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Gliptin-associated Bullous Pemphigoid and the Expression of Dipeptidyl Peptidase-4/CD26 in Bullous Pemphigoid

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Dipeptidyl peptidase-4 inhibitors (DPP-4i or gliptins) increase the risk of developing bullous pemphigoid. To clarify, whether gliptin-associated bullous pemphigoid has special features, we analyzed the clinical, histopathological and immunological features of 27 bullous pemphigoid patients, 10 of which previously used gliptin medication. Compared to those who had not previously received gliptins, subjects who had, showed higher BP180-NC16A ELISA (enzyme-linked immunosorbent assay) values, fewer neurological co-morbidities and shorter time to remission, but differences were not statistically significant. The HLA-DQB1*03:01 allele was more commonly present among the bullous pemphigoid patients than the control population, but was not more common in those with gliptin history. To determine the effect of gliptins on the expression of the DPP-4/CD-26 protein we performed immunohistochemistry, which showed that the skin expression of DPP-4/CD-26 was increased in bullous pemphigoid patients, but not affected by prior gliptin treatment. We conclude that DPP-4i medication is common among bullous pemphigoid patients and prior gliptin treatment may be associated with some specific features.

SIGNIFICANCE

Bullous pemphigoid is a blistering skin disease, generally seen in the elderly patients. Bullous pemphigoid is caused by an autoimmune reaction against the BP180 protein, but the exact reason is currently unknown. Several epidemiological studies have shown that the use of gliptins increases the risk for bullous pemphigoid, but it is unclear whether gliptin-associated bullous pemphigoid has specific clinical or immunological characteristics. Our study demonstrates that compared to "classical" bullous pemphigoid, gliptin-associated bullous pemphigoid may have some specific features, but the differences are not as striking as previously reported in Japanese patients. The anti-diabetic medications in bullous pemphigoid patients must be monitored carefully and gliptins should be replaced by another anti-diabetic medication.

Bullous pemphigoid (BP) is the most frequently occurring autoimmune blistering skin disease. It is mainly seen in elderly people and manifests as severe pruritus, blisters, erosions and crusts on the skin (1). BP autoantibodies target the BP180 protein (also known as collagen XVII), the non-collagenous (NC) 16A domain being its main epitope (2). A diagnosis of BP is based on the clinical criteria, direct immunofluorescence (DIF) analysis of a perilesional skin, serological assays, including BP180-NC16A enzyme-linked immunosorbent assay (ELISA), and indirect immunofluorescence analysis (1). The incidence of BP is growing (3–5) and neurological diseases, especially multiple sclerosis and dementias, increase the risk for subsequent BP (6–8).

Since BP typically affects the elderly and has several comorbidities, polypharmacy is common among BP patients (9) and over 50 drugs have been reported to induce BP (10). Both case reports and registry studies have added to a growing body of evidence, showing an association between dipeptidyl peptidase-4 inhibitors (DPP-4i or gliptin) and BP (11–20). In addition to being an aminopeptidase, DPP-4 acts also as CD26, which is a cell surface antigen present on T lymphocytes (21). This is of interest with regard to the association between DPP-4i use and BP because autoreactive T cells have been associated to BP pathomechanism (1, 2).

It has been shown that in gliptin-associated BP autoantibodies target parts of the BP180 other than the NC16A domain (20, 22, 23). Furthermore, gliptin-associated cases have been suggested to represent a "non-inflammatory" phenotype with less erythema and fewer urticarial lesions on the skin, fewer eosinophils infiltrating the skin lesions and a strong association with the HLA-DQB1*03:01 allele (20, 24). However, gliptin-associated BP cases that lacked any of the aforementioned special characteristics have been described (17, 18, 25, 26).

The aim of our study was to analyze the clinical and immunological features of BP in patients with a history of DPP-4i use and those without. We also explored the expression of DPP-4/CD26, BP180 and its binding partner laminin-γ2 in the skin of BP patients as well as the effect of gliptins on their expression in cultured keratinocytes.

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METHODS

Patients

The data, blood samples and skin biopsies were obtained from BP patients diagnosed as previously described (4) in the Department of Dermatology, Oulu University Hospital between January 2015 and March 2018. Patients' BP disease area index (BPDAI) (27) score at the time of diagnosis was assessed retrospectively from their records. All patients were of Caucasian origin. Patients were stratified as 'gliptin-treated' (DPP-4i-BP) or 'non-gliptin-treated', based on their gliptin treatment status at the time of the BP diagnosis. The study was performed according to the principles of the Declaration of Helsinki and approved by the Ethical Committee of the Northern Ostrobothnia Hospital District (Approval number 20/2015). All samples were taken after obtaining written informed consent.

