu sequencing core (Oulu, Finland) and by Fragment Analyzer (Advanced Analytical Technologies, Ankeny, IA), with 29 of 30 passing quality control (RNA quality number ≥ 7.0).

cDNA synthesis was performed by High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific) according to the manufacturer’s instructions. Gene expression was analyzed by qPCR amplification using the CFX Connect Real-time analyzer (Bio-Rad Laboratories), So Advanced Universal SYBR green supermix (Bio-Rad Laboratories), and commercial gene-specific primers (Bio-Rad Laboratories) (Supplementary Table S8). B2M, GUSB, and RPL13A were used as reference genes (Supplementary Table S8).

Data analysis
In Luminex analysis, the arithmetic means of two technical replicates were used. The zero/absolute values (concentrations below the assay sensitivity) were replaced by half of the extrapolated minimum value below the lowest standard. A few values over the maximum exceeding MAGPIX program prediction were imputed by linear extrapolation.

Because skin extracts and RNAs from nonlesional and lesional samples were from the same individual (dependence), the differential expression between rBP, DPP4i-BP, and healthy control groups was tested separately for nonlesional and lesional samples of patients with BP using Kruskal–Wallis statistical analysis and Dunn–Bonferroni posthoc tests. Spearman’s rank correlation analysis was used for bivariate correlation analysis. All calculation was performed using the IBM SPSS statistics program package. P = 0.05 was considered the limit of statistical significance.

Human Studies
See Supplementary Materials and Methods.

Data availability statement
No extra datasets on top of those shown in this study were generated during this study.

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CONFLICT OF INTEREST
LH has received educational grants from Takeda, Janssen-Cilag, Novartis, AbbVie, Pfizer, and LEO Pharma; has received honoraria from Lilly, Sanofi Genzyme, Novartis, Abbvie, Boehringer Ingelheim, and Orionpharma for consulting and/or speaking; and is an investigator for Abbvie. KT has received educational grants from Sanofi Genzyme and honoraria from Abbvie, Leo Pharma, Novartis, Sanofi Genzyme, Janssen-Cilag, Bristol-Myers Squibb, and UCB Pharma for consulting and/or speaking. The remaining authors state no conflict of interest.

AUTHOR CONTRIBUTIONS
Conceptualization: JT, NK, VG, JM, KT; Formal Analysis: JT, NK; Funding Acquisition: KT; Investigation: JT, AM, SR; Resources: LH, OV; Writing — Original Draft Preparation: JT; Writing — Review and Editing: JT, NK, OV, IM, KT

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SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2022.07.006

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Supplementary Figure S1. Expression of IL-6, CCL1, CCL17, and CCL26 is exceptionally high in a few lesional skin samples. The values of highly expressed cytokines determined by the Luminex assay (shown in Figure 1d, h, j, and l) were further analyzed in a more detailed manner. The concentration distributions of the highest Q4 of these cytokines in the lesional skin of rBP and DPP4i-BP and in healthy controls are shown. Ctrl, control; DPP4i-BP, dipeptidyl peptidase 4 inhibitor–associated bullous pemphigoid; Q, quartile; rBP, regular bullous pemphigoid.

