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Antti Nätynki

AUTOIMMUNIZATION AGAINST COLLAGEN XVII IN PATIENTS WITH HIGH RISK FOR BULLOUS PEMPHIGOID AND THE EFFECT OF VILDAGLIPTIN ON THE MOUSE SKIN PROTEOME

UNIVERSITY OF OULU GRADUATE SCHOOL;
UNIVERSITY OF OULU,
FACULTY OF MEDICINE;
MEDICAL RESEARCH CENTER OULU;
OULU UNIVERSITY HOSPITAL

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Academic dissertation to be presented with the assent of the Doctoral Programme Committee of Health and Biosciences of the University of Oulu for public defence in Auditorium 9 of Oulu University Hospital (Kajaanintie 50), on 19 April 2024, at 12 noon

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Abstract

Bullous pemphigoid (BP) is an autoimmune blistering skin disease characterized by intense itching and tense fluid-filled bullae and erythema located typically on the trunk and extremities. BP affects mainly patients over 70 years of age and has up to 40% two-year mortality. IgG autoantibodies against collagen XVII or BP180, a protein essential for dermo-epidermal junction structure, are demonstrated to be central in the pathogenesis of BP.

Dermatitis herpetiformis (DH), an autoimmune dermatosis of patients with celiac disease (CD), and the use of dipeptidyl peptidase-4 inhibitors (DPP 4is or gliptins), drugs to treat type 2 diabetes (T2D), markedly increase the risk of BP. However, it is currently not clear how these conditions promote the development of BP.

This study aimed to clarify the mechanisms of the associations between DH, the use of DPP-4is, and BP by analyzing the prevalence and target epitopes of anti-BP180 autoantibodies in patients with CD, DH, BP, or T2D. Transglutaminase antibodies characteristic for DH were also analyzed from CD, DH, and BP sera.

The study population comprised 43 CD and 46 DH patients, 61 BP patients, 136 DPP 4i-using and 136 non-DPP-4i-using T2D patients, and 82 control samples.

This study showed a low prevalence of BP180 antibodies in patients with CD, DH, and T2D. Autoantibodies from DH and DPP-4-using T2D patients reacted towards intra-cellular and mid-extracellular epitopes of BP180 which are atypical in BP.

Stromal cell-derived factor 1 (SDF-1, a main DPP-4 substrate) staining was shown to be increased in the BP epidermis and its levels were increased in the plasma of T2D, BP and aged controls, and the use of DPP-4i decreased its levels.

The use of vildagliptin causes the highest risk for BP among all the drugs studied. Exposure of mice to vildagliptin induced a change in the level of 165 proteins in mice skin without any cutaneous morphological changes. Many of the proteins were linked to actin and biological processes, including transport and movement of cell or subcellular components, organization of cytoskeleton and cell organelles, and myeloid cell homeostasis.

The results of this study do not support epitope spreading from transglutaminase autoantibodies to BP180 as an explanation for the increased risk of BP in DH. Analysis of mouse skin proteome revealed potential candidate proteins whose further study could shed light on the pathogenesis of BP.

Keywords: autoantibodies, bullous pemphigoid, celiac disease, collagen XVII, dermatitis herpetiformis, dipeptidyl peptidase-4 inhibitor, type 2 diabetes mellitus

Nätynki, Antti, Kollageeni XVII -autovasta-aineet ihokeliakiapotilailla ja gliptiinilääkitystä käyttävillä diabeetikoilla ja vildagliptiinin vaikutus hiiren ihon proteomiin
Oulun yliopiston tutkijakoulu; Oulun yliopisto, lääketieteellinen tiedekunta; Medical Research Center Oulu; Oulun yliopistollinen sairaala
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Tiivistelmä

Rakkulainen pemfigoidi on lähinnä iäkkäillä esiintyvä ihon autoimmuunisairaus, johon liittyy voimakas kutina, suurten nestetäytteisten rakkuloiden ja punoittavien muutosten ilmaantuminen vartalon, vatsan ja raajojen iholle sekä lisääntynyt kuolleisuus. Pemfigoidin patogeneesissä ihon tyvikalvon rakenneproteiini kollageeni XVII:ää (BP180) vastaan muodostuneet IgG-luokan vasta-aineet ovat keskeisiä.

Ihokeliakia ja tyypin 2 diabeteksen (T2D) hoidossa käytettävät dipeptidyyli peptidaasi-4 estäjät (DPP 4i) eli gliptiinit lisäävät merkittävästi pemfigoidin riskiä mekanismilla, jota ei tällä hetkellä tiedetä.

Tällä tutkimuksella haluttiin selvittää ihokeliakian, gliptiinien ja pemfigoidin välistä yhteyttä analysoimalla BP180 autovasta-aineiden esiintyvyys ja kohde-epitoopit keliakia-, ihokeliakia-, pemfigoidi- ja T2D-potilailla. Myös transglutaminaasivasta-aineet määritettiin keliakia-, ihokeliakia- ja pemfigoidipotilailta.

Tutkimusaineistoon sisältyi 43 keliakia-, 46 ihokeliakia- ja 61 pemfigoidipotilasta, 136 gliptiinejä käyttävää ja 136 gliptiinejä käyttämätöntä T2D-potilasta sekä 82 kontrollinäytettä.

Tutkimuksessa havaittiin matala BP180 vasta-aineiden esiintyvyys keliakia-, ihokeliakia- ja T2D-potilailla sekä vasta-aineiden pemfigoidille epätyypillisiä kohde-epitooppeja BP180:n solunsisäisiä ja solunulkoisten osien keskialueita kohtaan ihokeliakiapotilailla ja gliptiinejä käyttävillä T2D-potilailla.

Merkittävän DPP-4 substraatin SDF-1:n ekspression osoitettiin lisääntyneen pemfigoidipotilaiden epidermiksessä ja sen pitoisuus verenkierrossa oli kohonnut T2D- ja pemfigoidipotilailla ja iäkkäillä verrokeilla. Gliptiinejä käyttäneillä osoitettiin alemmat SDF-1-tasot.

Hiirten altistus eniten pemfigoidiriskiä lisäävälle lääkkeelle, vildagliptiinille, muutti 165 proteiinin ilmentymistä hiiren ihossa ilman histologisia muutoksia. Monet näistä proteiineista liittyivät solun tukirangan ja solukomponenttien järjestäytymiseen, aktiiniin, solujen liikkumiseen, solun komponenttien kuljetukseen ja myeloidien solujen homeostaasiin.

Tutkimuksen tulokset eivät tue vasta-aineiden epitoopin leviämistä transglutaminaasista BP180:een selityksenä kohonneelle pemfigoidiriskille ihokeliakiassa. Gliptiinien käyttö vaikutti BP180 autovasta-aineiden epitooppeihin mutta ei niiden prevalenssiin. Hiiren ihon proteomista paljastui proteiineja, joiden lisätutkimus voi lisätä ymmärrystä pemfigoidin patogeneesistä.

Asiasanat: autovasta-aineet, dipeptidyylipeptidaasi 4:n salpaaja, ihokeliakia, keliakia, rakkulainen pemfigoidi, tyypin 2 diabetes, tyypin XVII kollageeni

--To the making of many books there is no end, and much devotion to them is wearisome to the flesh.
-New World Translation of the Holy Scriptures, 2013,
Ecclesiastes 12:12

--paljolla kirjojen tekemisellä ei ole loppua, ja paljo omistautuminen niille väsyttää ihmisen.
-Pyhä Raamattu - Uuden maailman käännös, 2018,
Saarnaaja 12:12

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Oulu, November 2023 Antti Nätynki

Abbreviations

aa	amino acids
BM	basement membrane
BMZ	basement membrane zone
BP	bullous pemphigoid
BP180	bullous pemphigoid 180 kDa antigen, collagen XVII
BP230	bullous pemphigoid 230 kDa antigen, dystonin
CD	celiac disease
CI	confidence interval
CXCR4	CXC chemokine receptor 4
DEJ	dermal-epidermal junction
DH	dermatitis herpetiformis
DPP-4	dipeptidyl peptidase-4
DPP-4i	dipeptidyl peptidase-4 inhibitor
ELISA	enzyme-linked immunosorbent assay
GST	glutathione-S-transferase
HLA	human leukocyte antigen
IgG	immunoglobulin class G
IL	interleukin
kDa	kilodalton
NC16A	non-collagenous 16th A domain
SDF-1	stromal cell-derived factor-1, CXC chemokine ligand 12
STRING	Search tool for retrieval of interacting genes
TG2	tissue transglutaminase
TG3	epidermal transglutaminase
Th	T helper cell
T2D	type 2 diabetes mellitus
2D-DIGE	two-dimensional difference gel electrophoresis

Publications

This thesis is based on the following publications which are referred to throughout the text by their Roman numerals:

I Nätynki, A., Tuusa, J., Hervonen, K., Kaukinen, K., Lindgren, O., Huilaja, L., Kokkonen, N., Salmi, T., & Tasanen, K. (2020) Autoantibodies against the immunodominant bullous pemphigoid epitopes are rare in patients with dermatitis herpetiformis and coeliac disease. *Frontiers in Immunology*. 11:575805. https://doi: 10.3389/fimmu. 2020.575805

II Nätynki, A., Leisti, P., Tuusa, J., Varpuluoma, O., Huilaja, L., Izumi, K., Herukka, S. K., Ukkola, O., Junttila, J., Kokkonen, N., & Tasanen, K. (2022) Use of gliptins reduces levels of SDF-1/CXCL12 in bullous pemphigoid and type 2 diabetes, but does not increase autoantibodies against BP180 in diabetic patients. *Frontiers in Immunology*. 13:942131. https://doi: 10.3389/fimmu.2022.942131

III Nätynki, A., Kokkonen, N., Tuusa, J., Ohlmeier, S., Bergmann, U., & Tasanen, K. (2024) Proteomic changes related to actin cytoskeleton function in the skin of vildagliptin-treated mice. *Journal of Dermatological Science*. In press. https://doi.org/10.1016/j.jdermsci.2024.01.003

Contents

1 Introduction

Autoimmune diseases are conditions where the body develops an abnormal immune response against its own tissues that leads to damage or dysfunction of those tissues (Murphy & Weaver, 2017, pp. 654–657). An autoimmune reaction is mediated by auto-reactive B and T cells against normal components of the body, i.e., autoantigens, which in most cases of autoimmune diseases are proteins (Murphy & Weaver, 2017, p. 669). Activated B cells produce antibodies and if antibodies recognize autoantigens, they are called autoantibodies. Autoantibodies are the mainstay of diagnosis of autoimmune diseases and provide biomarkers for studying disease activity and their underlying mechanisms. However, all healthy individuals also carry numerous autoantibodies and have autoreactive T cells that cause no clinical symptoms, and their prevalence increases with age (Dillon et al., 2020; Haller-Kikkatalo et al., 2017; Nagele et al., 2013; Shome et al., 2022). The function of these "naturally" occurring autoantibodies is unclear (Shome et al., 2022).

Autoimmune diseases are usually chronic progressive diseases, but their symptoms can be transient, appearing suddenly as a flare-up, and resolve spontaneously and stay in remission for years. In most cases, no single factor can be pinpointed as a cause of an autoimmune disease; instead, a combination of genetic predisposition and certain environmental factors like infections are known to trigger them, but not all predisposed individuals develop autoimmune disease after coming into contact with triggering factors (Murphy & Weaver, 2017, pp. 634, 669). More than 80 different autoimmune diseases are known; they vary in severity and can be tissue-specific, like type 1 diabetes and multiple sclerosis, or systemic, like systemic lupus erythematosus, and can affect almost any part of the body including the skin (Gutierrez-Arcelus et al., 2016; Murphy & Weaver, 2017, p. 653). One major group of autoimmune diseases of the skin are autoimmune blistering skin diseases of the pemphigus group, pemphigoids, and dermatitis herpetiformis (DH), which is a dermatosis affecting gluten-sensitive celiac disease (CD) patients (Salmi, 2019; Schmidt & Zillikens, 2013).

Bullous pemphigoid (BP) is the most common autoimmune subepidermal blistering skin disease and it mostly affects the elderly. (Bağcı et al., 2017; Borradori et al., 2022). The highest increased risk for BP development is associated with neurodegenerative diseases, DH, and the use of certain drugs, especially dipeptidyl peptidase-4 inhibitors (DPP-4is, also known as gliptins) that are used to control blood glucose levels in type 2 diabetes (T2D) (Försti et al., 2016; Varpuluoma et al., 2018, 2019).

BP was described already in the 1950s (Lever, 1953, pp. 26–31) and the presence of BP autoantibodies was demonstrated in the 1960s (Beutner & Jordon, 1964; Jordon et al., 1967). Even though BP pathogenesis is relatively well characterized, not much is known about what causes the break of immune tolerance towards DEJ proteins and the time frame in which the autoantibodies are formed relative to the clinical disease. Also, there is no explanation why DH and the use of DPP-4is increase the risk of BP. These unanswered questions inspired this study.

2 Review of the literature

2.1 Clinical presentation of bullous pemphigoid

Bullous pemphigoid (BP) is the most common subepidermal blistering skin disease that usually affects patients over 70 years of age but may be seen at all ages (Hammers & Stanley, 2020; Schmidt & Zillikens, 2013; Waisbourd-Zinman et al., 2008). BP typically presents with intense itch and fluid-filled tense bullae and erythema, often with urticarial plaques (Schmidt & Zillikens, 2013) (Figure 1). Bullae may rise locally, but most often generalized blistering is seen in the trunk and extremities (Borradori et al., 2022). Erupted blisters leave erosions and crusts that heal without scarring. Sometimes bullae are absent, and itch is the only symptom, and it can precede blistering by weeks or sometimes by several months. 10–20% of BP patients also present blistering of the mucous membranes, mainly in the oral cavity (Bağcı et al., 2017).

Fig. 1. Patient with bullous pemphigoid (Department of Dermatology, Oulu University Hospital). Generalized blistering on erythematous skin and erosions can be seen.

2.2 Incidence and mortality of bullous pemphigoid

BP is a rare disease with a relatively low incidence. In a recent meta-analysis, the annual BP incidence was estimated to be 34.2 cases per million person-years in Europe and North America (Persson et al., 2022). In children, BP is very rare, with a prevalence of 4.9 per million children in Germany (Hübner et al., 2020; Schwieger-Briel et al., 2014). There seems to be no gender differences in the BP incidence (Brick et al., 2014; Försti et al., 2014). The incidence of BP has been rising over the last decades: In the years 1980–1989 the cumulative global incidence of BP was 5.0, in 1990–1999 it was 5.9, in 2000–2009 it was 9.3, and from 2010 onwards, 10.1 (Persson et al., 2022). Similar doubling or tripling of the BP incidence during the past two decades was reported in the United Kingdom, France, Germany, and Israel (Kridin & Ludwig, 2018). In Finland, the age-corrected incidence of BP was 14 new cases per million person-years during the years 1985–2009 and 20 new cases per million person-years during the years 2005–2009 with a mean patient age of 77 years (Försti et al., 2014). Age increases the incidence of BP dramatically, up to 190–312 cases per million person-years among patients 80 years and older (Kridin & Ludwig, 2018). The rising incidence of BP is explained by the aging populations, an increase in age-related BP risk factors, especially neurodegenerative diseases, increased exposure to trigger drugs, and improved diagnostics and awareness of BP (Försti et al., 2014; Kridin & Ludwig, 2018; Rozenblat et al., 2019; Schmidt & Zillikens, 2013).

BP is a severe chronic disease with significant mortality and morbidity. BP patients carry high 1-year mortality of 20–40%, which is 2–3 times higher than mortality in age- and sex-matched controls (Bağcı et al., 2017; Borradori et al., 2022). In a recent systematic review and meta-analysis, 1-year mortality of BP globally was estimated to be 23.5% (95% confidence interval (CI) 20.2–26.8), being higher in Europe (26.7%; 95% CI 22.2–31.2) than in Asia (20.5%; 95% CI 13.8–27.3) or USA (15.1%; 95% CI 7.9–22.3) (Kridin et al., 2019). In the Israeli population, 1-year mortality of BP was estimated to be 26.9% and the risk of death was 3.4 times higher than that in the general population (Kridin et al., 2019). Persson et al. reported a mortality rate of 2.7 times that in the general population in the first two years following the BP diagnosis in the population of the United Kingdom (Persson et al., 2021). BP patients in general are in poorer health condition and suffer more frequently from neurodegenerative diseases and T2D than age-matched controls (Försti et al., 2016; Guo et al., 2023). The main contributing factors to the mortality rate of BP are not the severity or extent of the disease itself, but rather, the age of the patients at disease onset and probably the common adverse effects from the long-term use of glucocorticoids (Deotto et al., 2021; Zeng et al., 2022).

2.3 Diagnosis of bullous pemphigoid

In addition to clinical examination by a dermatologist, the golden standard of BP diagnostics is the direct immunofluorescence staining of the skin biopsy taken from a perilesional skin area 1–2 cm next to the bullae (Borradori et al., 2022). In a positive BP case, direct immunofluorescence reveals linear staining of immunoglobulin class G (IgG) antibodies and/or complement C3 at the dermal-epidermal junction (DEJ) of the skin (Figure 2). These antibodies are formed against BP antigen 180 (BP180 or collagen XVII, or BP antigen 2 (BPAg2)) and BP antigen 230 (BP230 or BPAG1e, or dystonin) (Diaz et al., 1990; K. Li et al., 1993; Uitto & Christiano, 1992; H. Ujiie, 2023). Indirect immunofluorescence on salt-split skin can be used to distinguish BP from other clinically closely related dermatoses like epidermolysis bullosa acquisita. Patient serum is applied on salt-split normal human skin, where incubation of the skin section in 1 molar NaCl solution has separated the epidermis from the dermis, creating an artificial subepidermal blister. BP autoantibodies directed against the BP180 appear as linear staining on the epidermal side of the blister (Figure 2) (Nishie, 2014). Histology of the biopsy taken directly from a small bulla typically reveals subepidermal bullae with eosinophils in the blister fluid and on the upper dermis at the epidermal-dermal border (Figure 2). To a lesser extent, neutrophils can be seen in the blister fluid and upper dermis (Borradori et al., 2022; Hammers & Stanley, 2020; Schmidt & Zillikens, 2013).

Circulating anti-BP180 and anti-BP230 autoantibodies from the patient sera can also be measured using commercial enzyme-linked immunosorbent assays (ELISA), but values over the positivity cut-off in ELISA do not alone warrant the BP diagnosis (Keller et al., 2016; Z. Liu et al., 2019; Thoma-Uszynski et al., 2004). Circulating anti-BP180 autoantibodies are present in 80–90% of BP patient sera (H. Ujiie, 2023) and 0–4.2% in healthy persons (Mai, Izumi, Mai, et al., 2022; Prüßmann et al., 2015; Sitaru et al., 2007; Wieland et al., 2010). Of the patients with other dermatoses, 0–14.3% are reported to have anti-BP180 autoantibodies (Z. Liu et al., 2019). Measuring levels of circulating BP autoantibodies by BP180-NC16A ELISA can be used to monitor disease progression. For research purposes, BP autoantibodies are analyzed to determine their target epitopes and the sequence and timing of the break of immunotolerance against BP antigens (E. H. Lee et al., 2012; Mai, Izumi, Mai, et al., 2022; Sitaru et al., 2007).

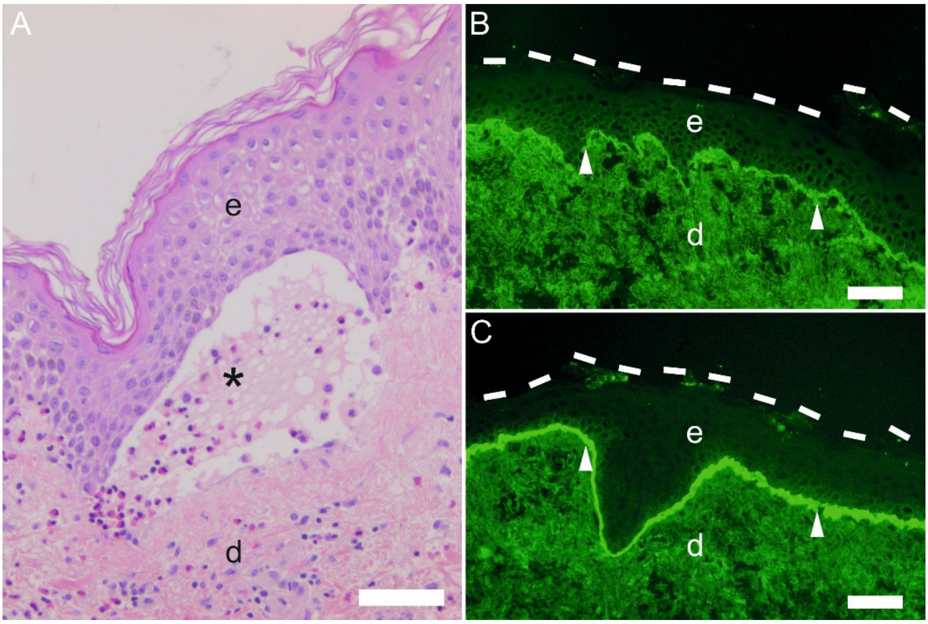

Fig. 2. Typical histological findings of a bullous pemphigoid patient diagnostic perilesional skin sample. (A) A subepidermal blister containing blister fluid and infiltrated inflammatory cells is visible in hematoxylin-eosin staining of the skin. (B) Linearly deposited immunoglobulin G indicated by the arrow heads is visible at the dermal-epidermal junction of the skin in direct immunofluorescence (C) Strong linear staining of complement C3 is seen in the dermal-epidermal junction as indicated by the arrow heads in direct immunofluorescence. A dashed line marks the epidermal edge. 20x magnification. Scalebars = 50 µm. Abbreviations: d, dermis; e, epidermis; *, blister

2.4 Treatment of bullous pemphigoid

The mainstay treatment for BP is topically administered high-potency glucocorticoids (0.05% clobetasol propionate cream) or alternatively, systemic glucocorticoids (initial dose of 0.5 mg/kg/day prednisone) (Borradori et al., 2022). If glucocorticoids are contraindicated due to comorbidities such as diabetes, severe osteoporosis or cardiovascular diseases or extensive BP unresponsive to glucocorticoids, other immunosuppressive drugs such as methotrexate, azathioprine, mycophenolate mofetil, and mycophenolate acid may be used as adjuvant therapy (Borradori et al., 2022; Guignant et al., 2022). The use of these drugs leads to disease control, in other words, a stop of an eruption of new bullae and healing of existing bullae usually in 3 weeks in 90% of the cases. Unfortunately, relapses are common and around 40% of patients relapse during the first year after the initial BP diagnosis (Joly et al., 2009; I. Ujiie et al., 2021).