Histology and immunohistochemistry

Three µm sections from skin lesion samples were stained with the hematoxylin-eosin stain. In each sample, representative hot spots were identified, and the number of eosinophils was counted using a $\times 40$ high power field (HPF) objective. The results were deemed negative (–) if < 5, (+) when 5–20, (++) when 21–50, and (+++) when there were > 50 eosinophils/HPF. Diagnostic DIF was performed at the Department of Pathology, Oulu University Hospital, Oulu, Finland.

Sections were incubated with monoclonal DPP-4/CD26 antibody (ab215711, Abcam, Cambridge, UK), monoclonal BP180 (ab184996, Abcam) and monoclonal laminin-γ2 antibody (E-6) (sc-28330, Santa Cruz Biotechnology, Dallas, TX, USA) for immunostaining. The histological slides were scanned and transformed into digital images (Aperio AT2, Leica Biosystems, Biobank Borealis, Oulu University Hospital, Finland). QuPath software was used to perform a semi-quantitative analysis of the proportion of positive cells in the analyzed area of the epidermis in the immunostained sections (28).

ELISA and immunoblotting

The BP180-NC16A ELISA assay was performed in the HUSLAB, (Helsinki, Finland). Based on the observations from our previous publication (29), two glutathione-S-transferase fusion proteins, FP4 and FP5, which correspond to human BP180 amino acids 377–455 and 489–567, respectively, were immunoblotted with a subset of BP sera ($n=24$) as previously described (29).

Human leukocyte antigen analysis

A subset of BP samples ($n=23$) were genotyped for HLA DR-DQ and analyzed using a method, where a full-house DQB1 analysis was extended, in a stepwise fashion, with DQA1 when relevant and with DR4 subtyping in the case of DQA1*03-DQB1*03:01 and DQB1*03:02 haplotypes. This methodology, which uses lanthanide- labeled sequence specific oligonucleotides, has been previously described (30, 31). Haplotype and allele frequencies, in the reference population, were derived from the non-transmitted haplotypes of family trios of two healthy parents and a child with type 1 diabetes (32).

Cell cultures

HaCaT cells cultured in CnT-Prime medium (CELLnTEC, Bern, Switzerland) were treated for 24 h with differernt concentrations of linagliptin, sitagliptin or vildagliptin (Cayman Chemical, Ann Arbor, MI, USA) in the presence of 50 µg/ml of ascorbic acid. The culture media were collected, cells were lysed in a radioimmuno-

precipitation assay buffer, and immunoblotting was performed, as previously described (33), except that the polyclonal BP180 anti-NC16A antibody (34), polyclonal anti-GAPDH, monoclonal anti-laminin-γ2 (Santa Cruz Biotechnology) and polyclonal anti-DPP-4/CD26 (ab129060, Abcam) were used as primary antibodies and horseradish peroxidase conjugated goat anti-mouse IgG (Abliance, Compiegne, France) and goat anti-rabbit IgG (Sigma-Aldrich, Manassas, VA, USA) as secondary antibodies. Specific protein bands were detected with ECL Prime substrates (GE Healthcare, Buckinghamshire, UK) and an LAS-3000 Imager Analyzer system (Fujifilm, Tokyo, Japan), and quantified with Image J software (NIH, Bethesda, MA, USA).

Statistics

Statistical analyses were performed, using the IBM SPSS Statistics version 24.0 software for Windows (IBM, Chicago, IL). The analyses were: Chi^2 test (nominal data), Chi^2 test with Yates correlation (HLA), variance analysis (normal distributed data) or Mann-Whitney U test (cell stainings and ELISA). A *p*-value of ≤ 0.05 was considered to be significant.

RESULTS

Clinical characteristics

Ten of the 27 BP patients had received DPP-4i medication prior to their BP diagnosis (**Table I**). The mean duration of the gliptin treatment was 24.1 months and the mean latency was 13.4 months. Within the BP group, gliptin-treated patients had a slightly higher mean age (80.3 years) than the non-treated (77.0 years), but the sex ratio did not differ between the two subgroups. Only one gliptin-treated BP patient (DPP-4i-BP10) had a mild vascular dementia, whereas 8 of the 17 patients, who had no gliptin history, had a neurological comorbidity (Table I).