Supplementary Figure S2. The highly increased expression levels of eosinophilic cytokines are found among patients with severe symptoms. Patient groups with rBP and DPP4i-BP were classified according to disease severity (mild, <10% of skin affected; moderate, 10–30% of the skin affected; severe, >30% of the skin affected). The distributions of BAFF, IL-6, CCL4, CXCL12, CCL1, CCL17, CCL26, and IL-5 expression levels measured by Luminex or ELISA are shown by dot plots.
Supplementary Figure S3. Cytokine expression is independent of disease duration. Patients groups with rBP and DPP4i-BP were classified according to disease duration (time between the appearance of symptoms and sampling) as shown. The distributions of BAFF, IL-6, CCL4, CXCL12, CCL1, CCL17, CCL26, and IL-5 expression levels measured by Luminex or ELISA are shown by dot plots. DPP4i-BP, dipeptidyl peptidase 4 inhibitor–associated bullous pemphigoid; mo, month; rBP, regular bullous pemphigoid.
Supplementary Figure S4. Gene expression of IL6, CCL1, and CCL26 is upregulated in DPP4i-BP skin compared with that in the healthy controls. qPCR analysis was performed from total RNA samples isolated from nonlesional and lesional skin biopsies from six patients with rBP and six patients with DPP4i-BP and from six healthy control subjects. The gene expression data are shown for cytokines implicated by biased expression in Luminex analysis. CXCL11 is a reference without change at the protein level, whereas TNF-α was upregulated both in rBP and DPP4i-BP at the protein level. Statistical analysis is presented in Supplementary Table S6. BP, bullous pemphigoid; Ctrl, control; DPP4i-BP, dipeptidyl peptidase 4 inhibitor-associated bullous pemphigoid; rBP, regular bullous pemphigoid; R.U., relative unit.
Supplementary Figure S5. A few cytokines show a positive correlation between their concentration in the skin and the levels of anti-BP180 NC16A auto-IgG autoantibodies in the patient’s serum. The concentration of lesional skin cytokines measured by Luminex was compared with serum anti-BP180 NC16A autoantibodies determined by ELISA. The bivariate correlations were studied using Spearman’s rank correlation analysis. A significant positive correlation was found for indicated cytokines in (a–e) rBP and (f–j) DPP4i-BP skin. (k–o) Examples of nonexistent correlation. DPP4i-BP, dipeptidyl peptidase 4 inhibitor–associated bullous pemphigoid; NC, noncollagenous; rBP, regular bullous pemphigoid.
Supplementary Figure S6. The difference in expression of prolymphocytic and eosinophilic cytokines is not determined by anti-BP180 NC16A auto-IgG status. The cytokine levels of pooled patients with BP are shown against the anti-BP180 NC16A auto-IgG ELISA values. The dashed line marks 9 U/ml considered as the threshold of autoantibody positivity. BP, bullous pemphigoid; NC, noncollagenous.
### Supplementary Table S1. Characteristics of Patients with BP and Controls

| Characteristics                             | Controls | rBP   | DPP4i-BP | Controls | rBP   | DPP4i-BP |
|---------------------------------------------|----------|-------|----------|----------|-------|----------|
| n                                           | 12       | 18    | 17       | 6        | 6     | 6        |
| Age, y, mean ± SD)                          | 79.8 ± 9.5 | 80.1 ± 8.2 | 77.6 ± 8.9 | 75.0 ± 10.6 | 81.2 ± 8.3 | 72.8 ± 9.8 |
| Sex (%F)                                    | 41.7     | 44.4  | 41.2     | 16.7     | 50.0  | 33.3     |
| Mean BP180-NC16A ELISA (relative units)     | n.d.     | 69 ± 52 | 80 ± 66  | n.d.     | 54 ± 40 | 91 ± 74  |

*Abbreviations: DPP4i-BP, dipeptidyl peptidase 4 inhibitor-associated bullous pemphigoid; F, female; NC, noncollagenous; n.d., not determined; rBP, regular bullous pemphigoid.*

1RNA isolated both from nonlesional and lesional skin.