The use of glucocorticoids and steroid-sparing immunosuppressive drugs has been a mainstay treatment for autoimmune diseases for over half a century. Even though glucocorticoids are highly effective in suppressing autoimmune reactions, their long-term use poses a high risk of adverse effects such as potentially fatal infections, blood cytopenia, osteoporosis, diabetes mellitus, hypertension, and gastrointestinal ulcers, which are thought to contribute to the high mortality of BP (Joly et al., 2002; Saag & Furst, 2023; Zeng et al., 2022).

New biological drugs with more precise targeting of the immune system and fewer side effects have shown promising results and may be considered in treating refractory BP cases, although their efficacy and safety are not yet validated in clinical trials (Borradori et al., 2022). These new biologics include rituximab, an antibody that depletes autoantibody-producing CD20^{+} B cells (Ly et al., 2023; Thomas et al., 2020), interleukin (IL) 4 and IL-13 blocking antibody dupilumab, and circulating IgE-depleting antibody omalizumab (Borradori et al., 2022; James et al., 2019; Zhou et al., 2021).

2.5 Dermo-epidermal junction

The dermo-epidermal junction (DEJ) or cutaneous basement membrane zone (BMZ) is the interface of the epidermis and dermis and attaches these layers to each other. Components of the BMZ are synthesized by keratinocytes located in the epidermis and fibroblasts, the major cellular component of the dermis. The basement membrane (BM) situated between the epidermis and the dermis provides a platform for complex signaling to enable dynamic shaping of the tissue structure during embryogenesis, growth, and wound healing (Pozzi et al., 2017). BM is attached to the underlying dermis via anchoring fibrils composed of type VII collagen. Basal cells, i.e., keratinocytes located in the basal layer of the epidermis are attached to the underlying BM with structures called hemidesmosomes (Figure 3). Hemidesmosomes are multiprotein complexes consisting of α6β4 integrins, plectin, laminin-332, BP180, BP230, and CD151/tetraspanin-24 (Roig-Rosello & Rousselle, 2020; Shih et al., 2020; Walko et al., 2015). Hemidesmosomes are necessary for maintaining epidermal homeostasis but they are also dynamically disassembled and reassembled during cell migration and wound healing (Walko et al., 2015). Mutations in several BMZ components, such as BP180, laminin-332, and/or α6β4 integrin, result in a group of congenital blistering skin diseases called epidermolysis bullosa, which involve skin fragility and blistering (Has et al., 2018).

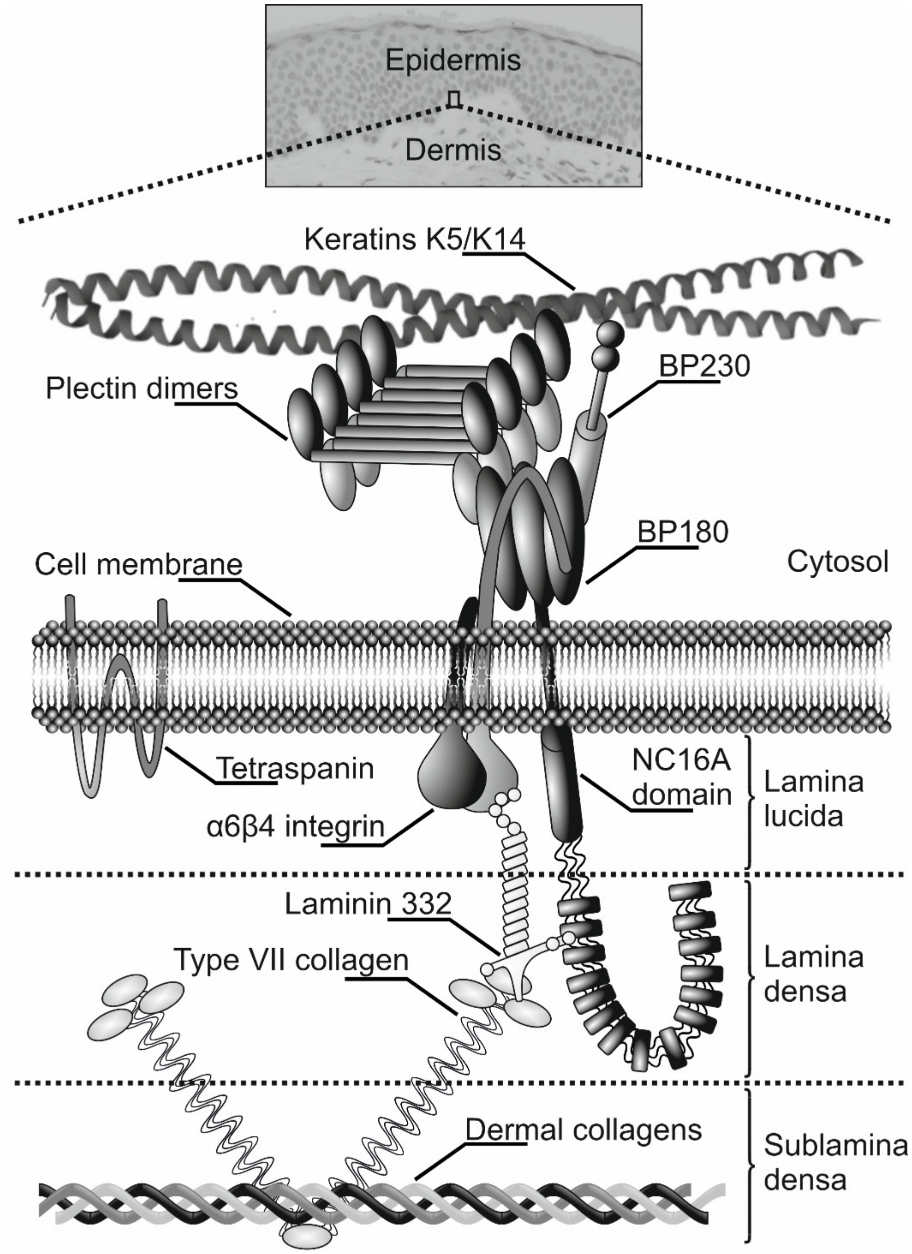

Fig. 3. A schematic representation of dermo-epidermal junction and hemidesmosomal proteins. Proteins and structures are not in scale. Modified from Schmidt & Zillikens 2013, Walko et al., 2015, and Shih et al., 2020)

2.6 BP180/collagen XVII

BP180 is a type II transmembrane protein that is an essential part of hemidesmosomes containing multiple binding sites for other hemidesmosomal proteins. The globular amino-terminal end of BP180 binds to hemidesmosomal plaque proteins BP230, plectin, and integrin β4 in the cell cytosol. The extracellular carboxyterminal end spans lamina lucida and binds to type VII collagen anchoring fibers in lamina densa via α6 integrin, type IV collagen, and laminin-332 (Figure 3) (Goletz et al., 2017; Roig-Rosello & Rousselle, 2020; Tuusa et al., 2021). In this way, BP180 interconnects intracellular keratin filaments of the basal layer keratinocytes to the underlying BM laminin network (Tuusa et al., 2021). BP180 is also expressed in several other tissues, such as the kidneys, intestines, and the brain, where its function is largely unknown (Tuusa et al., 2021).

BP180 extracellular part consists of 15 collagenous domains interspersed by 16 non-collagenous domains. Juxtamembranous 16th non-collagenous domain A (NC16A) is the immunodominant region of the BP autoantibodies (Nishie, 2020). BP180 is natively expressed in keratinocytes as a homotrimer, made up of three identical 180 kilodalton (kDa) α1-chains (Nishie, 2020). BP180 collagenous triple helix is cleaved at the NC16A domain by matrix metalloproteinases forming a 120 kDa soluble “ectodomain”. Proper BP180 cleavage is required for keratinocyte detachment and mobility during normal skin regeneration and wound healing (Franzke et al., 2009; Tuusa et al., 2021).

2.7 Autoimmunization and pathomechanism of bullous pemphigoid

Autoimmunization is a process in which the body produces autoantibodies against its own tissue constituents (Pisetsky, 2023). Autoimmunization typically develops through stages of initiation and propagation and may fluctuate between periods of resolution and exacerbation, clinically seen as phases of disease remission and flares, respectively. Healthy individuals have T and B cells that are able to react to both autoantigens and pathogens, and these cells are considered beneficial for immunity and regulatory mechanisms exist to prevent their activation against autoantigens (Boehncke & Brembilla, 2019; Z. W. Sun et al., 2021). Autoimmunization together with defective control of self-reactive T and B cells is thought to be a fundamental mechanism behind autoimmune diseases in which both genetic and environmental factors play a role (Genovese et al., 2019; Rosenblum et al., 2015).

Autoimmunization is thought to occur by one of three main mechanisms: Molecular mimicry, where foreign and self-epitopes share similar structures that antibodies cross-react with; epitope spreading, where prolonged inflammation leads to formation of new epitopes; and bystander activation, where T or B cells can be activated without encountering their specific antigens by strong enough indirect signals such as cytokines, chemokines, and pathogen-associated molecular patterns released during an infection (Fujinami et al., 2006; Pacheco et al., 2019). Combined with failure in their control systems, these three non-mutually exclusive normal mechanisms of the immune system can together lead to autoimmune disease over time (Pacheco et al., 2019).

After the initiation phase of the autoimmune disease with subclinical symptoms, the propagation phase starts presenting progressive tissue-specific or systemic inflammation. Chronic inflammation eventually damages tissues, which could introduce new antigens/neoepitopes by unmasking cryptic epitopes or altering the antigenicity of the damaged tissue components (Chan et al., 1998; Rosenblum et al., 2015; Tofigh et al., 2023; Vanderlugt & Miller, 2002). Lymphocytes can naturally produce clones that recognize new secondary epitopes on the initiating antigen to defend against mutating pathogens and increase the affinity of existing antibodies. This phenomenon of switching/spreading antigen targets of antibodies over time is called epitope spreading. Progression of the autoimmune disease is often accompanied by epitope spreading, which can start a vicious cycle where neoepitopes activate new lymphocytes with different specificities that target additional inter- or intramolecular epitopes furthering inflammation reaction, which leads to more tissue damage and neoepitope formation, which again activates a new set of lymphocytes (Rosenblum et al., 2015). This process can be active for years before clinical symptoms of the autoimmune disease transpire, and pre-clinical autoantibodies have been found to exist years before clinical symptoms in autoimmune diseases such as systemic lupus erythematous (Arbuckle et al., 2003) and type 1 diabetes (Jacobsen et al., 2020), making it very difficult to pinpoint the initial cause of the disease (Rosenblum et al., 2015). Intramolecular epitope spreading from extracellular BP180-NC16A to other BP180 epitopes and intermolecular epitope spreading between BP180 and BP230 has been observed both in BP patients and murine BP models (Di Zenzo et al., 2011; Didona & Di Zenzo, 2018; Hashimoto et al., 2011; H. Ujiie, 2023).

2.7.1 Breakage of immune tolerance

Knowledge about the breakage of immune tolerance in BP is scarce. In general, it is thought that age-related immunosenescence leads to loss of control mechanisms that prevent activation of T and B cell clones against self-antigens. Immunosenescence is a process of immune system dysregulation, characteristic of elderly patients. It leads to weakened immune responses, an increase in production of low-affinity IgG1 and IgG4 autoantibodies, and chronic low-level inflammation (Deotto et al., 2021). Increased T helper (Th) 2 cell and Th17 cytokine-mediated chronic inflammation seen in BP together with compromised skin barrier function could be speculated to lead to DEJ damage and epitope spreading in the elderly (Y. I. Lee et al., 2021). Reduced immunity can be hypothesized to lead to persistent infections that would increase the likelihood of the production of antibodies directed against pathogens' epitopes that could be cross-reactive with DEJ proteins, thus increasing the risk of BP in the elderly by molecular mimicry mechanism (Moro et al., 2020).

The two systems called central and peripheral tolerance limit the generation and activation of autoreactive T and B lymphocytes. Central tolerance encompasses the elimination of autoreactive T cells in the thymus and B cells in the bone marrow by a process of negative selection, where apoptosis is induced in lymphocytes expressing high affinity self-antigen-binding T- and B-cell receptors (Nemazee, 2017; Xing & Hogquist, 2012). CD4$^+$Foxp3$^+$ regulatory T cells have an essential role in maintaining peripheral tolerance by suppressing the activation of other immune cell types in secondary lymphoid organs (Sakaguchi et al., 2020; H. Ujiie, 2023; Vignali et al., 2008). A mouse model lacking regulatory T cells spontaneously develops anti-BP230 antibodies (Haeberle et al., 2018). However, the role of regulatory T cells in BP pathogenesis is unclear (H. Ujiie, 2019).

2.7.2 Bullous pemphigoid autoantibodies

The relative proportion of IgG1, IgG4, and IgE class of autoantibodies directed against the hemidesmosomal proteins BP180 and BP230 at the DEJ is thought to be central in the heterogeneous pathogenesis of BP (Ellebrecht et al., 2022). Circulating anti-BP180 IgG autoantibodies are present in 80–90% of BP patient sera and their levels correlate with the disease activity, pruritus severity, peripheral blood eosinophil counts, and disease duration, but no similar connection has been shown for anti-BP230 autoantibodies (Hashimoto et al., 2017; E. H. Lee et al., 2012). It seems that anti-BP230-only BP cases present a less severe BP phenotype with a greater proportion of IgG4 autoantibodies which are unable to activate the complement system (H. Ujiie, 2022).

In a subgroup of BP patients, IgA antibodies are present at the DEJ, but their role in BP pathogenesis is still unclear (Genovese et al., 2019). In another disease of pemphigoids called linear IgA dermatosis, IgA autoantibodies are directed against a 97 kDa fragment called LABD97, which is cleaved by plasmin from the cleaved soluble 120 kDa "ectodomain" form of BP180. Plasmin has also been suggested to function in BP pathogenesis, as inhibiting plasmin alleviates symptoms in experimental BP mouse model (Hofmann et al., 2009).

In 1995, Liu and co-workers showed that the classical complement pathway activation is required for subepidermal blister formation in an experimental BP mouse model (Z. Liu et al., 1995). Using a BP180-humanized mouse model, it was later established that BP patient serum anti-BP180 IgG antibodies are able to cause blistering in neonatal BP180-humanized mice (Nishie et al., 2007). Many BP animal models have been created to elucidate the role of different anti-BP180 and anti-BP230 Ig isotypes, subclasses, and immune system cells in BP pathogenesis (Fairley et al., 2007; Q. Li et al., 2010; Papara et al., 2022; Zone et al., 2007). Recently, anti-BP180 IgE (Lin et al., 2018) and anti-BP230 IgG antibodies (Makita et al., 2021) have been shown to be able to initiate blistering in experimental BP mouse models, but their relevance to BP pathology in humans is still controversial (Di Zenzo et al., 2017; Fairley et al., 2005).

2.7.3 Bullous pemphigoid pathomechanisms

BP pathomechanisms can be summarized into four partially overlapping events: 1) activation of the complement system, 2) recruitment of the inflammatory cells, 3) release of the proteolytic enzymes by the inflammatory cells, and 4) disruption of the hemidesmosome structure directly by the autoantibodies (Deotto et al., 2021; Lo Schiavo et al., 2013). In the majority of BP cases, direct immunofluorescence analysis of perilesional skin biopsy reveals complement protein C3 staining at the DEJ, indicating the activation of the complement system (Lo Schiavo et al., 2013; Messingham et al., 2019; Romeijn et al., 2017).

Complement-independent mechanisms are also capable of causing loss of dermal-epidermal adhesion. For example, when anti-BP180 IgG and IgE autoantibodies bind to the extracellular part of the transmembrane BP180 on the surface of the basal layer keratinocytes, the BP180-antibody complex is internalized into the keratinocytes (Iwata et al., 2016; Messingham, Holahan, & Fairley, 2014), which has been shown to decrease the amount of BP180 and to weaken keratinocyte adhesion to the basement membrane *in vivo* (Hiroyasu et al., 2013; Papara et al., 2022). In addition, anti-BP180 autoantibodies have been shown *in vitro* to stimulate keratinocytes to secrete proinflammatory IL-6 and IL-8, and increased levels of these interleukins have been found in the BP skin (Y. Liu et al., 2017; Tuusa et al., 2023). IL-6 and IL-8 are chemoattractants that lure circulating macrophages, eosinophils, mast cells, and neutrophils to the upper dermis. Dermal-infiltrated inflammatory cells start a well-characterized inflammatory cascade: First, mast cells bind complement proteins C3a and C5a and via FcγRI and FcεRI receptors they are also able to bind IgG and IgE, respectively (Messingham et al., 2019; Messingham, Holahan, Frydman, et al., 2014; Y. Tanaka et al., 1995). Binding complement proteins or only IgE is enough to trigger mast cell degranulation releasing proinflammatory factors such as histamine, tumor necrosis factor α, IL-6, platelet-activating factor, and vascular endothelial growth factor. These factors induce vasodilation, increase vascular permeability, and recruit more T cells, neutrophils, and eosinophils, thus amplifying the inflammation (Freire et al., 2017; Lo Schiavo et al., 2013; Maglie et al., 2023).

Recruited and activated inflammatory cells are capable of secreting various proteinases that can cleave BP180 and other extracellular matrix proteins at the DEJ (Le Jan et al., 2014; Z. Liu et al., 1998, 2000; Riani et al., 2017; Shimanovich et al., 2004; Verraes et al., 2001). These proteinases include neutrophil-secreted neutrophil elastase and eosinophil-secreted matrix metalloproteinases, plasmin, and granzyme B (Hiroyasu et al., 2019; Hofmann et al., 2009; Nishie, 2020). The number of activated eosinophils and the amount of their secreted proteinases and other factors present in the circulation, blister fluid, and perilesional skin parallel BP disease activity (Amber et al., 2018). Proteolytic cleavage of hemidesmosomal proteins leads to subepidermal separation through the lamina lucida of the BMZ (Cozzani et al. 2018).

2.8 Risk factors of bullous pemphigoid

So far, no single causative factor has been found to trigger BP; hence, it is almost certain that a combination of various predisposing and trigger factors is required for BP manifestation. Predisposing factors like genetic susceptibility, other autoimmune diseases, aging-related co-morbidities, and the use of certain drugs have been found to contribute to the increased risk of BP (Moro et al., 2020).

2.8.1 Neurological diseases

Neurological diseases have a high prevalence ranging between 28–56% among patients with BP and they have been established to be independent risk factors for BP (Kridin & Ludwig, 2018). These diseases include different types of dementias, Parkinson's disease, multiple sclerosis, and stroke (Bastuji-Garin et al., 2011; Cordel et al., 2007; Försti et al., 2016, 2017; Langan et al., 2011; Martin et al., 2022; Taghipour et al., 2010). Patients with multiple sclerosis are 5.9–15.4 times more likely to develop BP than control populations (Försti et al., 2016; Kibsgaard et al., 2017; Langan et al., 2011). Dementia increases subsequent BP risk by 3.4–9.9-fold (Langan et al., 2011; Martin et al., 2022; Papakonstantinou et al., 2019), Parkinson's disease by 3-fold, and stroke by 1.8–4.1-fold (Langan et al., 2011; Martin et al., 2022; Papakonstantinou et al., 2019). The severity or clinical presentation of BP seems not to be affected by the co-occurring neurological diseases. Patients suffering from neurological diseases without BP carry a higher proportion of circulating anti-BP180 autoantibodies than neurologically healthy age- and sex-matched controls (Foureur et al., 2006; Kokkonen et al., 2017; Messingham et al., 2016; Tuusa et al., 2019).

2.8.2 Drugs as risk factors for bullous pemphigoid

Currently, over 90 different drugs have been suggested to aggravate or induce BP (Verheyden et al., 2020). However, in most cases the evidence for a particular drug to induce BP is weak. When considering elderly patients suffering from several comorbidities and receiving multiple medications it is difficult to pinpoint any single causative factor behind the onset of BP, especially when the time between the exposure to certain drugs and the development of symptoms can span several months or years. However, there is strong evidence of drug-induced BP for the use of DPP-4is, immune checkpoint inhibitors (programmed cell death protein-1 and programmed cell death ligand-1 inhibitors), loop diuretics, penicillin and its derivatives (Kridin & Cohen, 2021; Lopez et al., 2018; Niebel et al., 2022; Verheyden et al., 2020).

Of all the BP-associated drugs, DPP-4is carry the highest risk for BP (García et al., 2016; Tasanen et al., 2019) and of the more than 10 different DPP-4is in use, the users of vildagliptin have the highest, up to more than 10-fold increased risk for the development of BP (Kridin et al., 2023; Kridin & Bergman, 2018; Varpuluoma et al., 2018). Currently, the mechanism of how gliptins increase the risk of BP is not known. The target of gliptins is DPP-4 (also known as T cell activation antigen CD26), a multifunctional transmembrane protein expressed in most cell types including epithelial and endothelial cells and various inflammatory cells, and it is also found in soluble form in body fluids. DPP-4 is a peptidase that catalyzes the cleavage of amino-terminal X-proline and X-alanine dipeptides from the penultimate position of various polypeptide substrates, including hormones, chemokines, and neuropeptides (Patel et al., 2021). DPP-4 inactivates incretin hormones glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide and its inhibition with DPP-4is prolongs incretin-dependent insulin secretion, thereby normalizing blood glucose levels in type 2 diabetes (Ahrén, 2021; Rosenstock & Zinman, 2007). DPP-4 inhibition has many other effects including regulation of inflammatory cells, such as T cells that express DPP-4 on their surface, which is essential for their T-cell receptor-mediated activation (Durinx et al., 2000; Ikushima et al., 2000; Ohnuma et al., 2007).