Sixteen out of 17 non-gliptin treated patients had blisters, and all but two of the 10 patients with gliptin history had bullous lesions (Table I). Of all the BP patients nine had no erythema and/or urticarial-like lesions and four of them were gliptin-treated patients (Table I). The data for BPDAI were available only for 11 patients (Table I). The mean time to remission for the non-gliptin-treated (8.9 months) was slightly longer than that for gliptin-treated patients (6.9 months).

Gliptin medication was withdrawn in 7 of the 10 patients and all but one achieved remission with the use of systemic treatment (Table I). Despite the cessation of vildagliptin, one case (DPP-4i-BP1) developed a chronic disease with several relapses (Table I). Three patients (DPP-4i-BP-2, -3 and -5) who stayed on gliptins, eventually achieved remission, one of them (DPP-4i-BP5) using topical glucocorticoids only (Table I).

Immunological and histological characteristics

The incidence of positive BP180-NC16A ELISA values between the gliptin-treated (70%) and non-gliptin-treated cases (82%) was similar, but the mean ELISA value

Table I. Clinical characteristics of patients with bullous pemphigoid

Patient[a]	Sex/age, years	Blisters/erythema	Neurological disease	Gliptin/Duration of gliptin treatment (months)/Cessation (yes or no)	Latency (months)[b]	Systemic treatment[c]	Clinical course	Remission after gliptin cessation (months)[d]	BPDAI
DPP-4i-BP1	F/81	+/+	No	Vildagl./29/Yes	6	Pred, AZA (cessation after 2 months of treatment due to side effects)	Chronical course, relapses during treatment	No	42/10/7/0
DPP-4i-BP2	F/88	+/−	No	Linagl./35/No	10	Pred, AZA (cessation after 1 month of treatment due to side effects)	Remission after 1 month of treatment	–	9/1/0/0/6
DPP-4i-BP3	M/79	+/+	No	Linalg./33.5/No	9.5	Pred, MMF	Remission after 12 month of treatment	–	NA
DPP-4i-BP4	F/84	+/+	No	Linagl./21/Yes	21	Pred, DOX (cessation after 1 week due to side effects)	Remission after 7 months of treatment	7	14/11/0/0
DPP-4i-BP5	M/77	−/−	No	Vildagl./29/No	11	Topical only	Remission after 3 months of treatment	–	0/0/0/0
DPP-4i-BP6	F/78	+/−	No	Linagl./18.5/Yes	7	Pred, AZA (cessation after 1 month of treatment due to side effects)	Remission after 13 months of treatment	1.5	NA
DPP-4i-BP7	F/88	−/+	No	Vildagl./19/Yes	18	AZA, Pred	Remission after 12 months of treatment	11	0/0/0/4
DPP-4i-BP8	M/82	+/+	No	Vildagl./5/Yes	5	Pred, AZA	Remission after 7 months of treatment	7	4/1/0/0/[f]
DPP-4i-BP9	M/63	+/+	No	Sitagl./27/Yes	22	Pred, AZA	Remission after 5 months of treatment	0	NA
DPP-4i-BP10	F/83	+/−	Dementia	Linagl./24/Yes	24	Pred, DOX	Remission after 2 months of treatment	2	23/3/0/1
BP1	M/76	+/+	No	–		Pred	Remission after 2 months of treatment		NA
BP2	F/64	+/+	No	–		Pred	Remission after 2 months of treatment		NA
BP3	M/67	+/+	Epilepsy	–		LC (cessation after 3 months due to lack of response), Pred, AZA	Remission after 19 months of treatment		2/4/0/0
BP4	F/82	+/−	Dementia	–		Topical only	Remission after 2–3 weeks of treatment		NA
BP5	F/80	+/+	Dementia	–		TCN (cessation after 3 week due to lack of response), Pred, AZA	Remission after 2 months of treatment		NA
BP6	M/91	+/−	Dementia	–		Pred	Remission after initiation of treatment		NA
BP7	M/63	+/+	Epilepsy	–		Pred, AZA (cessation after 1 months due to side effects), MTX	Remission after 39 months of treatment, relapses		NA
BP8	F/83	+[e]/+	No	–		Pred, DOX (cessation after 2 months due to lack of response), AZA (cessation after 1 month due to side effects)	Remission after 18 months of treatment		NA
BP9	M/89	+/+	Parkinson's disease	–		Pred, AZA (cessation after 2 months due to side effects)	Remission after 9 months of treatment		NA
BP10	M/69	+/+	No	–		Pred	Remission after initiation of treatment		15/4/0/0
BP11	M/70	+/+	No	–		Topical only	Local symptoms, no data of clinical course available		NA
BP12	F/81	+/+	No	–		DOX	Remission after initiation of treatment		NA
BP13	F/71	+/+	No	–		DOX	Remission after 3 months of treatment		5/2/0/0
BP14	F/83	+/−	Dementia	–		Pred	No data available		NA
BP15	M/74	+/+	No	–		Topical only	Remission after 1 month of treatment		7/0/0/0
BP16	F/91	+/−	Dementia	–		DOX	Remission after 2 months of treatment		NA
BP 17	F/76	−/−	No	–		Pred, AZA (cessation after 2 months due to side effects), MTX	Symptoms alleviated after 4 months of treatment, no further data available		NA