### Supplementary Table S2. Comparison of Clinical Characteristics of Patients with rBP and DPP4i-BP

| Characteristics                             | rBP          | DPP4i-BP    |
|---------------------------------------------|--------------|-------------|
| **n (%)**                                   | Mean anti-BP180 NC16A Ab (U/ml) | Mean anti-BP180 NC16A Ab (U/ml) |
| Severity                                   | 6 (33.3)     | 3 (17.6)    | 10.8      |
| Mild                                       | 5 (38.9)     | 5 (29.4)    | 52.5      |
| Moderate                                   | 5 (27.8)     | 9 (52.9)    | 117.3     |
| Severe                                     |              |             |           |
| Duration                                   |              |             |           |
| <1 mo                                      | 6 (33.3)     | 6 (35.3)    | 92.1      |
| 1–3 mo                                     | 6 (33.3)     | 7 (41.2)    | 69.0      |
| 3–6 mo                                     | 3 (16.7)     | 0 (0)       | —         |
| 6–12 mo                                    | 4 (22.2)     | 3 (17.6)    | 104.3     |
| >12 mo                                     | 3 (16.7)     | 1 (5.9)     | 2.50      |
| EOS E9/ml4                                  | 1.02 ± 1.21  | 0.83 ± 0.84 |
| EOS %5                                     | 10.1 ± 10.1  | 9.1 ± 9.6   | 9.1 ± 9.6 |

*Abbreviations: Ab, antibody; DPP4i-BP, dipeptidyl peptidase 4 inhibitor-associated bullous pemphigoid; EOS, eosinophil; NC, noncollagenous; rBP, regular bullous pemphigoid.*

1Nine Patients with DPP4i-BP were treated with linagliptin (mean = 26.9 mo [7–49 mo]), two with sitagliptin (mean = 51 mo [27–75 mo]), and five with vildagliptin (mean = 46.4 mo [5–103 mo]). Altogether, three patients had longer than five years of continuous gliptin treatment.

2The severity of the clinical picture was categorized by the patient record data as follows: <10% of skin affected: mild, 10–30% of the skin affected: moderate, >30% of the skin affected: severe.

3Duration is the time from the appearance of the symptoms to the skin and serum sampling.

4Absolute counts of EOSs.

5Relative share (%) of EOSs in blood leukocytes (mean ± SD).
Supplementary Table S3. Four-Plex Testing of the Suitability of Luminex Technology for the Use of Skin Extracts

| Dilution          | MMP1 (pg) | IL-6 (pg) | IL-1β (pg) | CXCL16 (pg) |
|-------------------|-----------|-----------|------------|-------------|
|                   | 1:2       | 1:4       | 1:2        | 1:4         | 1:2        | 1:4         |
| Subject 1 (4.3 mg/ml)¹ |           |           |            |             |            |             |
| Unspiked (pg)     | >10,740   | >10,740   | 249.2      | 106.5       | 563.6      | 252.9       | 180.3       | 88.9        |
| Spiked² (pg)      | >10,740   | >10,740   | 259        | 118.3       | 621.6      | 288.3       | 210.1       | 109.6       |
| Subject 2 (2.1 mg/ml)¹ |           |           |            |             |            |             |
| Unspiked (pg)     | >10,740   | 9,146.9   | 82.6       | 38.5        | 7.9        | 1.8         | 104.8       | 58.8        |
| Spiked² (pg)      | >10,740   | 6,999.6   | 99.3       | 45.5        | 92         | 45.2        | 137.6       | 76          |
| Subject 3 (3.8 mg/ml)¹ |           |           |            |             |            |             |
| Unspiked (pg)     | >10,740   | >10,740   | 68.3       | 30.8        | 146.4      | 67.6        | 96.2        | 49.3        |
| Spiked² (pg)      | >10,740   | >10,740   | 88.2       | 41          | 220.2      | 105.3       | 132.4       | 70.4        |
| Spike alone³      | 166.9     | 81.1      | 19.4       | 9.6         | 72.6       | 35.7        | 45          | 22          |

Average recovery (%)⁴: nd nd 80 101 99 109 73 89

Abbreviations: BP, bullous pemphigoid; MMP1, matrix metalloproteinase 1; nd, not determined.

Skin extracts were prepared into tissue extraction reagent—buffer as described in Materials and Methods from skin biopsies from lesional/inflamed skin from patients without BP. The measurements of two dilutions are shown.