One of the best characterized DPP-4 substrates is stromal cell-derived factor-1 (SDF-1), also known as CXC chemokine ligand 12, a lymphocyte- and monocyte-attracting chemokine (Janssens et al., 2018a). SDF-1 and its ubiquitously expressed receptor CXC chemokine receptor 4 (CXCR4) participate in maintaining homeostasis in tissues, for example, during inflammation, angiogenesis, and wound healing (Janssens et al., 2018a), and their cutaneous expression is up-regulated in atopic dermatitis (Z. W. Sun et al., 2021), psoriasis (Abdelaal et al., 2020; Quan et al., 2015), and keratinocyte-originating basal and squamous cell carcinomas (Quan et al., 2015). Ischemia upregulates SDF-1 via hypoxia-inducible factor 1 alpha but in diabetes, SDF-1 levels decrease despite hypoxia, which is thought to contribute to impaired wound healing in diabetes (Ceradini et al., 2004; Whittam et al., 2019).

Other reported drugs with a less certain association with BP onset mentioned in recently published European guidelines for BP management are non-steroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, and tumor necrosis factor alpha inhibitors (Borradori et al., 2022). The mechanism behind drug-associated BP is largely unknown.

2.8.3 Celiac disease and dermatitis herpetiformis

Our group has previously found a link between celiac disease (CD) and DH and increased BP incidence. CD increases the risk of subsequent BP by 2-fold and patients with DH have a substantially high, 22-fold elevated risk of developing BP (Varpuluoma et al., 2019).

CD is an inflammatory disease of the small intestines caused by gluten sensitivity in genetically susceptible individuals with the human leukocyte antigen (HLA) DQ2 or DQ8 haplotypes (Salmi, 2019). Gluten contains gliadin-peptides, which are modified by tissue transglutaminase (TG2) enzyme in small intestines. In CD, TG2-modified gliadin peptides induce the production of IgA autoantibodies directed against TG2-modified gliadin peptides and TG2, resulting in damage to small intestine epithelium (Nguyen & Kim, 2021).

DH is a cutaneous manifestation of CD and shares the same HLA risk alleles as CD. In addition to anti-TG2 antibodies found in the gut mucosa and sera of both CD and DH patients, epidermal transglutaminase 3 (TG3) IgA autoantibodies are present at the upper papillary dermis of DH patients. Symptoms of DH are intense pruritus and small blisters, papules, and excoriations on flexor surfaces, typically in the knees, elbows, and buttocks (Salmi & Hervonen, 2020). DH is more prevalent in Finland than in any other country in the world, but it is still a very rare disease. DH incidence is 4–35 per million person-years and decreasing, recently being highest in individuals 50–69 years of age (Salmi & Hervonen, 2020).

2.8.4 Genetic predisposition and other trigger factors for bullous pemphigoid

In addition to preceding diseases and medication, studies have shown an association between certain HLA alleles and BP. HLA I and II genes have immune-related functions as they encode class II major histocompatibility proteins, which are crucial in antigen presentation (Murphy & Weaver, 2017, p. 163). Various human HLA alleles are over-represented amongst BP patient populations around the world and carry a 1.6–12.3-fold risk for BP (Zhang & Wang, 2020). HLA-DQB1*03:01 and HLA-DQA1 alleles have been associated with a non-inflammatory phenotype of DPP-4i-induced BP in a Japanese population (Ozeki et al., 2023; H. Ujiie, 2018). The HLA-DQB1*03:01 allele is shared with BP and some neurodegenerative diseases, implying a possible overlapping mechanism between these diseases (Amber et al., 2017; Y. Sun et al., 2018; Zhang & Wang, 2020).

Various additional risk and trigger factors for BP onset include physical trauma caused, for example, by surgical procedures, thermal burns, bone fracture, UV-radiation, and radiotherapy (Dănescu et al., 2016; Lo Schiavo et al., 2013; Mai, Izumi, Sawada, et al., 2022; H. Ujiie, 2023). Infections, vaccines, and transplantations have also been documented in case reports to be probable causative factors of BP (Moro et al., 2020). BP often develops locally to the traumatized site, for example, on the skin area that received radiation therapy or the site of a surgical procedure. BP lesions are usually first confined to the affected site and may later spread around the body surface. It has been speculated that physical trauma breaks the BMZ structure, revealing new antigens, that increase the antigenicity of the BMZ components in predisposed individuals (Dănescu et al., 2016). As research moves forward, new trigger and predisposing factors are expected to emerge, and hopefully, better treatment of these factors could alleviate the burden caused by BP at the same time.

3 Aims of the study

Previous epidemiological studies have identified an increased risk for BP in patients with CD, DH, and T2D treated with DPP-4is, but at the moment the prevalence of BP autoantibodies in these patient groups is unknown. Also, the current understanding of the pathomechanism behind the DPP-4i-associated BP is limited. The overall goal of this thesis was to examine the mechanisms behind the breakage of immune tolerance against BP180 by setting the following specific research targets:

1. To examine the prevalence and epitopes of circulating anti-BP180 autoantibodies in patients with CD and DH and in patients with T2D with and without DPP-4i treatment.
2. To measure levels of SDF-1 in the sera of patients with BP and patients with T2D with and without DPP-4i treatment.
3. To study how the administration of vildagliptin changes the mouse skin proteome.

4 Materials and methods

The materials and methods used in this thesis are summarized in Table 1. Full descriptions and references can be found in the original publications I, II, and III, and their online supplementary materials.

Table 1. Materials and methods used in the original publications.

Level	Method	Publication(s)
DNA	DNA extraction	I
	GST-BP180 cloning	I, II
RNA	Quantitative polymerase chain reaction	III
Protein	Two-dimensional difference gel electrophoresis (2D-DIGE)	III
	BP180 epitope mapping by immunoblotting	I, II
	BP180 plasmin digestion	I
	BP180-NC16A Enzyme-linked immunosorbent assay (ELISA)	I, II, III
	Full-length BP180 ELISA	I, II
	Label-free shotgun mass spectrometry	III
	Tandem mass spectrometry fingerprinting	III
	Sodium-dodecyl-sulphate polyacrylamide gel electrophoresis	I, II, III
	Total protein concentration quantification	I, II, III
Cells	Cell culture and cell extract preparation	I, II
	Microscopy	I, II
	Transfection	I, II
Tissue	Skin collection	III
	Skin extract preparation	III
	Histological analysis	III
	Immunohistochemistry	III
	Indirect immunofluorescence	I, II, III
Animals	Vildagliptin administration to mice	III
Digital data	Densitometric image analysis	I, II, III
	Statistical analysis	I, II, III
	Gene enrichment analysis	III
	2D-DIGE analysis	III
	Tandem mass spectrometry fingerprinting analysis	III
	Label-free shotgun mass spectrometry analysis	III
	STRING analysis	III

4.1 Study populations and samples

Patient serum samples studied in Study I and Study II are listed in Tables 2 and 3.

Table 2. Patient group characteristics of Study I.

Variable	DH[1]	CD[2]	BP[3]	Control
Number of serum samples	46	43	34	48
Mean age ± SD[4] in years	48.7±15.1	48.8±13.4	79.9±7.9	49.4±4.6
Number of females/total (%)	16/46 (35)	28/43 (65)	14/34 (41)	13/48 (65)

[1] Dermatitis herpetiformis, [2] Celiac disease, [3] Bullous pemphigoid, [4] Standard deviation

Table 3. Patient group characteristics of Study II.

Variable	T2D[1]	T2D+g[2]	Control group 1[3]	BP[4]	BP+g[5]	Control group 2[6]
Number of serum samples	136	136	18	27	20	20
Mean age ± SD[7] in years	66.7±8.0	67.2±7.8	62.7±13.1	81.6±8.4	77.7±8.8	79.4±2.1
Number of females/total (%)	47/136 (35)	48/136 (35)	8/18 (44)	13/27 (48)	7/20 (35)	10/20 (50)

[1] Type 2 diabetes, [2] Type 2 diabetes with gliptins, [3] Control for type 2 diabetes, [4] Bullous pemphigoid, [5] Bullous pemphigoid with gliptins, [6] Control for bullous pemphigoid, [7] Standard deviation

4.1.1 Mouse samples

In Study III, six eight-week-old C57BL/6JOlaHsd strain male mice were exposed to a daily dose of 40 mg/kg vildagliptin. Six sex- and age-matched mice without vildagliptin treatment were used as controls. After 12 weeks, the mice were euthanized, and dorsal skin was collected.

4.2 Ethical aspects and permissions

The research ethics committees of Tampere University Hospital, Finland, Kuopio University Hospital, Finland, and the Northern Ostrobothnia Hospital District, Finland reviewed and approved the prospective collection of human DH, CD, BP, and control sera. Additional control samples were obtained from the Northern Finland Biobank Borealis, Oulu, Finland. The ARTEMIS and OPERA studies and the re-sampling of participants of the ARTEMIS study have been approved by the research ethics committee of the Northern Ostrobothnia Hospital District. All patients/participants provided their written informed consent to participate in this study. Studies were performed according to the principles of the Declaration of Helsinki. The Southern Finland Regional State Administrative Agency has approved the use of mice (license number ESAVI/10181/04.10.07/2017). The animal care principles and experimental procedures are in accordance with national Finnish legislation, the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS 123), and EU Directive 2010/63/EU. 3R principles were followed to minimize the suffering of experimental animals.

5 Results

5.1 BP180 and transglutaminase autoantibody profiles in patients with celiac disease, dermatitis herpetiformis, and bullous pemphigoid (I)

As DH carries a substantial risk for BP, we hypothesized that epitope spreading from TG autoantibodies which are pathognomonic for DH, to BP180 could explain the increased risk of BP in patients with DH. To test this hypothesis, we analyzed the prevalence of anti-TG2 and -TG3 autoantibodies in the BP sera and the prevalence of BP180 autoantibodies in CD and DH patient sera.

Using well-established commercial ELISA systems, we found that the prevalence of anti-BP180-NC16A autoantibodies was low and at a similar level in patients with DH and CD and in the control group. Furthermore, anti-TG2 and anti-TG3 autoantibodies were rare in BP patients (Study I, Figure 1). As expected, most of the DH and CD patients had anti-TG2 IgA autoantibodies (33/46, 71.7% and 31/43, 72.1%, respectively), but none of the BP samples and only a few of the control samples (3/48, 6.3%) were anti-TG2 IgA-positive. Additionally, anti-TG3 autoantibodies were frequently observed in DH patient sera (29/46, 63.0%), but only one CD (1/43, 2.3%) and one BP (1/34, 2.9%) serum and none of the control sera were TG3-positive (Study I, Figure 1, and Table 1).

Most of the BP sera (28/34, 82.4%) were classified as positive for anti-BP180-NC16A IgG antibodies, and only a few were found to be positive in the DH group (3/46, 6.5%), CD group (2/43, 4.7%), and control group (2/48, 4.2%) (Study I, Table 1). All three positive DH sera were also positive for anti-TG2 and anti-TG3 autoantibodies and the patients had not followed a gluten-free diet at the time when serum samples were drawn. None of the anti-BP180-NC16A-positive DH, CD, or control patients had BP-like skin symptoms.

Serum reactivity against full-length recombinant BP180 produced in COS-7 cells using immunoblotting was studied in all the anti-BP180-NC16A-positive sera and in addition, in randomly selected anti-BP180-NC16A-negative DH, CD, and control sera. A relatively high proportion of DH, CD, and control sera (11/20, 55.0%; 8/20, 40.0%; 8/21, 38.1%, respectively) contained IgG antibodies that recognized the full-length BP180 (Study I, Table 1). It is known that IgA antibodies are formed against plasmin-digested 97-kDa fragment of BP180 in patients with linear IgA dermatosis (Hofmann et al., 2009). We therefore also studied DH and

CD patients' serum IgA and IgG antibodies' reactivity against neoepitopes produced from partially plasmin-digested full-length and 120-kDa ectodomain of recombinant BP180. (Study I, Table 1). In general, a fainter signal in immunoblotting was detected for IgA than for IgG autoantibodies (Study I, Figure 2). Plasmin digestion decreased the recognition of BP180 and 120-kDa ectodomain by IgG autoantibodies compared to the recognition of intact full-length BP180. Plasmin-digested 120-kDa ectodomain was recognized by IgA autoantibodies in 25% of the CD and DH sera, in 30% of the BP sera, but also in 13.6% of the control sera (Study I, Table 1).

Comprehensive epitope mapping was performed for a subset of CD and DH patient sera using glutathione-S-transferase (GST)-BP180 fusion proteins spanning the whole BP180 polypeptide to determine the IgG antibodies' BP180 epitopes. Autoantibodies from none of the DH or control sera and from only one CD serum recognized immunodominant NC16A domain-containing fusion protein (Study I, Table 1). DH samples differed from CD and BP samples by recognition of amino-terminal and mid-extracellular portions of BP180 (amino acids (aa) 2–168 and 847–994 or 1116–1178, respectively), which are atypical epitopes for BP (Study I, Figure 3).

Indirect immunofluorescence analysis showed that IgA antibodies in CD (*n* = 9), DH (*n* = 13), or control group (*n* = 9) sera did not react with the DEJ. Only two CD and DH sera showed faint IgG depositions at DEJ (Study I, Figure 4).

5.2 Prevalence and characteristics of BP180 IgG autoantibody in patients with type 2 diabetes (I, II)

The use of DPP-4is has been frequently associated with an increase in BP incidence, but the mechanism behind this association is currently unknown. To examine whether the use of DPP-4is increases the prevalence of anti-BP180 autoantibodies among T2D patients we utilized the same ELISA and immunoblotting methods as in Study I and in addition, we also used full-length BP180 ELISA.

For Study II we obtained serum samples from the ARTEMIS (Junttila et al., 2018) and OPERA (Terho et al., 2019) research projects which had been previously conducted in the Oulu University Hospital. The ARTEMIS and OPERA patient cohorts contained 136 T2D patients who were using DPP-4i medication at the time the serum samples were collected. For control samples, we randomly selected 136 age- and sex-matched T2D controls not using DPP-4is. DPP-4is were often used in combination with metformin and sitagliptin was the most prescribed DPP-4i

(125/136, 92%), and a few patients were using either linagliptin, vildagliptin or saxagliptin.

IgG autoantibodies against the BP180-NC16A domain were found in 8/136, 5.9% of the DPP-4i group and 9/136, 6.6% of the non-DPP-4i group sera as determined by ELISA (Study II, Table 1). No differences were seen in the median circulating autoantibody levels between the groups. When we checked serum IgG autoantibody reactivity against the full-length BP180 in ELISA, we found out that 28% of the DPP-4i group were positive, but in the non-DPP-4i group, the positivity rate was even higher, 32% (Study II, Table 1). None of the positive cases had BP-like skin symptoms.

We invited patients for a follow-up visit where we collected serum samples and performed dermatological examination about nine years after the initial samples had been drawn. Only 57 out of 174 subjects (33%) accepted our invitation, probably due to the COVID-19 pandemic, the old age of the patients, or due to living far away from Oulu. No major changes in IgG seropositivity against the full-length BP180 or BP180-NC16A domain were observed during the follow-up period (Study II, Figure 1).

We also determined serum anti-BP180 IgG autoantibody epitope specificities of all the BP180-NC16A ELISA-positive T2D cases and age- and sex-matched ELISA-negative T2D cases and healthy controls using immunoblotting. The percentage of sera detecting full-length BP180 from whole cell COS-7 cell extract was 71% in the DPP-4i group, 89% in the non-DPP-4i group, and 46% in the age- and sex-matched healthy controls (Study II, table 2). IgG autoantibodies of the T2D patients recognized BP180-GST-fusion proteins containing amino-terminal polypeptides (aa 2–168, 261–401, or 377–455) more often than antibodies in healthy control sera. Serum autoantibodies of non-DPP-4i T2D patients recognized mid-extracellular (aa 661–865) and carboxy-terminal (aa 1278–1497) BP180 epitopes more frequently than autoantibodies from the healthy controls or DPP-4i T2D group (Study II, Figure 2, and Supplementary Tables 2–4).

5.3 Gliptin treatment reduces circulating SDF-1 levels in patients with type 2 diabetes and bullous pemphigoid (II)

SDF-1 is a chemokine and one of the best characterized DPP-4 substrates (Janssens et al., 2018a). We examined whether the use of DPP-4is affects the circulating levels of SDF-1 in patients with T2D by using an ELISA system that measures the most abundant isoform of SDF-1, the SDF-1α. In addition, we compared the level of SDF-1α between patients with regular and DPP-4i-associated BP. Age- and sex-matched healthy controls for the T2D group (mean age in years 62.7 ± 13.1) had lower levels of SDF-1α than T2D cases or older controls for the BP group (mean age in years 79.4 ± 2.1). DPP-4i usage decreased SDF-1α levels in both T2D and BP groups below those seen in the healthy controls matched for the T2D group (Study II, Figure 3). BP patients without DPP-4i usage had similar levels of SDF-1 as their age- and sex-matched controls.

5.4 The cutaneous expression of SDF-1 is increased in bullous pemphigoid patients (II)

We studied the expression and localization of SDF-1 by immunostaining SDF-1 in the perilesional skin of BP patients (*n* = 11) and healthy control skin (*n* = 5). SDF-1 was not detected in the healthy control skin. In the skin of BP patients, the upper layers of the epidermis were strongly stained, and a few SDF-1-positive cells were seen in the dermis. There was no difference in SDF-1 immunostaining between DPP-4i- and non-DPP-4i-associated BP skin. In the skin section containing a blister, in addition to the strongly stained upper layers of the epidermis, numerous SDF-1-positive cells were seen in the blister fluid and blister margins (Study II, Figure 4).

5.5 Vildagliptin treatment alters mouse skin proteome (III)

To elucidate the possible mechanisms and signaling pathways of how gliptins mediate the increased risk of BP we administered vildagliptin to mice for 12 weeks. During the experiment, vildagliptin-treated mice (*n* = 6) did not differ from the control group (*n* = 6) in terms of body weight, behavior, or clinical appearance.

We used two-dimensional difference gel electrophoresis (2D-DIGE) and label-free shotgun mass spectrometry methods to analyze the cutaneous proteome of the mice. Proteome analysis of the skin of the mice revealed significant changes in the levels of 165 proteins between vildagliptin-treated mice (*n* = 4) and controls (*n* = 5) (Study III, Figure 1). Out of these proteins, 18 showed increased levels and 40 showed decreased levels in the skin of vildagliptin-treated mice, and 101 were detected only in the skin of control mice and six only in the skin of vildagliptin-treated mice (Study III, Tables 1–3). Interestingly, galectin-1, a protein linked to the regulation of skin inflammation and cell-matrix adhesion (Castillo-González et al., 2021; Corrêa et al., 2017, 2022), had increased levels in shotgun mass spectrometry analysis.

Of the 165 proteins with changed protein levels between vildagliptin-treated mice and controls detected in either 2D-DIGE or shotgun mass spectrometry analysis, three had significantly differing levels observed in 2D-DIGE. Subsequent peptide mass fingerprinting identified these proteins as beta-actin, APOBEC2, and moesin; their levels were increased by vildagliptin (Study III, Figure 1). Both label-free shotgun mass spectrometry and 2D-DIGE analysis showed similar vildagliptin to control mice ratios for beta-actin (1.90 and 1.76, respectively) and for APOBEC2 (1.61 and 1.52, respectively) protein levels (Study III, Table 1, and Table 3). In addition, 2D-DIGE analysis showed increased moesin levels (ratio of 1.75) in the skin of vildagliptin-treated mice, but no difference was observed in label-free shotgun mass spectrometry analysis. Interestingly, two neighboring protein spots with the same molecular weight as moesin were visible in 2D-DIGE and further analysis revealed them also to be moesin with different isoelectric points, suggesting that the spots were some post-translational modifications of moesin (Study III, Supplementary Figure S2). We stained the gels with a phosphoprotein-specific fluorescent dye, but observed no signal from the two moesin spots, meaning that the moesin spots were not phosphorylated, and the post-translational modification of moesin remains unknown.

5.5.1 Biological processes associated with cytoskeleton organization are enriched in vildagliptin-treated mice (III)

The search tool for retrieval of interacting genes (STRING) (Szklarczyk et al., 2019) was used to predict potential biological processes that the proteins with significantly increased or decreased levels in the vildagliptin-treated mouse skin participate in. Biological processes with the strongest evidence of predicted interactions within proteins with increased levels in the vildagliptin-treated mice included transport and movement of cell or subcellular components as well as myeloid cell homeostasis (Study III, Supplementary Table S1). Several of the proteins were linked directly or indirectly to beta-actin (Study III, Supplementary Figure S5). The biological processes enriched with proteins with decreased levels in the vildagliptin-treated mice compared to controls or detected only in the control group included processes such as organization of the actin filaments, cytoskeleton or cell organelle organization, cell migration, and myeloid cell homeostasis (Study III, Supplementary Table S2).

5.5.2 Vildagliptin treatment affects protein localization in mouse skin (III)

Proteins found to be vildagliptin-induced in both 2D-DIGE and label-free shotgun mass spectrometry analysis (beta-actin and APOBEC2) were selected for additional analyses. We also included proteins linked to autoimmunization and skin diseases such as moesin, which regulates actin cytoskeleton and cell membrane remodeling (K. Kawaguchi et al., 2017; Solinet et al., 2013), and galectin-1, which is up-regulated in inflammatory skin diseases, and its protein levels were increased by vildagliptin (Study III, Table I). Furthermore, DPP-4 as the main vildagliptin target and BP180 as a main BP autoantigen were analyzed in detail. Of these selected proteins, we measured their mRNA levels with quantitative polymerase chain reaction, and cutaneous protein levels using immunoblotting and analyzed their localization in the skin using immunohistochemistry.

No morphological changes or presence of infiltrated inflammatory cells in the skin of vildagliptin-treated mice were seen. However, immunohistochemical staining of the skin revealed differences in the localization and amount of DPP-4, galectin-1, and moesin (Study III, Figure 2). DPP-4 was strongly stained in the BMZ of the skin of the control mice, but in the skin of the vildagliptin-treated mice no basal layer epidermal staining of DPP-4 was seen, and dermal staining of DPP-4 was lower than in the control mice. Galectin-1 was stained mainly in the cells of the dermis in the skin of the control mice, whereas in the vildagliptin-treated mice, galectin-1 was seen in individual basal layer cells of the epidermis. Moesin was observed in patches of basal layer epidermal cells in control mice and numerous strongly stained fibroblasts in the dermis. Vildagliptin-treated mice displayed a similar but weaker staining pattern of moesin (Study III, Figure 2). These differences were not detected at the mRNA level or the protein level using quantitative polymerase chain reaction and immunoblotting methods, respectively (Study III, Supplementary Figures S3–S4). Beta-actin, APOBEC2, and BP180 showed no differences between groups using immunohistochemistry (Study III, Supplementary Figure S6), immunoblotting, or quantitative polymerase chain reaction methods (Study III, Supplementary Figures S3–S4).