[a]DPP-4i-BP=patient with gliptin treatment at the time of BP diagnosis; [b]Latency from initiation of gliptin to BP diagnosis; [c]All patients used topical potent corticosteroids; [d]Time to remission after cessation of gliptins; [e]blisters in the mouth, [f]BPDAI partially unavailable.
NA: not applicable; Pred: prednisolone; AZA: azathioprine; DOX: doxycycline; LC: lymecycline; TCN: tetracycline; MMF: mycophenolate mofetil; MTX: methotrexate; BPDAI: Bullous Pemphigoid Disease Area Index.

of the non-gliptin-treated patients was lower (47.9 U/ml) compared to gliptin-treated patients (81.1 U/ml) ($p = 0.481$) (**Table II**).

In immunoblotting the NC16A domain (**Fig. 1**A) was recognized by the autoantibodies in sera from 5/9

(55.5%) of gliptin-treated cases and 11/15 (73.3%) of non-gliptin-treated cases (Fig. 1B, C). Both groups included patients, whose sera strongly recognized the NC16A domain and those with weak or missing immunoreactivity. Interestingly, compared to the non-gliptin

Table II. Immunological and histological characteristic of patients with bullous pemphigoid

Patient	DIF IgG/C3/IgG4	NC16A ELISA U/ml[b]	Eosinophils per bullous lesion/dermis	HLA-type DQB1*03:01
DPP-4i-BP1	+/+/–	150	++/+	–
DPP-4i-BP2	+/+/+	23	+++/+	NA
DPP-4i-BP3	+/+/+	8	–[a]/+	+
DPP-4i-BP4	+/+/–	130	+++/+++	+
DPP-4i-BP5	+/+/+	<5	–/–	–
DPP-4i-BP6	+/+/+	20	+++/+++	+
DPP-4i-BP7	+/–/+	<5	–[a]/++	–
DPP-4i-BP8	+/+/–	150	–/++	–
DPP-4i-BP9	+/+/–	140	+++/++	–
DPP-4i-BP10	+/+/+	190	+++/++	+
BP1	+/+/+	54	+++/+	–
BP2	+/+/+	47	+++/+++	NA
BP3	+/+/–	<5	+++/++	–
BP4	+/+/–	44	NA	+
BP5	+/+/+	48	–/+++	NA
BP6	–/+/–	46	+++/+++	NA
BP7	+/+/–	55	–/–	–
BP8	+/–/–	<5	–/–	+
BP9	+/+/+	120	+++/+++	–
BP10	+/+/–	29	–/++	–
BP11	+/+/–	120	+/–	–
BP12	+/+/–	56	–[a]/+	+
BP13	–/+/–	84	–/+	+
BP14	+/+/+	<5	++/–	+
BP15	–/–/+	15	NA	+
BP16	+/+/+	27	NA	–
BP17	+/+/–	69	NA	–

[a]Scale crust/cellular debris. [b]cut-off value 9 U/ml.
NA: not applicable; DIF: direct immunofluorescence.

users, the sera of DPP-4i-BP patients recognized weakly and less frequently another fusion protein, FP4, which corresponds to amino acids 377–455 of the intracellular domain of BP180 (Fig. 1B, C).

There were no major differences between the gliptin-treated and non-gliptin-treated patients in terms of eosinophil counts in skin biopsies taken from blisters (if present) or non-bullous lesions (Table II).

Human leukocyte antigen genetics

The HLA DR-DQ genotype analyses demonstrated that the phenotype frequency of HLA-DQB1*03:01 among the gliptin-treated ($n=9$) and non-gliptin-treated ($n=14$) patients was similar (44% and 42%, respectively) (Table II). In all 23 analyzed BP patients, the HLA-DQB1*03:01 allele was more frequent (12 out of 46 alleles, 26.1%) than in the control group (679 out of 5982, 11.4%) representing the Finnish background population ($p=0.004$). The frequency of the (DR11/12/13)-DQA1*05-DQB1*03:01 class II haplotype was also significantly greater in BP patients ($p=0.011$).