¹Total protein concentration of skin extract.
²The 3 μl of the standard pool of highest concentration was added into 1:2 diluted skin extract.
³The 3 μl of the standard pool of highest concentration was added into the dilution buffer.
⁴Recovery % = 100 (spiked skin extract — unspiked skin extract)/spike alone. Average of 1–3.
Supplementary Table S5. Comparison of the Expression of 32 Cytokines and Proteases between Patients with rBP, DPP4i-BP, and Healthy Controls

| Cytokine or Protease | Concentration in the Healthy Control, Mean ± SD (pg/mg) | Kruskal-Wallis P-Value | rBP Versus Healthy Control FC¹(adj. P-Value) | DPP4i-BP Versus Healthy Control FC¹(adj. P-Value) | DPP4i-BP Versus rBP FC¹(adj. P-Value) |
|---------------------|--------------------------------------------------------|------------------------|------------------------------------------|-----------------------------------------------|-----------------------------------|
| BAFF₁                | 29.49 ± 10.55                                          | L 0.0030²               | +1.8 (0.007)                              | +1.1 (1.000)                                  | −1.7 (0.022)                      |
| CCL1³                | 0.71 ± 0.53                                            | L 0.0007²               | +13.3 (0.0012)                            | +41.8 (0.0034)                                | +3.1 (1.000)                      |
| CCL2³                | 36.72 ± 11.95                                          | L <0.0001²               | +9.7 (<0.0001)                            | +9.4 (0.0013)                                 | −1.0 (0.591)                      |
|                      |                                                        | N 0.075                 | +1.3                                     | +6.5                                         | +5.2                             |
| CCL4³                | 45.90 ± 51.20                                          | L 0.0153²               | +3.1 (0.012)                              | +2.0 (0.350)                                  | +1.6 (0.457)                      |
| CCL8⁴                | 30.93±7.24                                             | L 0.0669                 | +1.7                                     | +1.4                                         | +1.2                             |
|                      |                                                        | N 0.114                 | +1.2                                     | +1.7                                         | +1.4                             |
| CCL11¹               | 22.21 ± 10.74                                          | L 0.362                 | +1.7                                     | +2.3                                         | +1.4                             |
|                      |                                                        | N 0.801                 | +1.1                                     | −1.1                                         | −1.2                             |
| CCL17²               | 54.76 ± 24.56                                          | L 0.0018                 | +6.9 (0.008)                              | +13.0 (0.003)                                 | +1.9 (1.000)                      |
| CCL22³               | 428.40 ± 204.96                                        | L 0.0413                 | +1.5 (0.458)                              | +1.3 (1.000)                                  | −1.1 (0.037)                      |
| CCL26³               | 38.46 ± 51.68                                          | L 0.0001                 | +9.4 (0.0006)                             | +13.0 (0.002)                                 | +1.4 (1.000)                      |
|                      |                                                        | N 0.008                  | +1.4 (0.563)                              | +8.2 (0.006)                                  | +6.0 (0.454)                      |
| CXCL9⁵               | 215.68 ± 154.76                                        | L 0.345                 | +1.4                                     | −1.1                                         | −1.6                             |
| CXCL10³              | 278.36 ± 633.14                                        | L 0.216                 | +2.2                                     | −5.3                                         | −11.7                            |
| CXCL11³              | 7.64 ± 12.40                                           | L 0.791                 | +1.8                                     | −1.8                                         | −3.1                             |
| CXCL16⁵,⁶             | 144.65 ± 41.90                                         | L 0.0365                 | +1.4 (0.033)                              | +1.2 (0.195)                                  | −1.2 (1.000)                      |
|                      |                                                        | N 0.371                 | +1.3                                     | +1.2                                         | −1.1                             |
| GM-CSF³              | 29.35 ± 38.87                                          | L 0.6413                 | +1.1                                     | −2.1                                         | −2.4                             |
|                      |                                                        | N 0.296                 | +1.4                                     | +3.7                                         | +5.0                             |
| GZMB⁴                | 70.52 ± 75.45                                          | L <0.0001                | +16.7 (<0.0001)                          | +12.8 (0.0005)                                 | +1.3 (1.000)                      |
|                      |                                                        | N 0.065                 | +1.4                                     | +2.5                                         | +1.8                             |
| IFN-γ⁴               | 1.78 ± 1.02                                            | L 0.7087                 | +1.6                                     | −1.1                                         | −1.8                             |
|                      |                                                        | N 0.603                 | +1.1                                     | 1.0                                          | +1.1                             |
| IL-1β³               | 17.27 ± 9.46                                           | L 0.1405                 | +4.8                                     | +4.0                                         | +1.2                             |
| IL-2²                | 3.98 ± 2.56                                            | L 0.0132                 | −1.4 (0.761)                              | −2.4 (0.013)                                  | −1.7 (0.160)                      |
|                      |                                                        | N 0.504                 | −1.4                                     | −1.4                                         | 1.0                              |
| IL-5⁵                | 0.90 ± 0.31                                            | L 0.0477                 | +2.8 (0.292)                              | +6.8 (0.042)                                  | +2.4 (1.000)                      |
|                      |                                                        | N 0.016                 | +1.1 (1.000)                              | +5.2 (0.012)                                  | +4.7 (0.320)                      |
| IL-6⁶,⁵               | 0.22 ± 0.17                                            | L <0.0001                | +322 (<0.0001)                            | +72 (0.0013)                                  | +4.5 (0.219)                      |
|                      |                                                        | N 0.0050                | +7.6 (0.046)                              | +45 (0.020)                                   | +6.0 (1.000)                      |
| IL-8⁷                | 4.71 ± 6.31                                            | L <0.0001                | +1291 (<0.0001)                          | +377 (0.0004)                                 | −3.4 (1.000)                      |
|                      |                                                        | N 0.094                 | +2.7                                     | +2.3                                         | +1.2                             |
| IL-10⁴               | 0.15 ± 0.05                                            | L 0.607                 | +3.0                                     | +1.5                                         | +2.0                             |
| IL-17⁴               | 1.16 ± 0.53                                            | L 0.0866                 | +4.8                                     | +1.8                                         | +2.8                             |
| IL-18⁴               | 35.63 ± 24.36                                          | L 0.2009                 | −1.3                                     | +1.3                                         | +1.7                             |
| IL-21⁴               | 6.39 ± 1.66                                            | L 0.0508                 | +2.0                                     | +1.4                                         | +1.5                             |
| IL-23⁴               | 285.66 ± 88.49                                         | L 0.6405                 | 1.0                                      | −1.1                                         | −1.1                             |
| IL-31⁴               | 22.20 ± 7.67                                           | L 0.7968                 | −1.2                                     | −1.2                                         | −1.0                             |
| IL-33⁴               | 446.74 ± 151.15                                        | L 0.0019                 | −1.9 (0.002)                              | −1.6 (0.018)                                  | +1.2 (1.000)                      |