6 Discussion

6.1 Prevalence of the BP180 autoantibodies in celiac disease, dermatitis herpetiformis, type 2 diabetes, and bullous pemphigoid patients

It is known that in many autoimmune diseases autoantibodies can be found years before the diagnosis (Arbuckle et al., 2003; Jacobsen et al., 2020; Ma et al., 2017; Raneses et al., 2022). Anti-BP180 autoantibodies are found in the majority of BP patients during the initial BP diagnosis (Kobayashi et al., 2002; E. H. Lee et al., 2012; Matsumura et al., 1996; Zillikens et al., 1997), but currently it is largely unknown how long before the development of cutaneous symptoms anti-BP180 autoantibodies can be detected and what is the prevalence of these autoantibodies in patients with increased risk for BP.

To the best of our knowledge, no larger studies involving anti-BP180 autoantibody measurement from CD and DH patients have been previously conducted. A few cases of concomitant BP and DH have been reported but without accompanying measurement of circulating or tissue-bound antibodies to both TG2/TG3 and BP180 (Didona & Di Zenzo, 2018; Schulze et al., 2013). Our study showed that only a minority of CD (4.7%), DH (6.5%), and T2D patients (5.9% for DPP-4is users and 6.5% for non-DPP-4is users) had anti-BP180-NC16A IgG autoantibodies and their levels measured in ELISA were much lower than in BP. Their prevalence was at a similar level to that of healthy controls (4.2%), and similar rates have been reported for elderly patients with pruritic disorders (Feliciani et al., 2009; Van Beek et al., 2014).

Studies examining circulating anti-BP180-NC16A IgG autoantibody prevalence and levels between “regular” BP and DPP-4i-associated BP have shown a lower number of seropositive (~80–90% and ~60%, respectively) and lower antibody levels in DPP-4i-associated BP (Horikawa et al., 2018; Y. Kawaguchi et al., 2019; Salemme et al., 2022; Ständer et al., 2021). The use of DPP-4is did not affect BP180-NC16A IgG antibody prevalence or levels in our T2D study population. Our results were similar to those of a Japanese study where no difference was found in anti-BP180-NC16A autoantibody prevalence in T2D patients using DPP-4is compared to T2D patients not using DPP-4is (Izumi et al., 2019).

DH patients in our study cohort (19/46) had been on a gluten-free diet for 23 years on average (Mansikka et al., 2019). The chronic inflammation that could damage BMZ in untreated DH was probably absent due to the patients' adherence to a gluten-free diet, and it could be speculated that in untreated DH the anti-BP180 autoantibody prevalence could be higher. The mean age of DH patients (~49 years) was 20 years younger than recently reported in BP patients with a preceding DH diagnosis (68.8 years) (Varpuluoma et al., 2019), which could explain why no BP cases with preceding DH or CD were found in our study cohort. BP patients with concomitant DH are rare and only few case reports describing few dozen individuals have been published (Didona & Di Zenzo, 2018; Schulze et al., 2013). Varpuluoma et al. (2019) reported that 1.2% of the BP patients in Finland had preceding DH (Varpuluoma et al., 2019). Aging is the main BP risk factor, and our group has previously shown that non-pathogenic anti-BP180 autoantibodies are more prevalent in aged Alzheimer's disease patients than in age-matched controls without neurological diseases and that they can bind to BMZ (Kokkonen et al., 2017). BP180 autoantibodies are also increased in other neurological diseases such as multiple sclerosis and Parkinson's disease (Messingham et al., 2016; Tuusa et al., 2019) where prolonged inflammation in the nervous system is speculated to lead to neoepitope formation and production of autoantibodies targeting neuronal BP180 which could be cross-reactive against cutaneous BP180 (Seppänen, 2013).

Epitope spreading from TG2 to TG3 likely takes place when CD develops into DH (Kárpáti et al., 2018). Similarly, intermolecular epitope spreading has been described between BP180 and BP230 in BP (Didona & Di Zenzo, 2018; H. Ujiie, 2023). Intermolecular epitope spreading from TG2/TG3 to BP180 offers a plausible explanation for DH developing into BP (Didona & Di Zenzo, 2018) which we tested in this study. However, all BP patients were negative for TG2 antibodies and only one had IgA anti-TG3 autoantibodies. On the other hand, the CD and DH patients who had TG2 and TG3 antibodies did not have more anti-BP180-NC16A-autoantibodies than healthy controls. Taken together, our current results do not support the hypothesis that the increased risk of BP in patients with DH and CD is due to intermolecular epitope spreading from TG2 or TG3 to BP180. However, our study does not fully exclude the possibility that with time, intramolecular epitope spreading could take place from the non-pathogenic BP180 epitopes to the immunodominant BP180-NC16A domain in elderly CD and DH patients. A much larger population of elderly CD and DH patients would be needed to test this.

In chronic inflammation, plasmin plays a role in neoepitope formation in linear IgA dermatosis disease. Plasmin cleaves BP180 ectodomain into 97 and 120 kDa fragments which are then targeted mainly by IgA autoantibodies (Hofmann et al., 2009) which are able to cause blistering in passive transfer mouse model (Zone et al., 2004). However, we found a similar number of positive sera (25–30%) in the BP, CD, and DH groups and a relatively high proportion (14.5%) of positive healthy control sera reacting towards plasmin-digested BP180. In addition, there were only a few CD and DH sera that simultaneously had IgA and IgG antibodies against the plasmin-treated BP180. We conclude that our results do not support the assumption that plasmin plays a role in producing BP180-derived neoepitopes recognized by anti-BP180 autoantibodies in DH.

Immunoblotting revealed IgG reactivity against full-length BP180 in over half of the DH sera, which is similar to the number of positives in patients with multiple sclerosis who have the highest risk of BP among neurological patients (Lai et al., 2016; Tuusa et al., 2019). IgG autoantibodies recognizing full-length BP180 were also found in a relatively large proportion of CD and DH and healthy controls and the majority of T2D sera. In the DPP-4i T2D group, the number of positives was lower than in the non-DPP-4i group (71% and 89%, respectively), and DPP-4i T2D group serum autoantibodies had weaker reactivity against full-length BP180. When BP180-NC16A IgG ELISA positive and negative T2D samples were compared, there was no difference in full-length BP180 reactivity. The number of sera from patients with CD, DH, or T2D containing IgG autoantibodies towards the full-length BP180 was relatively high, similar to our earlier findings from patients with Alzheimer's disease and multiple sclerosis (Kokkonen et al., 2017; Tuusa et al., 2019). Aging is presumed to affect the higher prevalence of full-length BP180 autoantibodies, and the Alzheimer's disease patients and T2D patients in our study population were older and presented higher anti-full-length BP180 autoantibody prevalence than the CD or DH patients.

We observed no difference in either anti-full-length BP180 autoantibody prevalence or serum levels between DPP-4i T2D and non-DPP-4i T2D patients using ELISA. Our results are in line with the work of Izumi et al. (2019) who reported more frequent but non-significant full-length BP180 ELISA positivity in DPP-4i-using than in non-DPP-4i-using T2D patients (10.9% vs 5.6%) and higher antibody titers in the DPP-4i T2D group (non-significant) (Izumi et al., 2019). Their study population was of similar age as ours and sitagliptin was also the most prescribed DPP-4i. Different genetic backgrounds can be a contributing factor to these observed differences.

The mean follow-up time of nine years in our study should be sufficiently long to reveal possible DPP-4i-induced BP cases as the mean latency between the initiation of DPP-4i usage and the BP diagnosis ranges from three to 27 months (Benzaquen et al., 2018; García et al., 2016; Kridin & Bergman, 2018; Lindgren et al., 2019; Plaquevent et al., 2019; Varpuluoma et al., 2018). Our study showed that up to a decade of sitagliptin use does not increase the prevalence of IgG autoantibodies against the BP180-NC16A or full-length BP180 as determined by ELISA among patients with T2D. T2D patients' age in the follow-up was ~73 years, so they were on average only four years younger than the typical DPP-4i-associated BP patients in Finland (Varpuluoma et al., 2018). Sitagliptin is the most used gliptin in Finland (Finnish Medicines Agency Fimea and Social Insurance Institution, 2011, 2021) and in our T2D study population over 90% of the DPP-4i users were prescribed sitagliptin. Sitagliptin has been shown to carry lower BP risk than vildagliptin (Arai et al., 2018; Kridin et al., 2023; S. G. Lee et al., 2019; Varpuluoma et al., 2018) and some studies have found no increase in BP risk related to sitagliptin use (Benzaquen et al., 2018; Kridin & Bergman, 2018). Interestingly, there was a single DPP-4i-using T2D case in the study cohort who developed BP during the follow-up. The patient had initially received sitagliptin but was later switched to vildagliptin before the occurrence of BP.

6.2 Characteristics of the BP180 autoantibodies in celiac disease, dermatitis herpetiformis, type 2 diabetes, and bullous pemphigoid patients

Intramolecular epitope spreading from non-pathogenic BP180 epitopes to immunodominant NC16A domain has been described in BP and suggested as a possible explanation for BP pathogenesis (Di Zenzo et al., 2004, 2011; Hashimoto et al., 2011; H. Ujiie, 2023). In the anti-BP180 autoantibody epitope mapping, we found that most individuals in the CD, DH, and T2D groups and the control samples had antibodies against intracellular and mid-extracellular BP180 epitopes, and the combination of recognized epitopes varied widely between individuals. When interpreting our results, it should be considered that control samples also recognized some BP180 epitopes. Currently, it is not known whether the presence of these autoantibodies is related to the increased risk of BP in these patient groups or by what mechanism they could increase the risk. These results are in line with our previous work where non-pathogenic IgG autoantibodies targeting the intracellular part (aa 377–455) and mid-extracellular part (aa 558–672, 802–907, and 847–994) of BP180 were found from a majority of Alzheimer's disease and multiple sclerosis patients and from healthy control without BP-like skin symptoms

(Tuusa et al., 2019). Furthermore, our group has shown that among the DPP-4i-associated BP patients, most sera contained IgG autoantibodies that targeted intracellular BP180 epitope (aa 377–455) and with weaker affinity than in the "regular" BP group (Lindgren et al., 2019). We observed similar trends with T2D sera reacting more frequently against the intra-cellular epitope (aa 377–455) than control sera and among the T2D group, sera of T2D patients using DPP-4is recognized this epitope less frequently and with lower affinity. Interestingly, Izumi et al. (2016) reported that around half of the DPP-4i-associated BP sera reacted more often to mid-extracellular BP180 epitopes and not NC16A domain, and this was linked to less severe non-inflammatory BP phenotype (Izumi et al., 2016). Our group has not observed this difference in clinical presentation between "regular" and DPP-4i-associated BP (Lindgren et al., 2019). Together with our previous findings of modified recognition of BP180 intra-cellular epitopes in multiple sclerosis, Alzheimer's disease, and DPP-4i-associated BP, and other research groups' studies on DPP-4i-associated BP, the differences seen in our T2D study population suggest that DPP-4is might modify BP180 degradation or immunogenicity of BP180.

Intramolecular epitope spreading from NC16A to BP180 intra-cellular epitopes and low pathogenicity of intra-cellular epitopes have been demonstrated in different BP animal models (Di Zenzo et al., 2010; H. Ujiie et al., 2019). It has also been shown that antibodies against carboxy-terminal epitopes of BP180 are common in BP and precede antibodies targeting NC16A (Di Zenzo et al., 2008; H. Ujiie, 2023). Mai et al. (2023) showed that immunizing mice with mouse BP180 protein leads to the production of antibodies targeting the extracellular NC13 domain of mouse BP180 and that these antibodies were naturally occurring IgM class antibodies that have not undergone somatic hypermutation (Mai et al., 2023). It seems that also elderly people have natural non-pathogenic antibodies toward certain BP180 epitopes (Desai et al., 2008; Kokkonen et al., 2017). These antibodies might target cryptic epitopes that need to be unmasked, for example, by tissue destruction or inflammation before the immune system can "see" them and start producing antibodies against them.

Because none of the DH, CD, T2D, or control patients had BP-like skin symptoms, the antibodies that recognized full-length BP180 or BP180 fusion proteins in denatured form in immunoblotting or in ELISA were probably non-pathogenic. This is in line with our results using indirect immunofluorescence that only a few of the CD or DH sera contained low titer IgG or IgA antibodies targeting the DEJ. In line with our observations, Izumi et al. (2019) reported that there were no BP skin symptoms among individuals with T2D carrying anti-BP180 autoantibodies (Izumi et al., 2019). Anti-BP180 IgG autoantibodies without concomitant skin symptoms have been reported among healthy controls (Mai, Izumi, Mai, et al., 2022). Another study where anti-BP180 autoantibodies from sera of normal healthy subjects were examined using immunoblotting and epidermal extracts showed that over half of the normal sera reacted against BP180 antigen, but not against immunodominant BP180-NC16A domain (Desai et al., 2008). Our results confirm this observation but offer no support to connect the pre-clinical BP180 autoantibodies found in our T2D study cohort with increased BP risk among T2D patients. It remains unclear whether low titer anti-BP180 autoantibodies in T2D are naturally occurring or possibly prognostic for future BP development. It seems that in addition to DPP-4is, exposure to additional trigger factors is required for BP to develop among T2D patients as only a fraction of DPP-4i users develop BP. A larger cohort of elderly T2D or DH patients would probably be needed to establish the link between pre-clinical anti-BP180-autoantibodies and the risk of BP.

6.3 The cutaneous localization of SDF-1 in bullous pemphigoid and its level in the sera of patients with type 2 diabetes and bullous pemphigoid with or without gliptin treatment

SDF-1 is a major DPP-4 substrate and has a role in inflammatory skin diseases (Quan et al., 2015; Z. W. Sun et al., 2021), the pathogenesis of T2D (Whittam et al., 2019), and lymphocyte trafficking (Janssens et al., 2018b). SDF-1 levels have been shown to change due to aging (Subramanian et al., 2014), which prompted us to examine the effects of DPP-4i use on the localization of SDF-1 in the BP skin and the levels of the most abundant SDF-1α isoform in BP and T2D sera.

Using immunohistochemistry, we observed strong staining of SDF-1 in the keratinocytes located in the BP perilesional skin and inflammatory cell infiltrate in the blister fluid. SDF-1 was not detected in healthy control skin. These findings are supported by a recent study of our group that showed higher SDF-1 levels in the perilesional skin of DPP-4i-associated BP patients compared to healthy controls (Tuusa et al., 2023). Recently, Fang et al. (2023) also reported increased SDF-1 levels in BP blister fluid and suggested that CD3+ T cells are the source of SDF-1 in BP skin (Fang et al., 2023). Furthermore, it was demonstrated that SDF-1 receptor CXCR4 is up-regulated in B cells at the lesional skin of BP patients (Fang et al., 2023). This suggests that cutaneous expression of SDF-1 could lure CXCR4$^+$

B cells into the dermis. We did not perform double staining to characterize the infiltrated cells in the blister fluid and blister margins, but based on their morphology, they were highly probably eosinophils or neutrophils. However, it has been pointed out that circulating eosinophils hardly express CXCR4, the receptor of SDF-1 (Nagase et al., 2000), and it is found only in a small population of circulating monocytes (Sandblad et al., 2015). In line with our findings, it has been shown that epidermal keratinocytes express SDF-1 during wound healing (Nishiguchi et al., 2018), and in psoriasis, staining of SDF-1 is strongly increased in hyperproliferative keratinocytes (Abdelaal et al., 2020). Quan et al. (2015) demonstrated convincingly that in normal human skin, dermal fibroblasts are the main source of SDF-1, and keratinocytes, but not fibroblasts, express CXCR4. They also showed that the addition of recombinant SDF-1 promotes keratinocyte proliferation and inhibition of CXCR4 reduces keratinocyte proliferation (Quan et al., 2015). The cleavage of SDF-1 by DPP-4 inhibits its binding to CXCR4 (Janssens et al., 2017), which implies that DPP-4is could change the trafficking of CXCR4$^+$ lymphocytes to skin or alter epidermal homeostasis. In addition, SDF-1 is known to upregulate matrix metalloproteinase 9, which is thought to be important in cleaving BP180 in BP (Franzke et al., 2009; Parmo-Cabañas et al., 2006). It is plausible that the increased epidermal SDF-1 we observed in the BP skin could increase matrix metalloproteinase activity and promote blistering in BP, which deserves further study.

Our results corroborate previous findings that SDF-1α levels are high in the elderly (Carbone et al., 2017; Subramanian et al., 2014). Our elderly control group had higher circulating SDF-1α levels than the younger control group (mean age 79.4 and 62.7 years, respectively). Using human-derived primary keratinocytes and keratinocyte organoid and mouse model, Nishiguchi et al. (2018) have shown that aging suppresses SDF-1 levels (Nishiguchi et al., 2018). Age-related decline in SDF-1 could be directly linked to a decline in the number of SDF-1-producing fibroblasts in aged skin (Wlaschek et al., 2021).

We found that circulating SDF-1α levels in T2D and BP sera taken from patients with DPP-4i-treatment were decreased compared to healthy age-matched control groups. This is in accordance with our recent work, which showed a similar decrease of cutaneous SDF-1 levels in DPP-4i-associated BP compared to healthy controls (Tuusa et al., 2023). We also found that in the non-DPP-4i T2D group, SDF-1α levels were increased compared to healthy age-matched controls and DPP-4i-treated T2D group, respectively. Our observation of increased circulating SDF-1α levels in T2D has been observed in other studies (Aso et al., 2015;

Derakhshan et al., 2012; Park et al., 2017). Complications of diabetes have been reported to increase plasma SDF-1 levels, for example, in patients suffering from diabetic nephropathy (Lu et al., 2021). A short period of four weeks of sitagliptin treatment of patients with poorly controlled T2D has been reported to increase plasma SDF-1α levels (Fadini et al., 2010). It is logical to assume that a short-term DPP-4 inhibition raises circulating SDF-1 levels, as soluble circulating DPP-4 rapidly cleaves SDF-1α in plasma (Janssens et al., 2018a). However, it is possible that some feedback mechanism down-regulates SDF-1α levels when its degradation by DPP-4 is inhibited for longer periods. Another explanation is that long-term use of DPP-4is reduces the concentration of SDF-1α by increasing the activity of another SDF-1 receptor, atypical chemokine receptor 3, that functions as a scavenging receptor for SDF-1 (Janssens et al., 2018b; Spinosa et al., 2017). Additional functional studies are warranted to further decipher the role of SDF-1 and its receptors in BP pathogenesis.

6.4 Mouse cutaneous proteome changes due to vildagliptin treatment

Since its launch in the year 2007, vildagliptin has been widely used to treat T2D and it has a low incidence of adverse effects (Mathieu et al., 2017). However, it carries the highest increased risk for BP of all the gliptins and in fact, of all the drugs associated with BP (Kridin et al., 2023; Kridin & Bergman, 2018; Tasanen et al., 2019; Varpuluoma et al., 2018). The mechanism by which gliptins increase the risk of BP is unknown; therefore, we studied how vildagliptin exposure affects the cutaneous proteome of mice to get clues of what effects long-term use of DPP-4is might have on the skin. Our study revealed that vildagliptin treatment changed the levels of 165 proteins in the mouse skin, many of which were associated with cytoskeleton and actin.

In the 2D-DIGE analysis of the mouse skin increased levels of beta actin, APOBEC2, and moesin were seen in the vildagliptin group compared to control animals. Not much is known about the function of APOBEC2 protein, especially related to the biology of the skin. It has been associated with hepatocyte proliferation in liver cancer or infection (A. Li et al., 2019), stimulation of retina regeneration (Powell et al., 2014), and negative regulation of myoblast differentiation (Ohtsubo et al., 2017). Moesin binds to actin filaments and microtubules and connects them to the plasma membrane (K. Kawaguchi et al., 2017; Solinet et al., 2013). Using 2D-DIGE we observed three moesin spots, one of which had increased intensity in vildagliptin-treated mice, but there was no change in label-free shotgun mass spectrometry analysis. We tried to further analyze the moesin protein spots with displayed charge shift in 2D-DIGE using phosphorylated protein dye, but it did not show any signal from the shifted spots. Acetylation is another alternative for post-translational modification behind the charge shift, but it remains to be tested.

Many inflammatory and immunological disorders are linked to the disruption of the actin cytoskeleton (Papa et al., 2021). For example, anti-desmoglein 1 and 3 antibodies in pemphigus vulgaris have been shown to disrupt keratinocyte attachment and actin dynamics (Gliem et al., 2010). BP180 binds to actin cytoskeleton via the actinin-4 linker protein complex which has been shown to affect keratinocyte migration and actin cytoskeleton (Hiroyasu et al., 2016). Further studies are needed to elucidate whether vildagliptin exposure could disrupt the actin dynamics of basal keratinocytes or other skin-resident cells and how this contributes to BP pathogenesis.

Immunohistochemical analysis showed that in vildagliptin treated mice cutaneous staining of DPP-4 was decreased. Twelve-week exposure to vildagliptin did not cause visible changes in mouse skin appearance or histology, which is in line with other studies using other murine models exposed to vildagliptin (Shimizu et al., 2012; Tsuboi et al., 2016). One preclinical study reported blistering on cynomolgus monkeys after 3 weeks of vildagliptin treatment that resolved two weeks after the treatment was stopped (Hoffmann et al., 2014). Based on these studies, T2D rodent models seem to be resistant to vildagliptin-induced skin symptoms. Still, a relatively short period of vildagliptin treatment in our study induced a change in the levels of several cutaneous proteins.

In closer analysis using label-free shotgun mass spectrometry, many of the proteins whose levels were changed were determined to be cytoskeleton components or associated with cell actin cytoskeleton and microtubule organization (Study III, Tables 1–3, and Supplementary Tables S1–S2). STRING analysis revealed that most of the cutaneous proteins with increased levels in the vildagliptin-treated mice were linked to actin and were associated with the transport and movement of subcellular components (Study III, Supplementary Figure S5, and Supplementary Table S1). Many of the proteins whose levels were decreased in the vildagliptin-treated mouse skin were associated with actin filament polymerization, cell migration, intracellular transport, and the organization of the cellular components and the cytoskeleton. Structural proteins of the cytoskeleton including actin and tubulin and cytoskeleton-interacting proteins like actinins were increased in the vildagliptin group (Study III, Table 2, and Supplementary Table S2).