The expression of dipeptidyl peptidase-4, BP180 and laminin-γ2 in skin and keratinocytes

The expression of the DPP-4/CD26 was significantly increased in the skin lesions of BP patients ($p=0.031$), especially in the acanthotic and spongiotic epidermis of
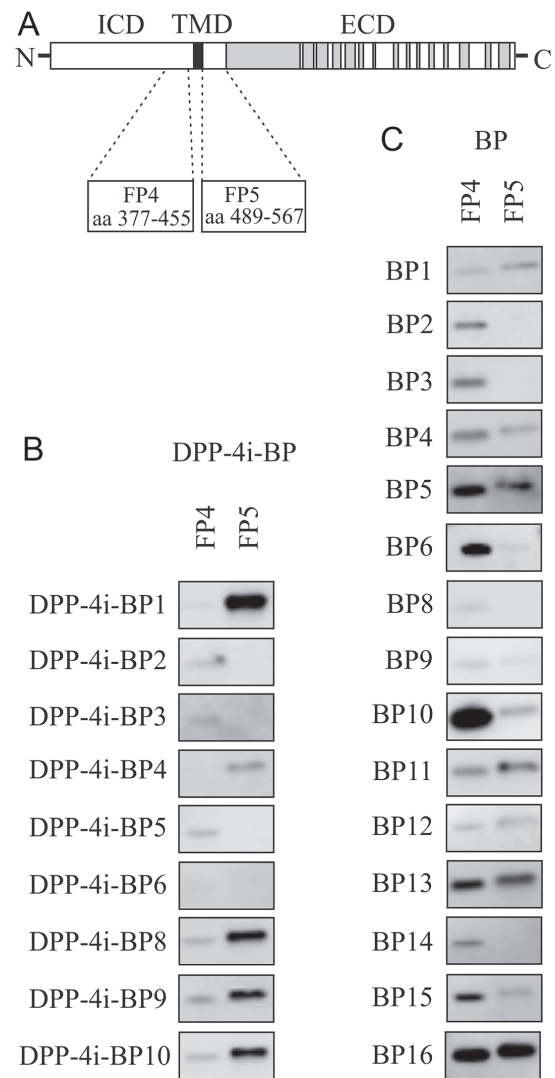
Fig. 1. Epitope mapping, using the serum samples of gliptin-treated and non-gliptin-treated bullous pemphigoid patients. (A) Glutathione-S-transferase fusion proteins FP4 and FP5, corresponding BP180 amino acids 377–455 (intracellular domain) and 489–567 (non-collagenous domain NC16A), respectively, were expressed in *E. coli* and purified. (B, C) The immunoblotting of 50 ng FP4 and FP5 with 1:100 diluted serum demonstrates differences between gliptin-treated DPP-4i-BP ($n=9$) and non-gliptin-treated BP ($n=15$) samples in the recognition of FP4 but not that of FP5.