(continued)
### Supplementary Table S5. Continued

| Cytokine or Protease | Concentration in the Healthy Control, Mean ± SD (pg/mg) | Kruskal–Wallis P-Value | rBP Versus Healthy Control FC (adj. P-Value)¹ | DPP4i-BP Versus Healthy Control FC (adj. P-Value)² | DPP4i-BP Versus rBP FC (adj. P-Value)³ |
|---------------------|-------------------------------------------------|------------------------|---------------------------------------------|--------------------------------------------------|----------------------------------------|
|                     | N 0.639                                         | −1.2                   | −1.1                                        | +1.1                                             |                                        |
| **Table S5 continues** |                                                |                        |                                             |                                                  |                                        |
| MIF⁴                | 187,554 ± 41,120 L                              | 0.130                  | +1.1                                        | +1.3                                             |                                         |
| MMP1⁵,⁶             | 26.77 ± 37.43 L                                 | <0.0001                | +309 (0.0001)                               | +111 (0.0006)                                    | −2.8 (0.569)                           |
|                     | N 0.958                                         | +1.1                   | +1.3                                        | +1.2                                             |                                        |
| TSLP⁴               | 0.11 ± 0.06 L                                   | 0.0005                 | +7.7 (0.0006)                               | +12.1 (0.005)                                    | +1.6 (1.000)                          |
|                     | N 0.193                                         | +5.2                   | +1.8                                        | +2.9                                             |                                        |
| TNF-α⁴              | 0.44 ± 0.20 L                                   | 0.0005                 | +4.9 (0.0003)                               | +4.3 (0.013)                                     | −1.1 (0.848)                          |
|                     | N 0.246                                         | +1.4                   | +1.7                                        | +1.2                                             |                                        |
| **ELISA measurements** |                                                |                        |                                             |                                                  |                                        |
| CXCL12              | 6622                                           | 0.0093                 | −1.1 (1.000)                                | −2.0 (0.011)                                     | −1.7 (0.088)                          |
|                     | N 0.090                                         | +1.5                   | +2.0                                        | +1.4                                             |                                        |
| CCL5                | 77.04                                          | 0.333                  | +1.5                                        | +1.2                                             | +1.3                                  |
|                     | N 0.380                                         | +1.5                   | +2.0                                        | +1.4                                             |                                        |