The actin cytoskeleton may have a role in biological processes leading to the loss of immunological tolerance and cell responses to autoantibodies. Actin-dependent processes like phagocytosis in antigen-presenting cells and macropinocytosis in several cell types including keratinocytes might alter proteolytic processing and presentation of neoantigens. Hiroyasu et al. (2016) have demonstrated that anti-BP180 autoantibodies mediate the internalization of BP180 via micropinocytosis from the surface of keratinocytes which weakens their attachment to the substrate (Hiroyasu et al., 2016). Moesin might also be linked to actin-mediated macropinocytosis and interestingly, SDF-1-CXCR4 signaling activates macropinocytosis (G. Tanaka et al., 2012; Yang et al., 2023).

Epidermal and circulating moesin expression has been demonstrated to increase in psoriasis patients (Maejima et al., 2014), whereas, in our study, moesin staining was reduced in the epidermis in vildagliptin-treated mice. We were unable to determine which signaling pathways might be affecting the decreased moesin expression in the mouse skin. Moesin deficiency has been linked to decreased CD8$^+$ regulatory T cell numbers and systemic lupus erythematosus-like autoimmunity (Satooka et al., 2017).

Galectin-1 levels were elevated in vildagliptin-treated mice skin compared to control mice. Galectin-1 was stained in dermal cells and basal layer epidermal cells. Galectin-1 is expressed in many immune cells such as dendritic cells and participates in regulating T helper cell populations and promotes Th2 type immune response (Sundblad et al., 2017). It also binds to B cell receptors and amplifies B cell activation (Sundblad et al., 2017). Inflammatory cells have been shown to express galectin-1 in lesional skin of atopic dermatitis and psoriasis patients and also in keratinocytes in psoriatic skin (Corrêa et al., 2022). The potential role of galectin-1 in promoting inflammation in DPP-4i-associated BP remains to be studied.

In vildagliptin-treated mice skin, DPP-4 staining was lost in the epidermis and decreased in the dermis. Lower DPP-4 expression is related to Th2 responses and BP is skewed towards Th2 responses, and DPP-4 inhibition suppresses Th1 cytokine production (Gurgel Penaforte-Saboia et al., 2021). Many DPP-4 substrates are cytokines, but no changes in cytokine levels were observed in 2D-DIGE or label-free shotgun mass spectrometry, which was expected as they are not constitutively expressed in the absence of inflammation and their isoelectric point fell outside the pH range used in our 2D-DIGE analysis.

Even though beta-actin and APOBEC2 protein levels were increased as determined by 2D-DIGE, we did not observe these differences by immunoblotting or immunohistochemical staining of mouse skin or at the messenger RNA level. A likely explanation is that 2D-DIGE can distinguish polypeptides of the same protein with different molecular weights or charges, but immunoblotting cannot distinguish signals originating from the polypeptides with equal molecular weights, missing those differentially expressed polypeptides found in 2D-DIGE. We ruled out differences in protein solubilities in 2D-DIGE and immunoblotting sample preparation as a cause of differing results between these methods by preparing new immunoblotting samples using the same protocol as with the 2D-DIGE samples. This did not change the results of the immunoblotting analyses and we chose to omit these findings. Also, differences in affinities of the used antibodies towards native and denatured forms of the proteins present in immunohistochemically stained skin sections and immunoblotting samples, respectively, can explain the different results between these methods.

In the clinical setting, only a small proportion of DPP-4i-treated patients with T2D develop BP (Ogura & Shiraishi, 2023; Plaquevent et al., 2019), meaning that additional genetic or environmental factors likely contribute to the breakage of immunotolerance against BP180 in BP. Our study of vildagliptin-treated mouse proteome revealed several proteins associated with inflammation and immune reactions (Study III, Supplementary Tables S1–S2). Their further study could bring new insights into the pathogenesis of BP.

6.5 Strengths and limitations of the study

CD and DH are both rare diseases. We had well-characterized CD and DH patient samples, but their number was too low, and the patients were too young for the development of BP symptoms to appear among the study population. Our study was still worth conducting because anti-BP180 autoantibodies have previously been found in a similar number of patients with neurological diseases (Kokkonen et al., 2017; Tuusa et al., 2019).

Our study populations in Study I and Study II contained only Finnish subjects, which limits the generalizability of our results to similar Caucasian populations.

We chose to examine only anti-full-length BP180 and anti-BP180-NC16A autoantibodies and might therefore have missed autoantibodies targeting other hemidesmosomal proteins that interact with BP180 such as BP230, laminin 322, and plectin, which could contribute to epitope spreading. However, autoantibodies against these other proteins are in general much rarer and have weaker affinity than anti-BP180 autoantibodies, and therefore, their prevalence would probably have been even lower than that of anti-BP180 autoantibodies in our study.

Due to the retrospective cross-sectional study design our study cannot explain possible causal relationships between BP180 autoantibodies and increased risk of BP in DH or T2D. Neither it is possible to establish a causal relationship between changes in SDF-1 levels or localization and BP or between SDF-1 levels and the use of DPP-4is. Measuring BP180 antibodies repeatedly, for example, in DH patients in a prospective study would be needed to verify a causal relationship between BP180 autoantibodies and possible epitope spreading, but it would not be feasible in practice because of the large number of DH patients and the long follow-up time required.

Epitope spreading from TG2/TG3 to BP180 could probably be more common in DH patients not following a gluten-free diet, but it would not be ethical to try to get DH patients to abstain from adhering to a gluten-free diet for long periods for research purposes.

In Study II, data on the duration of gliptin usage was incomplete for a large number of samples for the nine-year follow-up period to allow us to use the length of gliptin usage as a continuous variable in the analyses.

For future studies, a larger number of mice would be preferable to account for the high variation in protein levels observed in individual samples and to standardize the exact location of dorsal skin samples used for immunoblotting analysis.

As a relatively small portion of vildagliptin-treated T2D patients develop BP, other factors besides gliptins are probably needed to trigger BP. We could have used a T2D mouse model to study the effects of vildagliptin, but there are already published studies reporting no skin symptoms with gliptin-treated diabetic mice (Shimizu et al., 2012; Tsuboi et al., 2016). Vildagliptin has been reported to cause skin symptoms in cynomolcus monkeys (Hoffmann et al., 2014). This might reflect differences between rodent and primate physiology and the fact that in addition to vildagliptin, other trigger factors are needed to induce skin reactions in mice.

The strength of our study was the large number of well-characterized T2D samples in the previously published OPERA and ARTEMIS cohorts. This enabled us to form randomly selected age- and sex-matched control groups and achieve high statistical power in determining BP180 autoantibody levels.

Serum samples were taken several years ago so we were able to follow changes in the health state of patients. Unfortunately, we were able to collect follow-up samples from only ~20% of the patients.

We used well-established standard statistical tests and laboratory methodologies and carefully optimized them. For example, we used the necessary positive and negative control sera together with study samples to validate immunoblotting and ELISA protocols to minimize false positive and false negative findings. Different antigen retrieval protocols and antibody dilutions were tested to optimize the immunohistochemistry assays.

We adjusted the amount of protein in the mouse skin extracts to avoid false positive results in immunoblotting and used total protein normalization to increase the reliability of immunoblotting results.

6.6 Future prospects

BP and other autoimmune diseases have complex etiologies, and no single causative factor can probably be found behind the breakage of tolerance against proteins of BMZ. Advances in understanding the early stages of BP pathomechanisms could improve BP diagnostics and prognosis of patients. Better treatment and prevention of BP risk factors could help to slow down the increase of BP incidence in aging populations. Currently, BP is treated with systemic corticosteroids that carry many severe side effects, and more targeted therapies with fewer side effects are needed. Basic research in the field of immunology as well as systems biology approaches could reveal new candidate molecules for better targeted therapies for BP.

The results from Study III form a starting point for the planning of functional experiments to test, for example, the possible effects the knock-down, overexpression, or inhibition/stimulation of one of the differentially expressed proteins would have on *in vitro* cell culture models. It would be interesting to study the proteome of the skin of patients with regular and DPP-4i-associated BP and patients with T2D using DPP-4i-treatment and compare the results with current data on mouse skin proteome. Common changes in mice and human skin proteome could be the focus of further functional studies based on animal and cell culture models to elucidate the impact of these proteins on skin biology. Different omics approaches could be used to decipher the complex interaction of environmental and genetic factors behind the breakage of immunotolerance towards BMZ proteins in BP or in inflammatory dermatoses in general. In other autoimmune skin diseases, the gut-skin axis has recently been shown to be significant in their pathogenesis, and this field could be also studied in the context of BP.

7 Conclusions

The findings of this study increase the knowledge about the BP pathogenesis. As BP is challenging to treat and its incidence is increasing, better understanding of the disease pathogenesis and its risk factors is needed to improve the care of patients with BP and to raise awareness of BP development in high-risk groups. Research on BP might lead to new discoveries in other autoimmune diseases.

The following conclusions can be drawn based on Studies I–III:

1. The prevalence of anti-BP180-NC16A IgG autoantibodies was low in patients with CD, DH, and T2D. Epitope spreading was not observed from TG2/3 to BP180 in patients with CD, DH, or BP, but this does not exclude the possibility that epitope spreading might take place in more elderly patients. The study suggests that in addition to anti-BP180 autoantibodies, additional triggering factors are needed to lead to autoimmunization against BP180 in CD, DH, and T2D patients under 60 years of age.
2. Human serum contains non-pathogenic autoantibodies that recognize linear BP180 epitopes but not native BP180 epitopes at the DEJ.
3. DPP-4is might modulate BP180 antigenicity or degradation and presentation by immune cells.
4. Epidermal staining of SDF-1 is increased in the skin of patients with BP.
5. Plasma SDF-1α levels are increased in T2D, BP, and aged controls and decreased in DPP-4i-treated T2D and BP patient sera, which supports earlier studies. We could not establish a causal relationship between SDF-1α expression and blistering in BP or explain how DPP-4is lower plasma SDF-1α levels.
6. Vildagliptin exposure induced change in the levels of 165 different proteins in mouse skin, many of which were related to actin cytoskeleton organization.

References

Abdelaal, N. H., Elhefnawy, N. G., Abdulmonem, S. R., Sayed, S., Saleh, N. A., & Saleh, M. A. (2020). Evaluation of the expression of the stromal cell-derived factor-1 alpha (CXCL 12) in psoriatic patients after treatment with Methotrexate. *Journal of Cosmetic Dermatology*, *19*(1), 253–258. https://doi.org/10.1111/jocd.12994

Ahrén, B. (2021). Glucose-lowering action through targeting islet dysfunction in type 2 diabetes: Focus on dipeptidyl peptidase-4 inhibition. *Journal of Diabetes Investigation*, *12*(7), 1128–1135. https://doi.org/10.1111/jdi.13564

Amber, K. T., Valdebran, M., Kridin, K., & Grando, S. A. (2018). The role of eosinophils in bullous pemphigoid: A developing model of eosinophil pathogenicity in mucocutaneous disease. *Frontiers in Medicine*, *5*, 201. https://doi.org/10.3389/fmed.2018.00201

Amber, K. T., Zikry, J., & Hertl, M. (2017). A multi-hit hypothesis of bullous pemphigoid and associated neurological disease: Is HLA-DQB1*03:01, a potential link between immune privileged antigen exposure and epitope spreading? *HLA*, *89*(3), 127–134. https://doi.org/10.1111/tan.12960

Arai, M., Shirakawa, J., Konishi, H., Sagawa, N., & Terauchi, Y. (2018). Bullous pemphigoid and dipeptidyl peptidase 4 inhibitors: A disproportionality analysis based on the Japanese adverse drug event report database. *Diabetes Care*, *41*(9), e130–e132. https://doi.org/10.2337/dc18-0210

Arbuckle, M. R., McClain, M. T., Rubertone, M. V, Scofield, R. H., Dennis, G. J., James, J. A., & Harley, J. B. (2003). Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *New England Journal of Medicine*, *349*(16), 1526–1533. https://doi.org/10.1056/NEJMoa021933

Aso, Y., Jojima, T., Iijima, T., Suzuki, K., Terasawa, T., Fukushima, M., Momobayashi, A., Hara, K., Takebayashi, K., Kasai, K., & Inukai, T. (2015). Sitagliptin, a dipeptidyl peptidase-4 inhibitor, increases the number of circulating CD34+CXCR4+ cells in patients with type 2 diabetes. *Endocrine*, *50*(3), 659–664. https://doi.org/10.1007/s12020-015-0688-5

Bağcı, I. S., Horvath, O. N., Ruzicka, T., Sardy, M., Bağcı, I. S., Horváth, O. N., Ruzicka, T., & Sárdy, M. (2017). Bullous pemphigoid. *Autoimmunity Reviews*, *16*(5), 445–455. https://doi.org/10.1016/j.autrev.2017.03.010

Bastuji-Garin, S., Joly, P., Lemordant, P., Sparsa, A., Bedane, C., Delaporte, E., Roujeau, J. C., Bernard, P., Guillaume, J. C., Ingen-Housz-Oro, S., Maillard, H., Pauwels, C., Picard-Dahan, C., Dutronc, Y., & Richard, M. A. (2011). Risk factors for bullous pemphigoid in the elderly: A prospective case-control study. *Journal of Investigative Dermatology*, *131*(3), 637–643. https://doi.org/10.1038/jid.2010.301

Benzaquen, M., Borradori, L., Berbis, P., Cazzaniga, S., Valero, R., Richard, M. A., & Feldmeyer, L. (2018). Dipeptidyl peptidase IV inhibitors, a risk factor for bullous pemphigoid: Retrospective multicenter case-control study from France and Switzerland. *Journal of the American Academy of Dermatology*, *78*(6), 1090–1096. https://doi.org/10.1016/j.jaad.2017.12.038

Beutner, E. H., & Jordon, R. E. (1964). Demonstration of skin antibodies in sera of pemphigus vaulgaris patients by indirect immunofluorescence staining. *Proceedings of the Society for Experimental Biology and Medicine*, *117*(2), 505–510. https://doi.org/10.3181/00379727-117-29622

Boehncke, W., & Brembilla, N. C. (2019). Autoreactive T-lymphocytes in inflammatory skin diseases. *Frontiers in Immunology*, *10*, 1198. https://doi.org/10.3389/fimmu.2019.01198

Borradori, L., Van Beek, N., Feliciani, C., Tedbirt, B., Antiga, E., Bergman, R., Böckle, B. C., Caproni, M., Caux, F., Chandran, N. S., Cianchini, G., Daneshpazhooh, M., De, D., Didona, D., Di Zenzo, G. M., Dmochowski, M., Drenovska, K., Ehrchen, J., Goebeler, M., … Joly, P. (2022). Updated S2 K guidelines for the management of bullous pemphigoid initiated by the European Academy of Dermatology and Venereology (EADV). *Journal of the European Academy of Dermatology and Venereology*, *36*(10), 1689–1704. https://doi.org/10.1111/jdv.18220

Brick, K. E., Weaver, C. H., Lohse, C. M., Pittelkow, M. R., Lehman, J. S., Camilleri, M. J., Al-Hashimi, M., & Wieland, C. N. (2014). Incidence of bullous pemphigoid and mortality of patients with bullous pemphigoid in Olmsted County, Minnesota, 1960 through 2009. *Journal of the American Academy of Dermatology*, *71*(1), 92–99. https://doi.org/10.1016/j.jaad.2014.02.030

Carbone, L. D., Bůžková, P., Fink, H. A., Robbins, J. A., Bethel, M., Hamrick, M. W., & Hill, W. D. (2017). Association of plasma SDF-1 with bone mineral density, body composition, and hip fractures in older adults: The cardiovascular health study. *Calcified Tissue International*, *100*(6), 599–608. https://doi.org/10.1007/s00223-017-0245-8

Castillo-González, R., Cibrian, D., Fernández-Gallego, N., Ramírez-Huesca, M., Saiz, M. L., Navarro, M. N., Fresno, M., de la Fuente, H., & Sánchez-Madrid, F. (2021). Galectin-1 expression in CD8+ T lymphocytes controls inflammation in contact yypersensitivity. *Journal of Investigative Dermatology*, *141*(6), 1522–1532.e3. https://doi.org/10.1016/j.jid.2020.10.020

Ceradini, D. J., Kulkarni, A. R., Callaghan, M. J., Tepper, O. M., Bastidas, N., Kleinman, M. E., Capla, J. M., Galiano, R. D., Levine, J. P., & Gurtner, G. C. (2004). Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nature Medicine*, *10*(8), 858–864. https://doi.org/10.1038/nm1075

Chan, L. S., Vanderlugt, C. J., Hashimoto, T., Nishikawa, T., Zone, J. J., Black, M. M., Wojnarowska, F., Stevens, S. R., Chen, M., Fairley, J. A., Woodley, D. T., Miller, S. D., & Gordon, K. B. (1998). Epitope spreading: Lessons from autoimmune skin diseases. *Journal of Ivestigative Dermatology*, *110*(2), 103–109. https://doi.org/10.1046/j.1523-1747.1998.00107.x

Cordel, N., Chosidow, O., Hellot, M.-F., Delaporte, E., Lok, C., Vaillant, L., Bernard, P., D'Incan, M., Roujeau, J. C., & Joly, P. (2007). Neurological disorders in patients with bullous pemphigoid. *Dermatology*, *215*(3), 187–191. https://doi.org/10.1159/000106574

Corrêa, M. P., Andrade, F. E. C., Gimenes, A. D., & Gil, C. D. (2017). Anti-inflammatory effect of galectin-1 in a murine model of atopic dermatitis. *Journal of Molecular Medicine*, *95*(9), 1005–1015. https://doi.org/10.1007/s00109-017-1566-9

Corrêa, M. P., Correia-Silva, R. D., Sasso, G. R. S., D'Ávila, S. C. G. P., Greco, K. V., Oliani, S. M., & Gil, C. D. (2022). Expression pattern and immunoregulatory roles of Galectin-1 and Galectin-3 in atopic dermatitis and psoriasis. *Inflammation*, *45*(3), 1133–1145. https://doi.org/10.1007/s10753-021-01608-7

Dănescu, S., Chiorean, R., MacOvei, V., Sitaru, C., & Baican, A. (2016). Role of physical factors in the pathogenesis of bullous pemphigoid: Case report series and a comprehensive review of the published work. *The Journal of Dermatology*, *43*(2), 134–140. https://doi.org/10.1111/1346-8138.13031

Deotto, M., Spiller, A., Sernicola, A., & Alaibac, M. (2021). Bullous pemphigoid: An immune disorder related to aging (Review). *Experimental and Therapeutic Medicine*, *23*(1). https://doi.org/10.3892/etm.2021.10972

Derakhshan, R., Arababadi, M. K., Ahmadi, Z., Karimabad, M. N., Salehabadi, V. A., Abedinzadeh, M., Khorramdelazad, H., Balaei, P., Kennedy, D., & Hassanshahi, G. (2012). Increased circulating levels of SDF-1 (CXCL12) in type 2 diabetic patients are correlated to disease state but are unrelated to polymorphism of the SDF-1β gene in the Iranian population. *Inflammation*, *35*(3), 900–904. https://doi.org/10.1007/s10753-011-9391-8

Desai, N., Allen, J., Ali, I., Venning, V., & Wojnarowska, F. (2008). Autoantibodies to basement membrane proteins BP180 and BP230 are commonly detected in normal subjects by immunoblotting. *Australasian Journal of Dermatology*, *49*(3), 137–141. https://doi.org/10.1111/j.1440-0960.2008.00452.x

Di Zenzo, G., Borradori, L., & Muller, E. J. (2017). The pathogenesis of pemphigus: Controversy vs complexity. *Experimental Dermatology*, *26*(12), 1271–1273. https://doi.org/10.1111/exd.13176

Di Zenzo, G., Calabresi, V., Olasz, E. B., Zambruno, G., & Yancey, K. B. (2010). Sequential intramolecular epitope spreading of humoral responses to human BPAG2 in a transgenic model. *Journal of Investigative Dermatology*, *130*(4), 1040–1047. https://doi.org/10.1038/jid.2009.309

Di Zenzo, G., Grosso, F., Terracina, M., Mariotti, F., De Pità, O., Owaribe, K., Mastrogiacomo, A., Sera, F., Borradori, L., & Zambruno, G. (2004). Characterization of the anti-BP180 autoantibody reactivity profile and epitope mapping in bullous pemphigoid patients. *Journal of Investigative Dermatology*, *122*(1), 103–110. https://doi.org/10.1046/j.0022-202X.2003.22126.x

Di Zenzo, G., Thoma-Uszynski, S., Calabresi, V., Fontao, L., Hofmann, S. C., Lacour, J. P., Sera, F., Bruckner-Tuderman, L., Zambruno, G., Borradori, L., & Hertl, M. (2011). Demonstration of epitope-spreading phenomena in bullous pemphigoid: Results of a prospective multicenter study. *The Journal of Investigative Dermatology*, *131*(11), 2271–2280. https://doi.org/https://doi.org/10.1038/jid.2011.180

Di Zenzo, G., Thoma-Uszynski, S., Fontao, L., Calabresi, V., Hofmann, S. C., Hellmark, T., Sebbag, N., Pedicelli, C., Sera, F., Lacour, J. P., Wieslander, J., Bruckner-Tuderman, L., Borradori, L., Zambruno, G., & Hertl, M. (2008). Multicenter prospective study of the humoral autoimmune response in bullous pemphigoid. *Clinical Immunology*, *128*(3), 415–426. https://doi.org/10.1016/j.clim.2008.04.012

Diaz, L. A., Ratrie, H., Saunders, W. S., Futamura, S., Squiquera, H. L., Anhalt, G. J., & Giudice, G. J. (1990). Isolation of a human epidermal cDNA corresponding to the 180-kD autoantigen recognized by bullous pemphigoid and herpes gestationis sera: Immunolocalization of this protein to the hemidesmosome. *Journal of Clinical Investigation*, *86*(4), 1088–1094. https://doi.org/10.1172/JCI114812

Didona, D., & Di Zenzo, G. (2018). Humoral epitope spreading in autoimmune bullous diseases. *Frontiers in Immunology*, *9*, 779. https://doi.org/10.3389/fimmu.2018.00779

Dillon, C. F., Weisman, M. H., & Miller, F. W. (2020). Population-based estimates of humoral autoimmunity from the U.S. National Health and Nutrition Examination Surveys, 1960–2014. *PLoS ONE*, *15*(1), e0226516. https://doi.org/doi: 10.1371/journal.pone.0226516