both bullous and non-bullous lesions (**Fig. 2**A, B, G). Prior use of gliptins had no effect on the expression of the DPP-4/CD26 in BP samples (Fig. 2G). In healthy controls the BP180 immunostaining was strong on the basal keratinocytes (Fig. 2C). The immunoreactivity of the BP180 was consistently strong on the non-bullous areas of BP skin, but weak or even absent on the roof of, and the margins of, blisters (Fig. 2D). However, the difference between the controls and BP samples in terms of the proportion of BP180 positive cells in the analyzed epidermal area was not statistically significant (Fig. 2G), probably due to the consistently strong staining of the non-inflamed skin. The immunoreactivity of laminin-γ2 was weak on the basement membrane of the healthy skin,
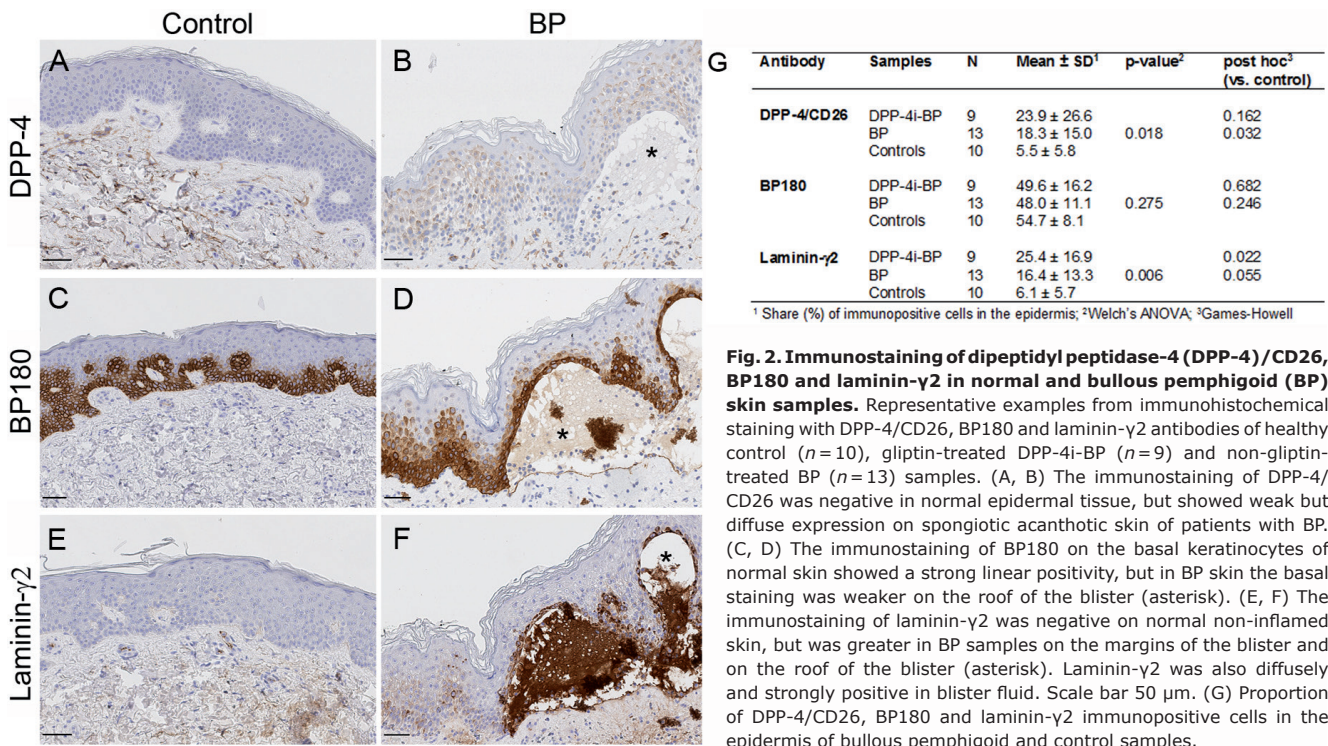
Fig. 2. Immunostaining of dipeptidyl peptidase-4 (DPP-4)/CD26, BP180 and laminin-γ2 in normal and bullous pemphigoid (BP) skin samples. Representative examples from immunohistochemical staining with DPP-4/CD26, BP180 and laminin-γ2 antibodies of healthy control ($n=10$), gliptin-treated DPP-4i-BP ($n=9$) and non-gliptin-treated BP ($n=13$) samples. (A, B) The immunostaining of DPP-4/CD26 was negative in normal epidermal tissue, but showed weak but diffuse expression on spongiotic acanthotic skin of patients with BP. (C, D) The immunostaining of BP180 on the basal keratinocytes of normal skin showed a strong linear positivity, but in BP skin the basal staining was weaker on the roof of the blister (asterisk). (E, F) The immunostaining of laminin-γ2 was negative on normal non-inflamed skin, but was greater in BP samples on the margins of the blister and on the roof of the blister (asterisk). Laminin-γ2 was also diffusely and strongly positive in blister fluid. Scale bar 50 µm. (G) Proportion of DPP-4/CD26, BP180 and laminin-γ2 immunopositive cells in the epidermis of bullous pemphigoid and control samples.

but strong in the basal keratinocytes and the margins of bullous lesions, and most strikingly positive in the blister fluid (Fig. 2E, F). The proportion of positive staining with laminin-γ2 was significantly elevated in both BP groups compared to the healthy controls ($p=0.022$, $p=0.055$, Fig. 2G).

The treatment of HaCaT keratinocytes with vildagliptin, sitagliptin or linagliptin elevated the amount of shed BP180 ectodomain up to threefold and laminin-γ2 twofold compared to the untreated control samples but the increases were not statistically significant (**Fig. 3**A,B). Gliptin treatments did not change the level of DPP-4/CD-26 either (Fig. 3C).