**Abbreviations:** adj., adjusted; DPP4i-BP, dipeptidyl peptidase 4 inhibitor–associated bullous pemphigoid; EOS, eosinophile; FC, fold change; GZMB, Granzyme B; L, lesional; MMP1, matrix metalloprotease 1; N, nonlesional; rBP, regular bullous pemphigoid.

The basal concentrations in the healthy control skin were normalized against the total protein concentration of the skin extract.

¹Fold difference of Amean versus Bmean: FC = Amean / Bmean if Amean > Bmean ; FC = −Bmean / Amean if Amean < Bmean
²Kruskal–Wallis pairwise posthoc test (Dunn–Bonferroni)
³Analytes were measured in the same 16-plex 1.
⁴Analytes were measured in the same 16-plex 2.
⁵This analyte was used in validation 4-plex.
⁶Values with statistical significance are in bold.
### Supplementary Table S6. Statistical Testing of Differential Gene Expression Analysed by qPCR

| Gene  | KW P-Value | DB P-Value | DB Adj. P-Value | FC
|-------|------------|------------|----------------|---
| BAFF lesion | 0.641 | | | |
| BAFF nonlesional | 0.331 | | | |
| IL-6 lesion | 0.006 | 0.005 | 0.015 | 14.7 |
| rBP vs Ctrl | 0.007 | 0.021 | 0.021 | 50.8 |
| DPP4i-BP vs rBP | 0.914 | 1.00 | 1.00 | 3.5 |
| IL-6 nonlesional | 0.198 | | | |
| CCL4 lesion | 0.623 | | | |
| CCL4 nonlesional | 0.003 | 0.002 | 0.005 | 2.9 |
| DB P-Value | 0.007 | 0.021 | 0.021 | 3.2 |
| DB Adj. P-Value | 0.017 | 0.052 | 0.052 | 6.2 |
| CCL1 lesion | 0.029 | 0.074 | 0.223 | 3.6 |
| rBP vs Ctrl | 0.009 | 0.028 | 0.028 | 33.5 |
| CCL1 nonlesional | 0.204 | | | |
| CCL17 lesion | 0.205 | | | |
| CCL17 non-lesional | 0.095 | | | |
| CCL26 lesion | 0.016 | 0.234 | 0.703 | 2.3 |
| rBP vs Ctrl | 0.004 | 0.012 | 0.012 | 12.9 |
| DPP4i-BP vs rBP | 0.994 | 0.281 | 0.281 | 5.7 |
| CCL26 nonlesional | 0.007 | 0.552 | 1.000 | 1.5 |
| rBP vs Ctrl | 0.003 | 0.009 | 0.009 | 9.2 |
| rBP vs rBP | 0.017 | 0.052 | 0.052 | 6.2 |
| IL-5 lesion | 0.152 | | | |
| IL-5 nonlesional | 0.067 | | | |
| CXCL11 lesion | 0.312 | | | |
| CXCL11 nonlesional | 0.032 | 0.011 | 0.033 | 5.9 |
| rBP vs Ctrl | 0.066 | 0.198 | 0.198 | 3.0 |
| DPP4i-BP vs rBP | 0.482 | 1.000 | 1.000 | 2.0 |
| TNF lesion | 0.128 | | | |
| TNF nonlesional | 0.019 | 0.160 | 0.479 | 1.3 |
| rBP vs Ctrl | 0.005 | 0.015 | 0.015 | 1.7 |
| DPP4i-BP vs rBP | 0.160 | 0.479 | 0.479 | 1.4 |