Durinx, C., Lambeir, A. M., Bosmans, E., Falmagne, J. B., Berghmans, R., Haemers, A., Scharpé, S., & De Meester, I. (2000). Molecular characterization of dipeptidyl peptidase activity in serum: Soluble CD26/dipeptidyl peptidase IV is responsible for the release of X-Pro dipeptides. *European Journal of Biochemistry*, *267*(17), 5608–5613. https://doi.org/10.1046/j.1432-1327.2000.01634.x

Ellebrecht, C. T., Maseda, D., & Payne, A. S. (2022). Pemphigus and pemphigoid: From disease mechanisms to druggable pathways. *Journal of Investigative Dermatology*, *142*(3 Pt B), 907–914. https://doi.org/10.1016/j.jid.2021.04.040

Fadini, G. P., Boscaro, E., Albiero, M., Menegazzo, L., Frison, V., De Kreutzenberg, S., Agostini, C., Tiengo, A., & Avogaro, A. (2010). The oral dipeptidyl peptidase-4 inhibitor sitagliptin increases circulating endothelial progenitor cells in patients with type 2 diabetes: Possible role of stromal-derived factor-1α. *Diabetes Care*, *33*(7), 1607–1609. https://doi.org/10.2337/dc10-0187

Fairley, J. A., Chang, L. F., & Giudice, G. J. (2005). Mapping the binding sites of anti-BP180 immunoglobulin E autoantibodies in bullous pemphigoid. *Journal of Investigative Dermatology*, *125*(3), 467–472. https://doi.org/10.1111/j.0022-202X.2005.23853.x

Fang, H., Xue, K., Cao, T., Li, Q., Dang, E., Liu, Y., Zhang, J., Qiao, P., Chen, J., Ma, J., Shen, S., Pang, B., Bai, Y., Qiao, H., Shao, S., & Wang, G. (2023). CXCL12/CXCR4 axis drives the chemotaxis and differentiation of B cells in bullous pemphigoid. *Journal of Investigative Dermatology*, *143*(2), 197–208.e6. https://doi.org/10.1016/j.jid.2022.08.041

Feliciani, C., Caldarola, G., Kneisel, A., Podstawa, E., Pfutze, M., Pfutzner, W., & Hertl, M. (2009). IgG autoantibody reactivity against bullous pemphigoid (BP) 180 and BP230 in elderly patients with pruritic dermatoses. *British Journal of Dermatology*, *161*(2), 306–312. https://doi.org/doi: 10.1111/j.1365-2133.2009.09266.x

Finnish Medicines Agency Fimea and Social Insurance Institution. (2011). *Finnish statistics on medicines 2010*. https://urn.fi/URN:NBN:fi-fe2016070722190

Finnish Medicines Agency Fimea and Social Insurance Institution. (2021). *Finnish statistics on medicines 2020*. https://urn.fi/URN:NBN:fi-fe2021122162417

Försti, A. K., Huilaja, L., Schmidt, E., & Tasanen, K. (2017). Neurological and psychiatric associations in bullous pemphigoid—more than skin deep? *Experimental Dermatology*, *26*(12), 1228–1234. https://doi.org/10.1111/exd.13401

Försti, A. K., Jokelainen, J., Ansakorpi, H., Seppänen, A., Majamaa, K., Timonen, M., & Tasanen, K. (2016). Psychiatric and neurological disorders are associated with bullous pemphigoid - a nationwide Finnish Care Register study. *Scientific Reports*, *6*, 37125. https://doi.org/10.1038/srep37125

Försti, A. K., Jokelainen, J., Timonen, M., & Tasanen, K. (2014). Increasing incidence of bullous pemphigoid in Northern Finland: A retrospective database study in Oulu University Hospital. *British Journal of Dermatology*, *171*(5), 1223–1226. https://doi.org/10.1111/bjd.13189

Foureur, N., Mignot, S., Senet, P., Verpillat, P., Picard-Dahan, C., Crickx, B., Labarre, C., Nicaise-Roland, P., & Descamps, V. (2006). Correlation between the presence of type-2 anti-pemphigoid antibodies and dementia in elderly subjects with no clinical signs of pemphigoid. *Annales de Dermatologie et de Vénéréologie*, *133*(5 Pt 1), 439–443. https://doi.org/10.1016/S0151-9638(06)70935-8

Franzke, C. W., Bruckner-Tuderman, L., & Blobel, C. P. (2009). Shedding of collagen XVII/BP180 in skin depends on both ADAM10 and ADAM9. *Journal of Biological Chemistry*, *284*(35), 23386–23396. https://doi.org/10.1074/jbc.M109.034090

Freire, P. C., Muñoz, C. H., & Stingl, G. (2017). IgE autoreactivity in bullous pemphigoid: eosinophils and mast cells as major targets of pathogenic immune reactants. *British Journal of Dermatology*, *177*(6), 1644–1653. https://doi.org/10.1111/bjd.15924

Fujinami, R. S., von Herrath, M. G., Christen, U., & Whitton, J. L. (2006). Molecular mimicry, bystander activation, or viral persistence: Infections and autoimmune disease. *Clinical Microbiology Reviews*, *19*(1), 80–94. https://doi.org/10.1128/CMR.19.1.80-94.2006

García, M., Aranburu, M. A., Palacios-Zabalza, I., Lertxundi, U., & Aguirre, C. (2016). Dipeptidyl peptidase-IV inhibitors induced bullous pemphigoid: A case report and analysis of cases reported in the European pharmacovigilance database. *Journal of Clinical Pharmacy and Therapeutics*, *41*(3), 368–370. https://doi.org/10.1111/jcpt.12397

Genovese, G., Zenzo, G. Di, Cozzani, E., Berti, E., Cugno, M., & Marzano, A. V. (2019). New insights into the pathogenesis of bullous pemphigoid: 2019 update. *Frontiers in Immunology*, *10*, 1506. https://doi.org/10.3389/fimmu.2019.01506

Gliem, M., Heupel, W. M., Spindler, V., Harms, G. S., & Waschke, J. (2010). Actin reorganization contributes to loss of cell adhesion in pemphigus vulgaris. *American Journal of Physiology - Cell Physiology*, *299*(3), 606–613. https://doi.org/10.1152/ajpcell.00075.2010

Goletz, S., Zillikens, D., & Schmidt, E. (2017). Structural proteins of the dermal-epidermal junction targeted by autoantibodies in pemphigoid diseases. *Experimental Dermatology*, *26*(12), 1154–1162. https://doi.org/10.1111/exd.13446

Guignant, M., Tedbirt, B., Murrell, D. F., Amagai, M., Aoki, V., Bauer, J., Ciancinni, G., Culton, D., Daneshpazhooh, M., De, D., Fairley, J., Hall, R., Kim, S.-C., Korman, N. J., Kowalewski, C., Mimouni, D., Patsatsi, A., Hebert, V., Saleh, M. A. M., … Joly, P. (2022). How do experts treat patients with bullous pemphigoid around the world? An international survey. *JID Innovations*, *2*(4), 100129. https://doi.org/10.1016/j.xjidi.2022.100129

Guo, W., Rathi, S., Marquez, J., Smith, H., Kuruvilla, A., Tonnesen, M. G., & Salvemini, J. N. (2023). Prevalence of diabetes mellitus in bullous pemphigoid patients in the absence of dipeptidyl peptidase-4 inhibitors: A systematic review and meta-analysis. *Archives of Dermatological Research*, *315*(8), 2207–2213. https://doi.org/10.1007/s00403-023-02562-0

Gurgel Penaforte-Saboia, J., Couri, C. E. B., Vasconcelos Albuquerque, N., Lauanna Lima Silva, V., Bitar da Cunha Olegario, N., Oliveira Fernandes, V., & Montenegro Junior, R. M. (2021). Emerging roles of dipeptidyl peptidase-4 inhibitors in delaying the progression of Type 1 diabetes mellitus. *Diabetes, Metabolic Syndrome and Obesity*, *14*, 565–573. https://doi.org/10.2147/DMSO.S294742

Gutierrez-Arcelus, M., Rich, S. S., Raychaudhuri, S., Hospital, W., Genetics, P., Sciences, H., & Institutet, K. (2016). Autoimmune diseases — connecting risk alleles with molecular traits of the immune system. *Nature Reviews Genetics*, *17*(3), 160–174. https://doi.org/10.1038/nrg.2015.33

Haeberle, S., Wei, X., Bieber, K., Goletz, S., Ludwig, R. J., Schmidt, E., Enk, A. H., & Hadaschik, E. N. (2018). Regulatory T-cell deficiency leads to pathogenic bullous pemphigoid antigen 230 autoantibody and autoimmune bullous disease. *Journal of Allergy and Clinical Immunology*, *142*(6), 1831–1842.e7. https://doi.org/10.1016/j.jaci.2018.04.006

Haller-Kikkatalo, K., Alnek, K., Metspalu, A., Mihailov, E., Metsküla, K., Kisand, K., Pisarev, H., Salumets, A., & Uibo, R. (2017). Demographic associations for autoantibodies in disease-free individuals of a European population. *Scientific Reports*, *7*, 44846. https://doi.org/10.1038/srep44846

Hammers, C. M., & Stanley, J. R. (2020). Recent advances in understanding pemphigus and bullous pemphigoid. *Journal of Investigative Dermatology*, *140*(4), 733–741. https://doi.org/doi: 10.1016/j.jid.2019.11.005

Has, C., Nyström, A., Saeidian, A. H., Bruckner-Tuderman, L., & Uitto, J. (2018). Epidermolysis bullosa: Molecular pathology of connective tissue components in the cutaneous basement membrane zone. *Matrix Biology*, *71–72*, 313–329. https://doi.org/10.1016/j.matbio.2018.04.001

Hashimoto, T., Ohzono, A., Teye, K., Numata, S., Hiroyasu, S., Tsuruta, D., Hachiya, T., Kuroda, K., Hashiguchi, M., Kawakami, T., & Ishii, N. (2017). Detection of IgE autoantibodies to BP180 and BP230 and their relationship to clinical features in bullous pemphigoid. *British Journal of Dermatology*, *177*(1), 141–151. https://doi.org/10.1111/bjd.15114

Hashimoto, T., Tsuruta, D., Dainichi, T., Hamada, T., Furumura, M., & Ishii, N. (2011). Demonstration of epitope spreading in bullous pemphigoid: Results of a prospective multicenter study. *Journal of Investigative Dermatology*, *131*(11), 2175–2177. https://doi.org/10.1038/jid.2011.276

Hiroyasu, S., Colburn, Z. T., & Jones, J. C. R. (2016). A hemidesmosomal protein regulates actin dynamics and traction forces in motile keratinocytes. *FASEB Journal*, *30*(6), 2298–2310. https://doi.org/10.1096/fj.201500160R

Hiroyasu, S., Ozawa, T., Kobayashi, H., Ishii, M., Aoyama, Y., Kitajima, Y., Hashimoto, T., Jones, J. C. R., & Tsuruta, D. (2013). Bullous pemphigoid IgG induces BP180 internalization via a macropinocytic pathway. *American Journal of Pathology*, *182*(3), 828–840. https://doi.org/10.1016/j.ajpath.2012.11.029

Hiroyasu, S., Turner, C. T., Richardson, K. C., & Granville, D. J. (2019). Proteases in pemphigoid diseases. *Frontiers in Immunology*, *10*, 1454. https://doi.org/10.3389/fimmu.2019.01454

Hoffmann, P., Bentley, P., Sahota, P., Schoenfeld, H., Martin, L., Longo, L., Spaet, R., Moulin, P., Pantano, S., Dubost, V., Lapadula, D., Burkey, B., Kaushik, V., Zhou, W., Hayes, M., Flavahan, N., Chibout, S. D., Busch, S., Morton, D., & Tomlinson, L. (2014). Vascular origin of vildagliptin-induced skin effects in cynomolgus monkeys: Pathomechanistic role of peripheral sympathetic system and neuropeptide Y. *Toxicologic Pathology*, *42*(4), 684–695. https://doi.org/10.1177/0192623313516828

Hofmann, S. C., Voith, U., Schönau, V., Sorokin, L., Bruckner-Tuderman, L., & Franzke, C. W. (2009). Plasmin plays a role in the in vitro generation of the linear IgA dermatosis antigen LADB97. *Journal of Investigative Dermatology*, *129*(7), 1730–1739. https://doi.org/10.1038/jid.2008.424

Horikawa, H., Kurihara, Y., Funakoshi, T., Umegaki-Arao, N., Takahashi, H., Kubo, A., Tanikawa, A., Kodani, N., Minami, Y., Meguro, S., Itoh, H., Izumi, K., Nishie, W., Shimizu, H., Amagai, M., & Yamagami, J. (2018). Unique clinical and serological features of bullous pemphigoid associated with dipeptidyl peptidase-4 inhibitors. *British Journal of Dermatology*, *178*(6), 1462–1463. https://doi.org/10.1111/bjd.16479

Hübner, F., König, I. R., Holtsche, M. M., Zillikens, D., Linder, R., & Schmidt, E. (2020). Prevalence and age distribution of pemphigus and pemphigoid diseases among paediatric patients in Germany. *Journal of the European Academy of Dermatology and Venereology*, *34*(11), 2600–2605. https://doi.org/10.1111/jdv.16467

Ikushima, H., Munakata, Y., Ishii, T., Iwata, S., Terashima, M., Tanaka, H., Schlossman, S. F., & Morimoto, C. (2000). Internalization of CD26 by mannose 6-phosphate/insulin-like growth factor II receptor contributes to T cell activation. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(15), 8439–8444. https://doi.org/10.1073/pnas.97.15.8439

Iwata, H., Kamaguchi, M., Ujiie, H., Nishimura, M., Izumi, K., Natsuga, K., Shinkuma, S., Nishie, W., & Shimizu, H. (2016). Macropinocytosis of type XVII collagen induced by bullous pemphigoid IgG is regulated via protein kinase C. *Laboratory Investigation*, *96*(12), 1301–1310. https://doi.org/10.1038/labinvest.2016.108

Izumi, K., Nishie, W., Beniko, M., & Shimizu, H. (2019). A cross-sectional study comparing the prevalence of bullous pemphigoid autoantibodies in 275 cases of type II diabetes mellitus treated with or without dipeptidyl peptidase-IV inhibitors. *Frontiers in Immunology*, *10*, 1439. https://doi.org/10.3389/fimmu.2019.01439

Izumi, K., Nishie, W., Mai, Y., Wada, M., Natsuga, K., Ujiie, H., Iwata, H., Yamagami, J., & Shimizu, H. (2016). Autoantibody profile differentiates between inflammatory and noninflammatory bullous pemphigoid. *Journal of Investigative Dermatology*, *136*(11), 2201–2210. https://doi.org/10.1016/j.jid.2016.06.622

Jacobsen, L. M., Bocchino, L., Evans-Molina, C., DiMeglio, L., Goland, R., Wilson, D. M., Atkinson, M. A., Aye, T., Russell, W. E., Wentworth, J. M., Boulware, D., Geyer, S., & Sosenko, J. M. (2020). The risk of progression to type 1 diabetes is highly variable in individuals with multiple autoantibodies following screening. *Diabetologia*, *63*(3), 588–596. https://doi.org/10.1007/s00125-019-05047-w

James, T., Salman, S., Stevenson, B., Bundell, C., Kelly, G., Nolan, D., & John, M. (2019). IgE blockade in autoimmunity: Omalizumab induced remission of bullous pemphigoid. *Clinical Immunology*, *198*, 54–56. https://doi.org/10.1016/j.clim.2018.12.015

Janssens, R., Mortier, A., Boff, D., Ruytinx, P., Gouwy, M., Vantilt, B., Larsen, O., Daugvilaite, V., Rosenkilde, M. M., Parmentier, M., Noppen, S., Liekens, S., Van Damme, J., Struyf, S., Teixeira, M. M., Amaral, F. A., & Proost, P. (2017). Truncation of CXCL12 by CD26 reduces its CXC chemokine receptor 4- and atypical chemokine receptor 3-dependent activity on endothelial cells and lymphocytes. *Biochemical Pharmacology*, *132*, 92–101. https://doi.org/10.1016/j.bcp.2017.03.009

Janssens, R., Struyf, S., & Proost, P. (2018a). The unique structural and functional features of CXCL12. *Cellular & Molecular Immunology*, *15*(4), 299–311. https://doi.org/10.1038/cmi.2017.107

Janssens, R., Struyf, S., & Proost, P. (2018b). Pathological roles of the homeostatic chemokine CXCL12. *Cytokine and Growth Factor Reviews*, *44*, 51–68. https://doi.org/10.1016/j.cytogfr.2018.10.004

Joly, P., Roujeau, J.-C., Benichou, J., Picard, C., Dreno, B., Delaporte, E., Vaillant, L., D’Incan, M., Plantin, P., Bedane, C., Young, P., & Bernard, P. (2002). A comparison of oral and topical corticosteroids in patients with bullous pemphigoid. *New England Journal of Medicine*, *346*(5), 321–327. https://doi.org/10.1056/NEJMoa011592

Joly, P., Roujeau, J. C., Benichou, J., Delaporte, E., D’incan, M., Dreno, B., Bedane, C., Sparsa, A., Gorin, I., Picard, C., Tancrede-Bohin, E., Sassolas, B., Lok, C., Guillaume, J. C., Doutre, M. S., Richard, M. A., Caux, F., Prost, C., Plantin, P., … Bernard, P. (2009). A comparison of two regimens of topical corticosteroids in the treatment of patients with bullous pemphigoid: A multicenter randomized study. *Journal of Investigative Dermatology*, *129*(7), 1681–1687. https://doi.org/10.1038/jid.2008.412

Jordon, R. E., Beutner, E. H., Witebsky, E., Blumental, G., Hale, W. L., & Lever, W. F. (1967). Basement zone antibodies in bullous pemphigoid. *JAMA: The Journal of the American Medical Association*, *200*(9), 751–756. https://doi.org/10.1001/jama.1967.03120220053008

Junttila, M. J., Kiviniemi, A. M., Lepojärvi, E. S., Tulppo, M., Piira, O. P., Kenttä, T., Perkiömäki, J. S., Ukkola, O. H., Myerburg, R. J., & Huikuri, H. V. (2018). Type 2 diabetes and coronary artery disease: Preserved ejection fraction and sudden cardiac death. *Heart Rhythm*, *15*(10), 1450–1456. https://doi.org/10.1016/j.hrthm.2018.06.017

Kárpáti, S., Sárdy, M., Németh, K., Mayer, B., Smyth, N., Paulsson, M., & Traupe, H. (2018). Transglutaminases in autoimmune and inherited skin diseases: The phenomena of epitope spreading and functional compensation. *Experimental Dermatology*, *27*(8), 807–814. https://doi.org/10.1111/exd.13449

Kawaguchi, K., Yoshida, S., Hatano, R., & Asano, S. (2017). Pathophysiological roles of Ezrin/Radixin/Moesin proteins. *Biological and Pharmaceutical Bulletin*, *40*(4), 381–390. https://doi.org/10.1248/bpb.b16-01011

Kawaguchi, Y., Shimauchi, R., Nishibori, N., Kawashima, K., Oshitani, S., Fujiya, A., Shibata, T., Ohashi, N., Izumi, K., Nishie, W., Shimizu, H., Arima, H., & Sobajima, H. (2019). Dipeptidyl peptidase-4 inhibitors-associated bullous pemphigoid: A retrospective study of 168 pemphigoid and 9,304 diabetes mellitus patients. *Journal of Diabetes Investigation*, *10*(2), 392–398. https://doi.org/10.1111/jdi.12877

Keller, J. J., Kittridge, A. L., Debanne, S. M., & Korman, N. J. (2016). Evaluation of ELISA testing for BP180 and BP230 as a diagnostic modality for bullous pemphigoid: A clinical experience. *Archives of Dermatological Research*, *308*(4), 269–272. https://doi.org/10.1007/s00403-016-1631-1

Kibsgaard, L., Rasmussen, M., Lamberg, A., Deleuran, M., Olesen, A. B., & Vestergaard, C. (2017). Increased frequency of multiple sclerosis among patients with bullous pemphigoid: A population-based cohort study on comorbidities anchored around the diagnosis of bullous pemphigoid. *British Journal of Dermatology*, *176*(6), 1486–1491. https://doi.org/10.1111/bjd.15405

Kobayashi, M., Amagai, M., Kuroda-Kinoshita, K., Hashimoto, T., Shirakata, Y., Hashimoto, K., & Nishikawa, T. (2002). BP180 ELISA using bacterial recombinant NC16a protein as a diagnostic and monitoring tool for bullous pemphigoid. *Journal of Dermatological Science*, *30*(3), 224–232. https://doi.org/10.1016/s0923-1811(02)00109-3

Kokkonen, N., Herukka, S. K., Huilaja, L., Kokki, M., Koivisto, A. M., Hartikainen, P., Remes, A. M., & Tasanen, K. (2017). Increased levels of the bullous pemphigoid BP180 autoantibody are associated with more severe dementia in Alzheimer's disease. *Journal of Investigative Dermatology*, *137*(1), 71–76. https://doi.org/10.1016/j.jid.2016.09.010

Kridin, K., Avni, O., Damiani, G., Tzur Bitan, D., Onn, E., Weinstein, O., & Cohen, A. D. (2023). Dipeptidyl-peptidase IV inhibitor (DPP4i) confers increased odds of bullous pemphigoid even years after drug initiation. *Archives of Dermatological Research*, *315*(1), 33–39. https://doi.org/10.1007/s00403-021-02317-9

Kridin, K., & Bergman, R. (2018). Association of bullous pemphigoid with dipeptidyl-peptidase 4 inhibitors in patients with diabetes: Estimating the risk of the new agents and characterizing the patients. *JAMA Dermatology*, *154*(10), 1152–1158. https://doi.org/10.1001/jamadermatol.2018.2352

Kridin, K., & Cohen, A. D. (2021). Dipeptidyl-peptidase IV inhibitor-associated bullous pemphigoid: A systematic review and meta-analysis. *Journal of the American Academy of Dermatology*, *85*(2), 501–503. https://doi.org/10.1016/j.jaad.2018.09.048

Kridin, K., & Ludwig, R. J. (2018). The growing incidence of bullous pemphigoid: Overview and potential explanations. *Frontiers in Medicine*, *5*, 220. https://doi.org/10.3389/fmed.2018.00220