DISCUSSION

Recent studies have reported that gliptin-associated BP has several specific characteristics, including: a "non-inflammatory" phenotype with smaller blisters, milder erythema and limited distribution of skin lesions, auto-antigens, targeting different immunodominant epitopes in BP180, lower lesion eosinophil populations and a strong association with the HLA-DQB1*03:01 allele (20, 22, 23). However, a recent registry study from Israel found no difference between gliptin-associated and 'regular' BP in terms of the involvement of body sites, severity or the appearance of atypical clinical symptoms (14). Similarly, a recent single center study from Northern Greece found no specific clinical or immunological features in BP patients with preceding gliptin medication use, except a significantly higher number of relapses in patients with

'regular' BP (26). Our findings concur with the Israeli and Greece studies: we could not detect significant differences in either clinical symptoms or eosinophil counts in BP between the patients with and without preceding gliptin medication. Furthermore, the majority of gliptin-treated patients had autoantibodies against the immunodominant NC16A domain, and the HLA-DQB1*03:01 allele was equally common among gliptin-treated and non-gliptin-treated BP cases. Unfortunately, we were not able to reliably estimate the severity of BP, since BPDAI data were available only for a few of our cases.

Interestingly, we found that neurological diseases, especially dementias, seems to be more common in our non-gliptin-treated BP patients even though they were slightly younger than the patients who had received gliptin treatment. Neither our previous nationwide registry study (16) nor a Japanese hospital record study (35) detected an impact of gliptin treatment on the patients age at BP diagnosis, although a case-control study from France and Switzerland demonstrated that gliptin treatment was more strongly associated with BP in patients older than 80 years (13).

The mean latency between the initiation of gliptin treatment and diagnosis of BP was approximately 13 months, which is in line with the findings of the recent registry studies (14, 16). As in most previous reports (17, 23, 36) gliptins were withdrawn from most of our patients when they were diagnosed with BP, and all but one, required systemic treatment. Discontinuation of DPP-4i treatment is supported by studies, which demonstrated that gliptin withdrawal was followed by
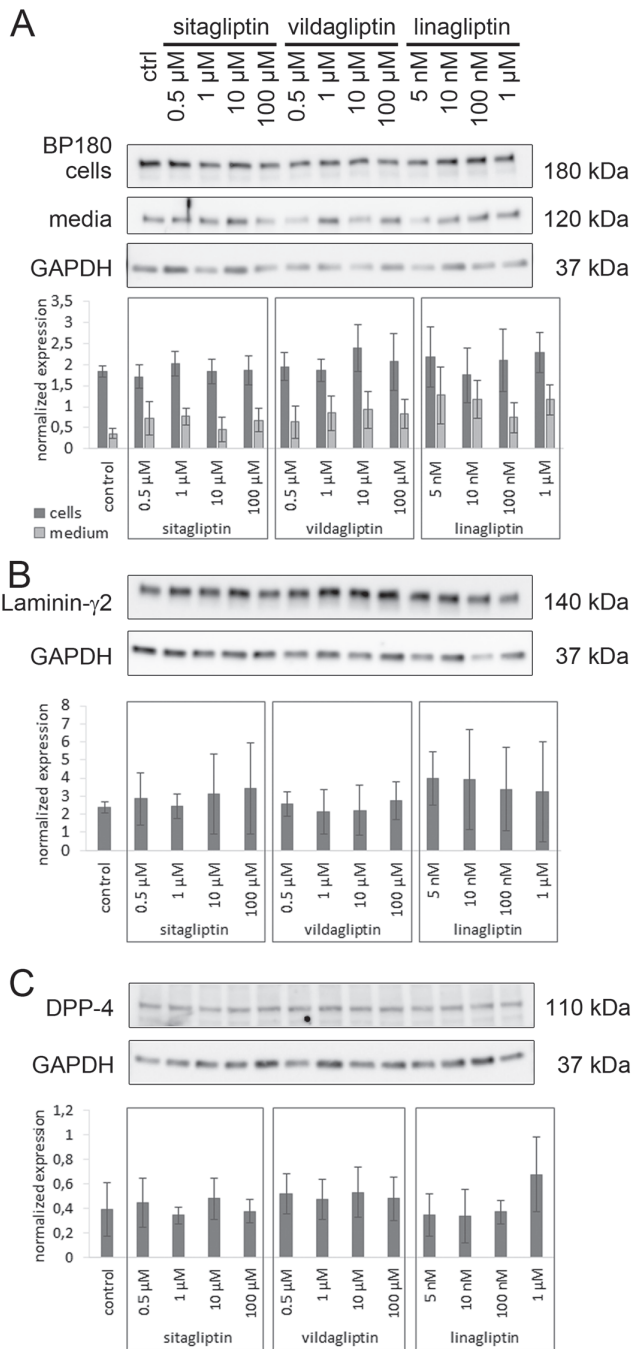
Fig. 3. The protein levels of BP180, laminin-γ2 and dipeptidyl peptidase-4 (DPP-4)/CD-26 in gliptin-treated cultured keratinocytes. Cultured HaCaT keratinocytes were treated for 24 h with differing concentrations of sitagliptin, vildagliptin or linagliptin and protein levels were analyzed with immunoblotting. (A) Gliptin treatments did not markedly change the amount of full-length BP180 protein in cell extracts (180 kDa) or that of shed ectodomain (120 kDa) in media. (B) The amounts of laminin-γ2 or (C) DPP-4/CD-26 in cell extracts did not change significantly during gliptin treatments. The results are means (±SD) of 5 samples. Protein levels in each sample were normalized against GAPDH.