Abbreviations: adj., adjusted; Ctrl, control; DB, Dunn–Bonferroni; DPP4i-BP, dipeptidyl peptidase 4 inhibitor–associated bullous pemphigoid; FC, fold change; KW, Kruskal–Wallis; rBP, regular bullous pemphigoid; vs, versus.

Differences in mRNA levels from nonlesional and lesional samples of rBP and DPP4i-BP skin were tested by the KW method and separately against each other and healthy controls with DB posthoc tests. Statistical significance (adj. P < 0.05) is indicated by bold font.

1Fold difference of Amean vs Bmean: FC = Amean / Bmean if Amean > Bmean; FC = − Bmean / Amean if Amean < Bmean.

### Supplementary Table S7. Cytokines with Implicated Association with BP in the Literature

| Target | Change | Reference |
|--------|--------|-----------|
| APRIL | Serum | up | Watanabe et al. (2007) |
| BAFF | Serum, B-cells | up | Asashima et al. (2006); Qian et al. (2014) |
| CCL1 | Serum, BF | up | Miyagaki et al. (2009) |
| CCL2 | Serum | up | Nakashima et al. (2007) |
| CCL11 | Serum, BF, skin | up | Günther et al. (2011); Kowalski et al. (2019) (meta-analysis); Nakashima et al., 2007; Shrikhande et al., 2000; Wakugawa et al., 2000 |
| CCL17 | Serum | up | Kowalski et al. (2019) (meta-analysis); Nin-Asai et al., 2016; Suzuki et al., 2021 |
| CCL18 | Serum, BF | up | Günther et al. (2009) |
| CCL26 | Serum, BF, skin | up | Günther et al. (2011); Kowalski et al. (2019) (meta-analysis) |
| CXCL9 | Serum | up | Nakashima et al. (2007) |
| CXCL10 | Serum | up | Nakashima et al. (2007) |
| IL-1α | BF | down | Kowalski et al. (2019) (meta-analysis) |
| IL-1β | BF | up | Le Jan et al. (2019) |
| IL-5 | Serum, BF | up | Endo et al. (1992); Engineer et al. (2001); Inaoki and Takehara (1998); Kowalski et al. (2019) (meta-analysis); Shrikhande et al., 2000; Wakugawa et al., 2000 |
| IL-6 | Serum, BF, cultured keratinocytes after auto-IgG induction | up | D’Auria et al. (1999); Inaoki and Takehara (1998); Kowalski et al. (2019) (meta-analysis); Shrikhande et al., 2000; Schmidt et al., 2000 |
| IL-8 | Serum, BF, cultured keratinocytes after auto-IgG induction | up | Inaoki and Takehara (1998); Kowalski et al. (2019) (meta-analysis); Schmidt et al. (2000) |
| IL-9 | Serum | up | Suzuki et al. (2021) |
| IL-10 | Serum | up | D’Auria et al. (1999) |
| IL-17 | Serum, BF, neutrophils, mast cells | up | Kowalski et al. (2019) (meta-analysis); Chakievska et al., 2019; Le Jan et al., 2014; Suzuki et al., 2021 |
| IL-21 | Serum | up | Li et al. (2013) |
| IL-36 | Serum | up | Žebrowska et al. (2017) |
| IFN-γ | Serum | down | Suzuki et al. (2021) |
| TNF-α | Serum, BF | up | D’Auria et al. (1999); Kowalski et al. (2019) (meta-analysis); Suzuki et al., 2021 |
| TSLP | Serum, BF, skin | up | Li et al. (2020) |
| TWEAK | Serum | up | Liu et al. (2017) |