Kridin, K., Shihade, W., & Bergman, R. (2019). Mortality in patients with bullous pemphigoid: A retrospective cohort study, systematic review and meta-analysis. *Acta Dermato-Venereologica*, *99*(1), 72–77. https://doi.org/10.2340/00015555-2930

Lai, Y. C., Yew, Y. W., & Lambert, W. C. (2016). Bullous pemphigoid and its association with neurological diseases: a systematic review and meta-analysis. *Journal of the European Academy of Dermatology and Venereology*, *30*(12), 2007–2015. https://doi.org/10.1111/jdv.13660

Langan, S. M., Groves, R. W., & West, J. (2011). The relationship between neurological disease and bullous pemphigoid: A population-based case-control study. *Journal of Investigative Dermatology*, *131*(3), 631–636. https://doi.org/10.1038/jid.2010.357

Le Jan, S., Plée, J., Vallerand, D., Dupont, A., Delanez, E., Durlach, A., Jackson, P. L., Edwin Blalock, J., Bernard, P., & Antonicelli, F. (2014). Innate immune cell-produced IL-17 sustains inflammation in bullous pemphigoid. *Journal of Ivestigative Dermatology*, *134*(12), 2908–2917. https://doi.org/10.1038/jid.2014.263

Lee, E. H., Kim, Y. H., Kim, S., Kim, S. E., & Kim, S. C. (2012). Usefulness of enzyme-linked immunosorbent assay using recombinant BP180 and BP230 for serodiagnosis and monitoring disease activity of bullous pemphigoid. *Annals of Dermatology*, *24*(1), 45–55. https://doi.org/10.5021/ad.2012.24.1.45

Lee, S. G., Lee, H. J., Yoon, M. S., & Kim, D. H. (2019). Association of dipeptidyl peptidase 4 inhibitor use with risk of bullous pemphigoid in patients with diabetes. *JAMA Dermatology*, *155*(2), 172–177. https://doi.org/10.1001/jamadermatol.2018.4556

Lee, Y. I., Choi, S., Roh, W. S., Lee, J. H., & Kim, T. G. (2021). Cellular senescence and inflammaging in the skin microenvironment. *International Journal of Molecular Sciences*, *22*(8), 3849. https://doi.org/10.3390/ijms22083849

Lever, W. F. (1953). PEMPHIGUS. *Medicine*, *32*(1), 1–123. https://doi.org/10.1097/00005792-195302000-00001

Li, A., Wu, J., Zhai, A., Qian, J., Wang, X., Qaria, M. A., Zhang, Q., Li, Y., Fang, Y., Kao, W., Song, W., Zhang, Z., & Zhang, F. (2019). HBV triggers APOBEC2 expression through miR-122 regulation and affects the proliferation of liver cancer cells. *International Journal of Oncology*, *55*(5), 1137–1148. https://doi.org/10.3892/ijo.2019.4870

Li, K., Tamai, K., Tan, E. M., & Uitto, J. (1993). Cloning of type XVII collagen. Complementary and genomic DNA sequences of mouse 180-kilodalton bullous pemphigoid antigen (BPAG2) predict an interrupted collagenous domain, a transmembrane segment, and unusual features in the 5‘-end of the gene and the 3‘. *Journal of Biological Chemistry*, *268*(12), 8825–8834. https://doi.org/10.1016/S0021-9258(18)52948-3

Lindgren, O., Varpuluoma, O., Tuusa, J., Ilonen, J., Huilaja, L., Kokkonen, N., & Tasanen, K. (2019). Gliptin-associated bullous pemphigoid and the expression of dipeptidyl peptidase-4/CD26 in bullous pemphigoid. *Acta Dermato-Venereologica*, *99*(6), 602–609. https://doi.org/10.2340/00015555-3166

Liu, Y., Li, L., & Xia, Y. (2017). BP180 is critical in the autoimmunity of bullous pemphigoid. *Frontiers in Immunology*, *8*, 1752. https://doi.org/10.3389/fimmu.2017.01752

Liu, Z., Chen, L., Zhang, C., & Xiang, L. F. (2019). Circulating bullous pemphigoid 180 autoantibody can be detected in a wide spectrum of patients with other dermatologic conditions: A cross-sectional study. *Journal of the American Academy of Dermatology*, *80*(3), 774–775. https://doi.org/10.1016/j.jaad.2018.06.006

Liu, Z., Shipley, J. M., Vu, T. H., Zhou, X., Diaz, L. A., Werb, Z., & Senior, R. M. (1998). Gelatinase B-deficient mice are resistant to experimental bullous pemphigoid. *Journal of Experimental Medicine*, *188*(3), 475–482. https://doi.org/10.1084/jem.188.3.475

Liu, Z., Zhou, X., Shapiro, S. D., Shipley, J. M., Twining, S. S., Diaz, L. A., Senior, R. M., & Werb, Z. (2000). The serpin alpha1-proteinase inhibitor is a critical substrate for gelatinase B/MMP-9 in vivo. *Cell*, *102*(5), 647–655. https://doi.org/10.1016/s0092-8674(00)00087-8

Lo Schiavo, A., Ruocco, E., Brancaccio, G., Caccavale, S., Ruocco, V., & Wolf, R. (2013). Bullous pemphigoid: Etiology, pathogenesis, and inducing factors: Facts and controversies. *Clinics in Dermatology*, *31*(4), 391–399. https://doi.org/10.1016/j.clindermatol.2013.01.006

Lopez, A. T., Khanna, T., Antonov, N., Audrey-Bayan, C., & Geskin, L. (2018). A review of bullous pemphigoid associated with PD-1 and PD-L1 inhibitors. *International Journal of Dermatology*, *57*(6), 664–669. https://doi.org/10.1111/ijd.13984

Lu, C.-F., Ma, J.-H., Su, J.-B., Wang, X.-Q., Liu, W.-S., & Ge, X.-Q. (2021). Serum stromal cell-derived factor-1 levels are associated with diabetic kidney disease in type 2 diabetic patients. *Endocrine Journal*, *68*(9), 1101–1107. https://doi.org/10.1507/endocrj.EJ21-0039

Ly, S., Nedosekin, D., & Wong, H. K. (2023). Review of an anti-CD20 monoclonal antibody for the treatment of autoimmune diseases of the skin. *American Journal of Clinical Dermatology*, *24*(2), 247–273. https://doi.org/10.1007/s40257-022-00751-7

Ma, W. T., Chang, C., Gershwin, M. E., & Lian, Z. X. (2017). Development of autoantibodies precedes clinical manifestations of autoimmune diseases: A comprehensive review. *Journal of Autoimmunity*, *83*, 95–112. https://doi.org/10.1016/j.jaut.2017.07.003

Maejima, H., Nagashio, R., Yanagita, K., Hamada, Y., Amoh, Y., Sato, Y., & Katsuoka, K. (2014). Moesin and stress-induced phosphoprotein-1 are possible sero-diagnostic markers of psoriasis. *PLoS ONE*, *9*(7), e101773. https://doi.org/10.1371/journal.pone.0101773

Maglie, R., Solimani, F., Didona, D., Pipitò, C., Antiga, E., & Di Zenzo, G. (2023). The cytokine milieu of bullous pemphigoid: Current and novel therapeutic targets. *Frontiers in Medicine*, *10*, 1128154. https://doi.org/10.3389/fmed.2023.1128154

Mai, Y., Izumi, K., Mai, S., Nishie, W., & Ujiie, H. (2023). Detection of a natural antibody targeting the shed ectodomain of BP180 in mice. *Journal of Dermatological Science*, *112*(1), 15–22. https://doi.org/10.1016/j.jdermsci.2023.07.009

Mai, Y., Izumi, K., Mai, S., & Ujiie, H. (2022). The significance of preclinical anti-BP180 autoantibodies. *Frontiers in Immunology*, *13*, 963401. https://doi.org/10.3389/fimmu.2022.963401

Mai, Y., Izumi, K., Sawada, K., Akasaka, E., Mai, S., Sawamura, D., Ihara, K., Nakaji, S., & Nishie, W. (2022). A 1,035-subject study suggesting a history of bone fracture as a possible factor associated with the development of anti-BP180 autoantibodies. *Journal of Investigative Dermatology*, *142*(3 Pt B), 984–987.e3. https://doi.org/10.1016/j.jid.2021.11.028

Mansikka, E., Hervonen, K., Kaukinen, K., Ilus, T., Oksanen, P., Lindfors, K., Laurila, K., Hietikko, M., Taavela, J., Jernman, J., Saavalainen, P., Reunala, T., & Salmi, T. (2019). Gluten challenge induces skin and small bowel relapse in long-term gluten-free diet–treated dermatitis herpetiformis. *Journal of Investigative Dermatology*, *139*(10), 2108–2114. https://doi.org/10.1016/j.jid.2019.03.1150

Martin, E., Mauer, I., Malzahn, U., Heuschmann, P. U., Goebeler, M., & Benoit, S. (2022). Comorbid diseases among bullous pemphigoid patients in Germany: New insights from a case-control study. *Journal Der Deutschen Dermatologischen Gesellschaft*, *20*(6), 798–805. https://doi.org/10.1111/ddg.14738

Mathieu, C., Kozlovski, P., Paldánius, P. M., Foley, J. E., Modgill, V., Evans, M., & Serban, C. (2017). Clinical safety and tolerability of vildagliptin - insights from randomised trials, observational studies and post-marketing surveillance. *European Endocrinology*, *13*(2), 68–72. https://doi.org/10.17925/EE.2017.13.02.68

Matsumura, K., Amagai, M., Nishikawa, T., & Hashimoto, T. (1996). The majority of bullous pemphigoid and herpes gestationis serum samples react with the NC16a domain of the 180-kDa bullous pemphigoid antigen. *Archives of Dermatological Research*, *288*(9), 507–509. https://doi.org/10.1007/BF02505245

Messingham, K. N., Aust, S., Helfenberger, J., Parker, K. L., Schultz, S., McKillip, J., Narayanan, N. S. S., & Fairley, J. A. A. (2016). Autoantibodies to collagen XVII are present in Parkinson's disease and localize to tyrosine-hydroxylase positive neurons. *Journal of Investigative Dermatology*, *136*(3), 721–723. https://doi.org/10.1016/j.jid.2015.12.005

Messingham, K. N., Crowe, T. P., & Fairley, J. A. (2019). The intersection of IgE autoantibodies and eosinophilia in the pathogenesis of bullous pemphigoid. *Frontiers in Immunology*, *10*, 2331. https://doi.org/10.3389/fimmu.2019.02331

Messingham, K. N., Holahan, H. M., & Fairley, J. A. (2014). Unraveling the significance of IgE autoantibodies in organ-specific autoimmunity: Lessons learned from bullous pemphigoid. *Immunologic Research*, *59*(1–3), 273–278. https://doi.org/10.1007/s12026-014-8547-7

Messingham, K. N., Holahan, H. M., Frydman, A. S., Fullenkamp, C., Srikantha, R., & Fairley, J. A. (2014). Human eosinophils express the high affinity IgE receptor, FcεRI, in bullous pemphigoid. *PLoS ONE*, *9*(9), e107725. https://doi.org/10.1371/journal.pone.0107725

Moro, F., Fania, L., Sinagra, J. L. M., Salemme, A., & Di Zenzo, G. (2020). Bullous pemphigoid: Trigger and predisposing factors. *Biomolecules*, *10*(10), 1432. https://doi.org/10.3390/biom10101432

Murphy, K. M., & Weaver, C. (2017). *Janeway's Immunobiology: Ninth International Student Edition* (9th ed.). Garland Science. https://books.google.fi/books?id=DmebDwAAQBAJ

Nagase, H., Miyamasu, M., Yamaguchi, M., Fujisawa, T., Ohta, K., Yamamoto, K., Morita, Y., & Hirai, K. (2000). Expression of CXCR4 in eosinophils: Functional analyses and cytokine-mediated regulation. *Journal of IImunology*, *164*(11), 5935–5943. https://doi.org/10.4049/jimmunol.164.11.5935

Nagele, E. P., Han, M., Acharya, N. K., DeMarshall, C., Kosciuk, M. C., & Nagele, R. G. (2013). Natural IgG autoantibodies are abundant and ubiquitous in human sera, and their number is influenced by age, gender, and disease. *PLoS ONE*, *8*(4), e60726. https://doi.org/10.1371/journal.pone.0060726

Nemazee, D. (2017). Mechanisms of central tolerance for B cells. *Nature Reviews Immunology*, *17*(5), 281–294. https://doi.org/10.1038/nri.2017.19

Nguyen, C. N., & Kim, S. J. (2021). Dermatitis ehrpetiformis: An update on diagnosis, disease monitoring, and management. *Medicina*, *57*(8), 843. https://doi.org/10.3390/medicina57080843

Niebel, D., Wilsmann-Theis, D., Bieber, T., Berneburg, M., Wenzel, J., & Braegelmann, C. (2022). Bullous pemphigoid in patients receiving immune-checkpoint inhibitors and psoriatic patients—focus on clinical and histopathological variation. *Dermatopathology*, *9*(1), 60–81. https://doi.org/10.3390/dermatopathology9010010

Nishie, W. (2014). Update on the pathogenesis of bullous pemphigoid: An autoantibody-mediated blistering disease targeting collagen XVII. *Journal of Dermatological Science*, *73*(3), 179–186. https://doi.org/10.1016/j.jdermsci.2013.12.001

Nishie, W. (2020). Collagen XVII processing and blistering skin diseases. *Acta Dermato-Venereologica*, *100*(5), adv00054. https://doi.org/10.2340/00015555-3399

Nishiguchi, M. A., Spencer, C. A., Leung, D. H., & Leung, T. H. (2018). Aging suppresses skin-derived circulating SDF1 to promote full-thickness tissue regeneration. *Cell Reports*, *24*(13), 3383–3392.e5. https://doi.org/10.1016/j.celrep.2018.08.054

Ogura, T., & Shiraishi, C. (2023). Comparison of adverse events occurred during administration of dipeptidyl peptidase-4 inhibitor in patients with diabetes using FDA adverse event reporting system. *Clinical Drug Investigation*, *43*(2), 129–140. https://doi.org/10.1007/s40261-022-01242-7

Ohnuma, K., Uchiyama, M., Yamochi, T., Nishibashi, K., Hosono, O., Takahashi, N., Kina, S., Tanaka, H., Lin, X., Dang, N. H., & Morimoto, C. (2007). Caveolin-1 triggers T-cell activation via CD26 in association with CARMA1. *Journal of Biological Chemistry*, *282*(13), 10117–10131. https://doi.org/10.1074/jbc.M609157200

Ohtsubo, H., Sato, Y., Suzuki, T., Mizunoya, W., Nakamura, M., Tatsumi, R., & Ikeuchi, Y. (2017). APOBEC2 negatively regulates myoblast differentiation in muscle regeneration. *International Journal of Biochemistry and Cell Biology*, *85*, 91–101. https://doi.org/10.1016/j.biocel.2017.02.005

Ozeki, T., Muramatsu, K., Yoshimoto, N., Ujiie, I., Izumi, K., Iwata, H., Mushiroda, T., & Ujiie, H. (2023). Association of genetic variants of HLA-DQA1 with bullous pemphigoid induced by dipeptidyl peptidase-4 inhibitors. *The Journal of Investigative Dermatology*, *143*(11), 2219–2225.e5. https://doi.org/10.1016/j.jid.2023.04.017

Pacheco, Y., Acosta-ampudia, Y., Monsalve, D. M., Chang, C., Gershwin, M. E., & Anaya, J. (2019). Bystander activation and autoimmunity. *Journal of Autoimmunity*, *103*, 102301. https://doi.org/10.1016/j.jaut.2019.06.012

Papa, R., Penco, F., Volpi, S., & Gattorno, M. (2021). Actin remodeling defects leading to autoinflammation and immune dysregulation. *Frontiers in Immunology*, *11*, 604206. https://doi.org/10.3389/fimmu.2020.604206

Papakonstantinou, E., Limberg, M. M., Gehring, M., Kotnik, N., Kapp, A., Gibbs, B. F., & Raap, U. (2019). Neurological disorders are associated with bullous pemphigoid. *Journal of the European Academy of Dermatology and Venereology*, *33*(5), 925–929. https://doi.org/10.1111/jdv.15444

Papara, C., Karsten, C. M., Ujiie, H., Schmidt, E., Schmidt-Jiménez, L. F., Baican, A., Freire, P. C., Izumi, K., Bieber, K., Peipp, M., Verschoor, A., Ludwig, R. J., Köhl, J., Zillikens, D., & Hammers, C. M. (2022). The relevance of complement in pemphigoid diseases: A critical appraisal. *Frontiers in Immunology*, *13*, 973702. https://doi.org/10.3389/fimmu.2022.973702

Park, K. S., Kwak, S. H., Cho, Y. M., Park, K. S., Jang, H. C., Kim, S. Y., & Jung, H. S. (2017). Vildagliptin reduces plasma stromal cell-derived factor-1α in patients with type 2 diabetes compared with glimepiride. *Journal of Diabetes Investigation*, *8*(2), 218–226. https://doi.org/10.1111/jdi.12572

Parmo-Cabañas, M., Molina-Ortiz, I., Matías-Román, S., García-Bernal, D., Carvajal-Vergara, X., Valle, I., Pandiella, A., Arroyo, A. G., & Teixidó, J. (2006). Role of metalloproteinases MMP-9 and MT1-MMP in CXCL12-promoted myeloma cell invasion across basement membranes. *Journal of Pathology*, *208*(1), 108–118. https://doi.org/10.1002/path.1876

Patel, P. M., Jones, V. A., Kridin, K., & Amber, K. T. (2021). The role of dipeptidyl peptidase-4 in cutaneous disease. *Experimental Dermatology*, *30*(3), 304–318. https://doi.org/10.1111/exd.14228

Persson, M. S. M., Begum, N., Grainge, M. J., Harman, K. E., Grindlay, D., & Gran, S. (2022). The global incidence of bullous pemphigoid: A systematic review and meta-analysis. *British Journal of Dermatology*, *186*(3), 414–425. https://doi.org/doi: 10.1111/bjd.20743

Persson, M. S. M., Harman, K. E., Vinogradova, Y., Langan, S. M., Hippisley-Cox, J., Thomas, K. S., & Gran, S. (2021). Incidence, prevalence and mortality of bullous pemphigoid in England 1998–2017: A population-based cohort study. *British Journal of Dermatology*, *184*(1), 68–77. https://doi.org/10.1111/bjd.19022

Pisetsky, D. S. (2023). Pathogenesis of autoimmune disease. *Nature Reviews Nephrology*, *19*(8), 509–524. https://doi.org/10.1038/s41581-023-00720-1

Plaquevent, M., Tétart, F., Fardet, L., Ingen-Housz-Oro, S., Valeyrie-Allanore, L., Bernard, P., Hebert, V., Roussel, A., Avenel-Audran, M., Chaby, G., D'Incan, M., Ferrier-Le-Bouedec, M. C., Duvert-Lehembre, S., Picard-Dahan, C., Jeudy, G., Collet, E., Labeille, B., Morice, C., Richard, M. A., … Joly, P. (2019). Higher frequency of dipeptidyl peptidase-4 inhibitor intake in bullous pemphigoid patients than in the French general population. *Journal of Investigative Dermatology*, *139*(4), 835–841. https://doi.org/10.1016/j.jid.2018.10.045

Powell, C., Cornblath, E., & Goldman, D. (2014). Zinc-binding domain-dependent, deaminase-independent actions of apolipoprotein B mRNA-editing enzyme, catalytic polypeptide 2 (apobec2), mediate its effect on zebrafish retina regeneration. *Journal of Biological Chemistry*, *289*(42), 28924–28941. https://doi.org/10.1074/jbc.M114.603043

Pozzi, A., Yurchenco, P. D., & Iozzo, R. V. (2017). The nature and biology of basement membranes. *Matrix Biology : Journal of the International Society for Matrix Biology*, *57–58*, 1–11. https://doi.org/10.1016/j.matbio.2016.12.009

Prüßmann, W., Prüßmann, J., Koga, H., Recke, A., Iwata, H., Juhl, D., Görg, S., Henschler, R., Hashimoto, T., Schmidt, E., Zillikens, D., Ibrahim, S. M., & Ludwig, R. J. (2015). Prevalence of pemphigus and pemphigoid autoantibodies in the general population. *Orphanet Journal of Rare Diseases*, *10*, 63. https://doi.org/10.1186/s13023-015-0278-x

Quan, C., Cho, M. K., Shao, Y., Mianecki, L. E., Liao, E., Perry, D., & Quan, T. (2015). Dermal fibroblast expression of stromal cell-derived factor-1 (SDF-1) promotes epidermal keratinocyte proliferation in normal and diseased skin. *Protein & Cell*, *6*(12), 890–903. https://doi.org/10.1007/s13238-015-0198-5

Raneses, E., McGinley Simpson, M., Rosenberg, A., Coffman, M., & Meyerle, J. (2022). A retrospective review of autoimmune bullous disease antibody positivity before clinical symptoms. *Journal of the American Academy of Dermatology*, *86*(1), 237–239. https://doi.org/10.1016/j.jaad.2021.02.003

Riani, M., Le Jan, S., Plée, J., Durlach, A., Le Naour, R., Haegeman, G., Bernard, P., & Antonicelli, F. (2017). Bullous pemphigoid outcome is associated with CXCL10-induced matrix metalloproteinase 9 secretion from monocytes and neutrophils but not lymphocytes. *Journal of Allergy and Clinical Immunology*, *139*(3), 863–872.e3. https://doi.org/10.1016/j.jaci.2016.08.012

Roig-Rosello, E., & Rousselle, P. (2020). The human epidermal basement membrane: A shaped and cell instructive platform that aging slowly alters. *Biomolecules*, *10*(12), 1607. https://doi.org/10.3390/biom10121607

Romeijn, T. R., Jonkman, M. F., Knoppers, C., Pas, H. H., & Diercks, G. F. H. (2017). Complement in bullous pemphigoid: Results from a large observational study. *British Journal of Dermatology*, *176*(2), 517–519. https://doi.org/10.1111/bjd.14822

Rosenblum, M. D., Remedios, K. A., & Abbas, A. K. (2015). Mechanisms of human autoimmunity. *Journal of Clinical Investigation*, *125*(6), 2228–2233. https://doi.org/10.1172/JCI78088