It has been suggested that the non-inflammatory phenotype of gliptin-associated BP is caused by autoantibodies, which target the extracellular domain of BP180 rather than the NC16A domain (20, 23). However, in a recent retrospective analysis, the NC16A autoantibodies were found in 65.6% of cases with gliptin-associated BP ($n=32$) (35). In our study the incidence of positive BP180-NC16A ELISA values in gliptin-treated patients was high (70%), similar to the incidence in the non-gliptin-treated patients. However, the mean ELISA value in the non-gliptin-treated patients was lower, although the difference was not statistically significant. Immunoblotting confirmed that the autoantibodies in gliptin users and non-users recognize the NC16A domain to a similar extent. We detected that the autoantibodies in the gliptin-treated patients recognized a juxtamembranous intracellular fragment weaker than those in the non-gliptin-treated patients, which could suggest a variation in the auto-antigen processing between gliptin and non-gliptin associated BP.

The expression of the CD26 protein is upregulated in T-cell lymphomas, psoriasis, lichen planus and atopic dermatitis (37), but its increased expression in BP lesions, to our knowledge, has not been previously reported. There were no differences in the immunostaining of DPP-4/CD26 between the samples taken from gliptin and non-gliptin-treated BP patients, which indicates that the cutaneous expression of DPP-4/CD26 in BP is independent of the endogenous DPP-4i. Gliptins are not substrate specific and may also inhibit other members of the large DPP family such as FAP, DPP-8, DPP-9 and DPP-II (38). Therefore, the different effects of DPP-4i can include tissue remodeling, macrophage activation, Th17 differentiation, and neutrophil chemotaxis (38). In our study the treatment of cultured keratinocytes with gliptins had no effect on the amount of BP180, its binding partner laminin-γ2 or CD-26/DPP-4, suggesting that gliptins may not directly affect keratinocytes but rather exert their influence via activation/inactivation of other cell types.

Previous findings, which state that DPP-4i-BP is associated with lower numbers of infiltrating eosinophils (20, 24, 39) are unexpected, since DPP-4 has its regulatory effects on the recruitment of eosinophils, and DPP-4i intake can lead to a chemokine-mediated increase in the recruitment of eosinophils *in vivo* (40). We could not detect any differences between the gliptin-treated and non-gliptin-treated groups in terms of the populations of eosinophils on bullous lesions or in the dermis.

The HLA-DQB1*03:01 allele has been associated with BP, especially the mucous membrane pemphigoid in several ethnicities (41–47). Based on the particularly strong association in Japanese patients, DQB1*03:01 has been suggested as a useful biomarker for gliptin-associated BP (39). Our study confirmed the association of DQB1*03:01 with BP in a Finnish population, but we could not detect a difference between gliptin-associated

an improvement of BP clinical outcomes (14). It should be noted that the mean BP remission time was clearly shorter in gliptin-treated BP patients, but the size of our study population limits the conclusions that may be drawn from this finding.

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and conventional BP based on the presence or absence of the allele.

In conclusion, while the use of gliptins has been shown to be associated with an elevated risk for BP, we found no apparent differences between the immunological features of BP patients who had received gliptins and those with no history of gliptin treatment. In our study of Finnish patients, we detected no evidence supporting the previously suggested idea of a non-inflammatory BP phenotype. We found that the expression of DPP-4/CD-26 is elevated in BP skin, regardless of previous gliptin intake, underlining the role of DPP-4/CD-26 in inflammatory processes in general.

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