Abbreviations: BF, blister fluid; down, downregulated; up, upregulated.
## Supplementary Table S8. qPCR Primers

| Name of the Gene | Bio-Rad Library Code |
|------------------|----------------------|
| B2M              | qHsaCID0015347       |
| GUSB             | qHsaCID0011706       |
| RPL13A           | qHsaCED0020417       |
| CCL1             | qHsaCID0008542       |
| CCL4             | qHsaCED0044260       |
| CCL17            | qHsaCID0022158       |
| CCL26            | qHsaCED003289        |
| CXCL11           | qHsaCED0001533       |
| CXCL12           | qHsaCID0012398       |
| IL5              | qHsaCID0012173       |
| IL6              | qHsaCID0020314       |
| TNF (TNF alpha)  | qHsaCED0037461       |
| TNFSF13B (BAFF)  | qHsaCED0042928       |

## Supplementary Table S9. Comparison of Cytokine Expression in Relation to the Anti-BP180-NC16A Auto-IgG Status

| Analyte            | Mean Rank NC16A Negative/Positive | P-Value | Analyte            | Mean Rank NC16A Negative/Positive | P-Value | Analyte            | Mean Rank NC16A Negative/Positive | P-Value |
|--------------------|-----------------------------------|---------|--------------------|-----------------------------------|---------|--------------------|-----------------------------------|---------|
| BAFF               | 13.6/18.73                        | 0.30    | CXCL11            | 15.9/18.35                        | 0.62    | IL-10              | 14.4/18.6                        | 0.395   |
| CCL1               | 9.6/19.4                          | 0.048   | CCL12             | 16.4/19.27                        | 0.073   | IL-17              | 11.4/19.1                        | 0.12    |
| CCL2               | 6.6/19.9                          | 0.007   | CCL16             | 14.4/18.6                         | 0.396   | IL-18              | 17.2/18.13                       | 0.85    |
| CCL4               | 9.4/19.43                         | 0.043   | GM-CSF            | 11.1/19.15                        | 0.104   | IL-21              | 8.5/19.58                        | 0.025   |
| CCL8               | 9.8/19.37                         | 0.053   | GZMB              | 6.8/19.87                         | 0.008   | IL-23              | 17.4/18.1                        | 0.888   |
| CCL11              | 12.4/18.93                        | 0.187   | INF-γ             | 18.70/17.88                       | 0.869   | IL-31              | 15.2/18.47                       | 0.509   |
| CCL17              | 10.8/19.2                         | 0.09    | IL-1β             | 14.0/18.67                        | 0.346   | IL-33              | 23.8/17.03                       | 0.172   |
| CCL22              | 15.6/18.4                         | 0.572   | IL-2              | 17.7/18.05                        | 0.944   | MIF                | 19.2/17.8                        | 0.777   |
| CCL26              | 13.0/18.83                        | 0.239   | IL-5              | 14.20/18.63                       | 0.37    | MMP-1              | 11.0/19.17                       | 0.099   |
| CXCL9              | 14.6/18.57                        | 0.423   | IL-6              | 8.8/19.53                         | 0.030   | TSLP               | 6.0/20.0                         | 0.005   |
| CXCL10             | 14.2/18.63                        | 0.37    | IL-8              | 12.6/18.9                         | 0.203   | TNF-β              | 6.1/19.98                        | 0.005   |

Abbreviations: BP, bullous pemphigoid; GZMB, Granzyme B; MMP1, matrix metalloproteinase 1; NC, noncollagenous.  
Nonparametric (Mann–Whitney U) testing of cytokine/protease expression difference between BP180 NC16A ELISA-negative and -positive BP (pooled groups) patient skin. $P < 0.05$ is considered statistically significant and shown in bold font.