Rosenstock, J., & Zinman, B. (2007). Dipeptidyl peptidase-4 inhibitors and the management of type 2 diabetes mellitus. *Current Opinion in Endocrinology, Diabetes, and Obesity*, *14*(2), 98–107. https://doi.org/10.1097/MED.0b013e3280a02f65

Rozenblat, M., Halaj, A., Rozenblat, T., Fisher, S., Sah, M., Dodiuk-Gad, R. P., & Ziv, M. (2019). Mortality and risk factors among Israeli bullous pemphigoid patients. *Archives of Dermatological Research*, *311*(1), 19–27. https://doi.org/10.1007/s00403-018-1875-z

Saag, K. G., & Furst, D. E. (2023). Major side effects of systemic glucocorticoids. *UpToDate*, Retrieved September 18, 2023, from. https://www.uptodate.com/contents/major-side-effects-of-systemic-glucocorticoids

Sakaguchi, S., Mikami, N., Wing, J. B., Tanaka, A., Ichiyama, K., & Ohkura, N. (2020). Regulatory T cells and human disease. *Annual Review of Immunology*, *38*, 541–566. https://doi.org/10.1146/annurev-immunol-042718-041717

Salemme, A., Fania, L., Scarabello, A., Caproni, M., Marzano, A. V., Cozzani, E., Feliciani, C., De Simone, C., Papini, M., Satta, R. R., Parodi, A., Mariotti, F., Lechiancole, S., Genovese, G., Passarelli, F., Festa, F., Bellei, B., Provini, A., Sordi, D., … Di Zenzo, G. (2022). Gliptin-associated bullous pemphigoid shows peculiar features of anti-BP180 and -BP230 humoral response: Results of a multicenter study. *Journal of the American Academy of Dermatology*, *87*(1), 56–63. https://doi.org/10.1016/j.jaad.2022.02.036

Salmi, T. (2019). Dermatitis herpetiformis. *Clinical and Experimental Dermatology*, *44*(7), 728–731. https://doi.org/10.1111/ced.13992

Salmi, T., & Hervonen, K. (2020). Current concepts of dermatitis herpetiformis. *Acta Dermato-Venereologica*, *100*(5), 115–121. https://doi.org/10.2340/00015555-3401

Sandblad, K. G., Jones, P., Kostalla, M. J., Linton, L., Glise, H., & Winqvist, O. (2015). Chemokine receptor expression on monocytes from healthy individuals. *Clinical Immunology*, *161*(2), 348–353. https://doi.org/10.1016/j.clim.2015.09.012

Satooka, H., Nagakubo, D., Sato, T., & Hirata, T. (2017). The ERM Protein Moesin Regulates CD8(+) Regulatory T Cell Homeostasis and Self-Tolerance. *Journal of Immunology*, *199*(10), 3418–3426. https://doi.org/10.4049/jimmunol.1700074

Schmidt, E., & Zillikens, D. (2013). Pemphigoid diseases. *Lancet*, *381*(9863), 320–332. https://doi.org/10.1016/S0140-6736(12)61140-4

Schulze, F., van Beek, N., Terheyden, P., Zillikens, D., & Schmidt, E. (2013). Concomitant bullous pemphigoid and dermatitis herpetiformis. *Dermatology*, *226*(3), 217–221. https://doi.org/10.1159/000349982

Schwieger-Briel, A., Moellmann, C., Mattulat, B., Schauer, F., Kiritsi, D., Schmidt, E., Sitaru, C., Ott, H., & Kern, J. S. (2014). Bullous pemphigoid in infants: Characteristics, diagnosis and treatment. *Orphanet Journal of Rare Diseases*, *9*, 185. https://doi.org/10.1186/s13023-014-0185-6

Seppänen, A. (2013). Collagen XVII: A shared antigen in neurodermatological interactions? *Clinical and Developmental Immunology*, *2013*, 240570. https://doi.org/10.1155/2013/240570

Shih, Y. chi, Wang, B., Yuan, H., Zheng, J., & Pan, M. (2020). Role of BP230 autoantibodies in bullous pemphigoid. *Journal of Dermatology*, *47*(4), 317–326. https://doi.org/10.1111/1346-8138.15251

Shimanovich, I., Mihai, S., Oostingh, G. J., Ilenchuk, T. T., Bröcker, E.-B., Opdenakker, G., Zillikens, D., & Sitaru, C. (2004). Granulocyte-derived elastase and gelatinase B are required for dermal-epidermal separation induced by autoantibodies from patients with epidermolysis bullosa acquisita and bullous pemphigoid. *Journal of Pathology*, *204*(5), 519–527. https://doi.org/10.1002/path.1674

Shimizu, S., Hosooka, T., Matsuda, T., Asahara, S. ichiro, Koyanagi-Kimura, M., Kanno, A., Bartolome, A., Etoh, H., Fuchita, M., Teruyama, K., Takahashi, H., Inoue, H., Mieda, Y., Hashimoto, N., Seino, S., & Kido, Y. (2012). DPP4 inhibitor vildagliptin preserves β-cell mass through amelioration of endoplasmic reticulum stress in C/EBPB transgenic mice. *Journal of Molecular Endocrinology*, *49*(2), 125–135. https://doi.org/10.1530/JME-12-0039

Shome, M., Chung, Y., Chavan, R., Park, J. G., Qiu, J., & LaBaer, J. (2022). Serum autoantibodyome reveals that healthy individuals share common autoantibodies. *Cell Reports*, *39*(9), 110873. https://doi.org/10.1016/j.celrep.2022.110873

Sitaru, C., Dähnrich, C., Probst, C., Komorowski, L., Blöcker, I., Schmidt, E., Schlumberger, W., Rose, C., Stöcker, W., & Zillikens, D. (2007). Enzyme-linked immunosorbent assay using multimers of the 16th non-collagenous domain of the BP180 antigen for sensitive and specific detection of pemphigoid autoantibodies. *Experimental Dermatology*, *16*(9), 770–777. https://doi.org/10.1111/j.1600-0625.2007.00592.x

Solinet, S., Mahmud, K., Stewman, S. F., El Kadhi, K. Ben, Decelle, B., Talje, L., Ma, A., Kwok, B. H., & Carreno, S. (2013). The actin-binding ERM protein Moesin binds to and stabilizes microtubules at the cell cortex. *Journal of Cell Biology*, *202*(2), 251–260. https://doi.org/10.1083/jcb.201304052

Spinosa, P. C., Luker, K. E., Luker, G. D., & Linderman, J. J. (2017). The CXCL12/CXCR7 signaling axis, isoforms, circadian rhythms, and tumor cellular composition dictate gradients in tissue. *PLoS ONE*, *12*(11), e0187357. https://doi.org/10.1371/journal.pone.0187357

Ständer, S., Schmidt, E., Zillikens, D., Ludwig, R. J., & Kridin, K. (2021). More severe erosive phenotype despite lower circulating autoantibody levels in dipeptidyl peptidase-4 inhibitor (DPP4i)-associated bullous pemphigoid: A retrospective cohort study. *American Journal of Clinical Dermatology*, *22*(1), 117–127. https://doi.org/10.1007/s40257-020-00563-7

Subramanian, S., Liu, C., Aviv, A., Ho, J. E., Courchesne, P., Muntendam, P., Larson, M. G., Cheng, S., Wang, T. J., Mehta, N. N., & Levy, D. (2014). Stromal cell-derived factor 1 as a biomarker of heart failure and mortality risk. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *34*(9), 2100–2105. https://doi.org/10.1161/ATVBAHA.114.303579

Sun, Y., Liu, H., Wang, Z., Fu, X., Wang, C., Mi, Z., Sun, L., Bao, F., Yu, G., Zhou, G., & Zhang, F. (2018). The HLA-DQB1*03:01 is associated with bullous pemphigoid in the Han Chinese population. *Journal of Investigative Dermatology*, *138*(8), 1874–1877. https://doi.org/10.1016/j.jid.2018.02.021

Sun, Z. W., Kim, J. H., Kim, S. H., Kim, H. R., Zhang, K. L., Pan, Y., Ko, M. K., Kim, B. M., Chu, H., Lee, H. R., Kim, H. L., Kim, J. H., Fu, X., Hyun, Y. M., Yun, K. N., Kim, J. Y., Lee, D. W., Song, S. Y., Lin, C. P., … Park, C. O. (2021). Skin-resident natural killer T cells participate in cutaneous allergic inflammation in atopic dermatitis. *Journal of Allergy and Clinical Immunology*, *147*(5), 1764–1777. https://doi.org/10.1016/j.jaci.2020.11.049

Sundblad, V., Morosi, L. G., Geffner, J. R., & Rabinovich, G. A. (2017). Galectin-1: A Jack-of-all-trades in the resolution of acute and chronic inflammation. *Journal of Immunology*, *199*(11), 3721–3730. https://doi.org/10.4049/jimmunol.1701172

Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N. T., Morris, J. H., Bork, P., Jensen, L. J., & Von Mering, C. (2019). STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Research*, *47*(D1), D607–D613. https://doi.org/10.1093/nar/gky1131

Taghipour, K., Chi, C. C., Vincent, A., Groves, R. W., Venning, V., & Wojnarowska, F. (2010). The association of bullous pemphigoid with cerebrovascular disease and dementia: A case-control study. *Archives of Dermatology*, *146*(11), 1251–1254. https://doi.org/10.1001/archdermatol.2010.322

Tanaka, G., Nakase, I., Fukuda, Y., Masuda, R., Oishi, S., Shimura, K., Kawaguchi, Y., Takatani-Nakase, T., Langel, Ü., Gräslund, A., Okawa, K., Matsuoka, M., Fujii, N., Hatanaka, Y., & Futaki, S. (2012). CXCR4 stimulates macropinocytosis: Implications for cellular uptake of arginine-rich cell-penetrating peptides and HIV. *Chemistry and Biology*, *19*(11), 1437–1446. https://doi.org/10.1016/j.chembiol.2012.09.011

Tanaka, Y., Takenaka, M., Matsunaga, Y., Okada, S., Anan, S., Yoshida, H., & Ra, C. (1995). High affinity IgE receptor (Fc epsilon RI) expression on eosinophils infiltrating the lesions and mite patch tested sites in atopic dermatitis. *Archives of Dermatological Research*, *287*(8), 712–717. https://doi.org/10.1007/BF01105794

Tasanen, K., Varpuluoma, O., & Nishie, W. (2019). Dipeptidyl peptidase-4 inhibitor-associated bullous pemphigoid. *Frontiers in Immunology*, *10*, 1238. https://doi.org/10.3389/fimmu.2019.01238

Terho, A., Bloigu, R., Bloigu, A., Niemelä, O., Tulppo, M., Kesäniemi, Y. A., & Ukkola, O. (2019). Life style habits, biochemical factors and their interaction in the prediction of incident hypertension during 21-year follow-up. *Blood Pressure*, *28*(1), 40–48. https://doi.org/10.1080/08037051.2018.1540923

Thoma-Uszynski, S., Uter, W., Schwietzke, S., Hofmann, S. C., Hunziker, T., Bernard, P., Treudler, R., Zouboulis, C. C., Schuler, G., Borradori, L., & Hertl, M. (2004). BP230- and BP180-specific auto-antibodies in bullous pemphigoid. *Journal of Investigative Dermatology*, *122*(6), 1413–1422. https://doi.org/10.1111/j.0022-202X.2004.22603.x

Thomas, R. M., Colon, A., & Motaparthi, K. (2020). Rituximab in autoimmune pemphigoid diseases: Indications, optimized regimens, and practice gaps. *Clinics in Dermatology*, *38*(3), 384–396. https://doi.org/10.1016/j.clindermatol.2019.07.023

Tofigh, R., Hosseinpourfeizi, M., Baradaran, B., Teimourian, S., & Safaralizadeh, R. (2023). Rheumatoid arthritis and non-coding RNAs; how to trigger inflammation. *Life Sciences*, *315*, 121367. https://doi.org/10.1016/j.lfs.2023.121367

Tsuboi, K., Mizukami, H., Inaba, W., Baba, M., & Yagihashi, S. (2016). The dipeptidyl peptidase IV inhibitor vildagliptin suppresses development of neuropathy in diabetic rodents: Effects on peripheral sensory nerve function, structure and molecular changes. *Journal of Neurochemistry*, *136*(4), 859–870. https://doi.org/10.1111/jnc.13439

Tuusa, J., Kokkonen, N., Mattila, A., Huilaja, L., Varpuluoma, O., Rannikko, S., Glumoff, V., Miettunen, J., & Tasanen, K. (2023). Dipeptidyl peptidase 4 inhibitor–associated bullous pemphigoid is characterized by an altered expression of cytokines in the skin. *Journal of Investigative Dermatology*, *143*(1), 78–86.e12. https://doi.org/10.1016/j.jid.2022.07.006

Tuusa, J., Kokkonen, N., & Tasanen, K. (2021). BP180/collagen XVII: A molecular view. *International Journal of Molecular Sciences*, *22*(22), 12233. https://doi.org/10.3390/ijms222212233

Tuusa, J., Lindgren, O., Tertsunen, H. M., Nishie, W., Kokkonen, N., Huilaja, L., Izumi, K., Herukka, S. K., Miettunen, J., Shimizu, H., Remes, A. M., & Tasanen, K. (2019). BP180 autoantibodies target different epitopes in multiple sclerosis or Alzheimer's disease than in bullous pemphigoid. *Journal of Investigative Dermatology*, *139*(2), 293–299. https://doi.org/10.1016/j.jid.2018.09.010

Uitto, J., & Christiano, A. M. (1992). Molecular genetics of the cutaneous basement membrane zone. Perspectives on epidermolysis bullosa and other blistering skin diseases. *Journal of Clinical Investigation*, *90*(3), 687–692. https://doi.org/10.1172/JCI115938

Ujiie, H. (2018). HLA-DQB1*03:01 as a biomarker for genetic susceptibility to bullous pemphigoid induced by DPP-4 inhibitors. *Journal of Investigative Dermatology*, *138*(5), 1201–1204. https://doi.org/10.1016/j.jid.2017.11.023

Ujiie, H. (2019). Regulatory T cells in autoimmune skin diseases. *Experimental Dermatology*, *28*(6), 642–646. https://doi.org/10.1111/exd.13535

Ujiie, H. (2023). What's new in the pathogeneses and triggering factors of bullous pemphigoid. *Journal of Dermatology*, *50*(2), 140–149. https://doi.org/https://doi.org/10.1111/1346-8138.16654

Ujiie, H., Yoshimoto, N., Natsuga, K., Muramatsu, K., Iwata, H., Nishie, W., & Shimizu, H. (2019). Immune reaction to type XVII collagen induces intramolecular and intermolecular epitope spreading in experimental bullous pemphigoid models. *Frontiers in Immunology*, *10*, 1410. https://doi.org/10.3389/fimmu.2019.01410

Ujiie, I., Iwata, H., Yoshimoto, N., Izumi, K., Shimizu, H., & Ujiie, H. (2021). Clinical characteristics and outcomes of bullous pemphigoid patients with versus without oral prednisolone treatment. *Journal of Dermatology*, *48*(4), 502–510. https://doi.org/10.1111/1346-8138.15816

Van Beek, N., Dohse, A., Riechert, F., Krull, V., Recke, A., Zillikens, D., & Schmidt, E. (2014). Serum autoantibodies against the dermal-epidermal junction in patients with chronic pruritic disorders, elderly individuals and blood donors prospectively recruited. *British Journal of Dermatology*, *170*(4), 943–947. https://doi.org/10.1111/bjd.12739

Vanderlugt, C. L., & Miller, S. D. (2002). Epitope spreading in immune-mediated diseases: Implications for immunotherapy. *Nature Reviews. Immunology*, *2*(2), 85–95. https://doi.org/10.1038/nri724

Varpuluoma, O., Försti, A. K., Jokelainen, J., Turpeinen, M., Timonen, M., Huilaja, L., & Tasanen, K. (2018). Vildagliptin significantly increases the risk of bullous pemphigoid: A Finnish nationwide registry study. *Journal of Investigative Dermatology*, *138*(7), 1659–1661. https://doi.org/10.1016/j.jid.2018.01.027

Varpuluoma, O., Jokelainen, J., Försti, A. K., Timonen, M., Huilaja, L., & Tasanen, K. (2019). Dermatitis herpetiformis and celiac disease increase the risk of bullous pemphigoid. *Journal of Investigative Dermatology*, *139*(3), 600–604. https://doi.org/10.1016/j.jid.2018.10.010

Verheyden, M. J., Bilgic, A., & Murrell, D. F. (2020). A systematic review of drug-associated bullous pemphigoid. *Acta Dermato-Venereologica*, *100*(15), 1–9. https://doi.org/10.2340/00015555-3457

Verraes, S., Hornebeck, W., Polette, M., Borradori, L., & Bernard, P. (2001). Respective contribution of neutrophil elastase and matrix metalloproteinase 9 in the degradation of BP180 (type XVII collagen) in human bullous pemphigoid. *Journal of Investigative Dermatology*, *117*(5), 1091–1096. https://doi.org/10.1046/j.0022-202x.2001.01521.x

Vignali, D. A. A., Collison, L. W., & Workman, C. J. (2008). How regulatory T cells work. *Nature Reviews. Immunology*, *8*(7), 523–532. https://doi.org/10.1038/nri2343

Waisbourd-Zinman, O., Ben-Amitai, D., Cohen, A. D., Feinmesser, M., Mimouni, D., Adir-Shani, A., Zlotkin, M., & Zvulunov, A. (2008). Bullous pemphigoid in infancy: Clinical and epidemiologic characteristics. *Journal of the American Academy of Dermatology*, *58*(1), 41–48. https://doi.org/10.1016/j.jaad.2007.08.010

Walko, G., Castañón, M. J., & Wiche, G. (2015). Molecular architecture and function of the hemidesmosome. *Cell & Tissue Research*, *360*(2), 363–378. https://doi.org/10.1007/s00441-014-2061-z

Whittam, A. J., Maan, Z. N., Duscher, D., Barrera, J. A., Hu, M. S., Fischer, L. H., Khong, S., Kwon, S. H., Wong, V. W., Walmsley, G. G., Giacco, F., Januszyk, M., Brownlee, M., Longaker, M. T., & Gurtner, G. C. (2019). Small molecule inhibition of dipeptidyl peptidase-4 enhances bone marrow progenitor cell function and angiogenesis in diabetic wounds. *Translational Research*, *205*, 51–63. https://doi.org/10.1016/j.trsl.2018.10.006

Wieland, C. N., Comfere, N. I., Gibson, L. E., Weaver, A. L., Krause, P. K., & Murray, J. A. (2010). Anti-bullous pemphigoid 180 and 230 antibodies in a sample of unaffected subjects. *Archives of Dermatology*, *146*(1), 21–25. https://doi.org/10.1001/archdermatol.2009.331

Wlaschek, M., Maity, P., Makrantonaki, E., & Scharffetter-Kochanek, K. (2021). Connective tissue and fibroblast senescence in skin aging. *Journal of Investigative Dermatology*, *141*(4S), 985–992. https://doi.org/10.1016/j.jid.2020.11.010

Xing, Y., & Hogquist, K. A. (2012). T-cell tolerance: Central and peripheral. *Cold Spring Harbor Perspectives in Biology*, *4*(6), a006957. https://doi.org/10.1101/cshperspect.a006957

Yang, C., Zhang, F., Chen, F., Chang, Z., Zhao, Y., Shao, D., Sun, W., Dong, W. F., & Wang, Z. (2023). Biomimetic nanovaccines potentiating dendritic cell internalization via CXCR4-mediated macropinocytosis. *Advanced Healthcare Materials*, *12*(5), e2202064. https://doi.org/10.1002/adhm.202202064

Zeng, F. A. P., Wilson, A., Sheriff, T., & Murrell, D. F. (2022). Side effects of steroid-sparing agents in patients with bullous pemphigoid and pemphigus: A systematic review. *JAAD International*, *9*, 33–43. https://doi.org/10.1016/j.jdin.2022.07.005

Zhang, J., & Wang, G. (2020). Genetic predisposition to bullous pemphigoid. *Journal of Dermatological Science*, *100*(2), 86–91. https://doi.org/10.1016/j.jdermsci.2020.05.010

Zhou, T., Peng, B., & Geng, S. (2021). Emerging biomarkers and therapeutic strategies for refractory bullous pemphigoid. *Frontiers in Immunology*, *12*, 718073. https://doi.org/10.3389/fimmu.2021.718073

Zillikens, D., Mascaro, J. M., Rose, P. A., Liu, Z., Ewing, S. M., Caux, F., Hoffmann, R. G., Diaz, L. A., & Giudice, G. J. (1997). A highly sensitive enzyme-linked immunosorbent assay for the detection of circulating anti-BP180 autoantibodies in patients with bullous pemphigoid. *Journal of Investigative Dermatology*, *109*(5), 679–683. https://doi.org/10.1111/1523-1747.ep12338088

Zone, J. J., Egan, C. A., Taylor, T. B., & Meyer, L. J. (2004). IgA autoimmune disorders: Development of a passive transfer mouse model. *Journal of Investigative Dermatology Symposium Proceedings*, *9*(1), 47–51. https://doi.org/10.1111/j.1087-0024.2004.00840.x

Original publications

I Nätynki, A., Tuusa, J., Hervonen, K., Kaukinen, K., Lindgren, O., Huilaja, L., Kokkonen, N., Salmi, T., & Tasanen, K. (2020) Autoantibodies against the immunodominant bullous pemphigoid epitopes are rare in patients with dermatitis herpetiformis and coeliac disease. *Frontiers in Immunology*. 11:575805. https://doi: 10.3389/fimmu. 2020.575805

II Nätynki, A., Leisti, P., Tuusa, J., Varpuluoma, O., Huilaja, L., Izumi, K., Herukka, S. K., Ukkola, O., Junttila, J., Kokkonen, N., & Tasanen, K. (2022) Use of gliptins reduces levels of SDF-1/CXCL12 in bullous pemphigoid and type 2 diabetes, but does not increase autoantibodies against BP180 in diabetic patients. *Frontiers in Immunology*. 13:942131. https://doi: 10.3389/fimmu.2022.942131

III Nätynki, A., Kokkonen, N., Tuusa, J., Ohlmeier, S., Bergmann, U., & Tasanen, K. (2024) Proteomic changes related to actin cytoskeleton function in the skin of vildagliptin-treated mice. *Journal of Dermatological Science*. In press. https://doi.org/10.1016/j.jdermsci.2024.01.003

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