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CHAPTER 7
Gene expression profile of lesional skin in bullous and nonbullous pemphigoid patients: an explorative pilot study

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Preliminary results

Introduction

Bullous pemphigoid (BP) is the most common autoimmune bullous disease that typically presents in elderly patients with severe pruritus, and tense blisters on erythematous skin.[1–3] One-in-five patients present with severe pruritus and a phenotype without blisters, a disease variant termed nonbullous pemphigoid (NBP).[4–6] The clinical presentation of NBP is heterogeneous and may mimic other pruritic dermatological diseases, causing misdiagnosis and long diagnostic delays.[7,8] While the pathogenesis of BP is only partly clarified, no studies have assessed the pathogenesis of NBP.

A higher susceptibility for pemphigoid diseases was observed in individuals expressing the major histocompatibility complex class II allele HLA-DQB1*03:01, which likely presents pemphigoid antigens to autoreactive T cells.[9–12] Subsequently, autoantibodies against hemidesmosome proteins BP180 and BP230 are formed.[13,14] Autoantibodies against BP180 were demonstrated to be pathogenic in animal and human studies.[15–19] The role of autoantibodies to the intracellular BP230 is less clear, and single BP230 autoantibody reactivity was associated with a less inflammatory BP phenotype, and with NBP.[4,15,20–22]

The disease mechanism of BP is not completely elucidated. Most studies support the hypothesis that autoantibodies to BP180 mediate blister formation by activation of the complement system through classical and alternative pathways, attracting inflammatory cells towards the skin.[23–25] Studies particularly found evidence for an important role of eosinophils and mast cells, whom upon activation release proteolytic enzymes that break down structural hemidesmosome proteins, causing subepithelial blister formation.[13,26–28] Complement independent mechanisms of blistering were also described, involving depletion of the BP180 molecule from the hemidesmosome by autoantibody induced pinocytosis.[29] So far, it is unknown why NBP patients do not develop blisters, while the autoantibody profile can be similar to that of BP.

In this study, we assessed the activation of the immune response in lesional skin of BP and NBP patients. By quantification of immune-response related gene transcripts, we aim to find differences in the immune response in BP and NBP.

Material and methods

Selection of patients and patient material

Patients diagnosed with BP (n=12) and NBP (n=12) at the outpatient clinic of our dermatology department were retrospectively selected. Diagnostic inclusion criteria consisted of the 2-out-of-3 rule, meaning patients had to meet two of the following three criteria; 1) pruritus and/or cutaneous blisters, 2) positive linear IgG or C3c staining by direct immunofluorescence (DIF) microscopy, 3) positive IgG staining on the epidermal side of salt-split skin by indirect immunofluorescence microscopy.[4] Another inclusion criterion was the performance of a lesional skin biopsy for histopathology for diagnostic purposes. Patients using systemic immunosuppressive drugs at the time of the biopsy were excluded. The use of topical corticosteroids was avoided, but allowed at lowest class if a more suitable biopsy was not available.

Patient characteristics were assessed by reviewing patient charts. Patient material utilized for analyzing gene transcripts consisted of histopathologic skin biopsies that were formalin fixed, and embedded in paraffin before stored at room temperature, according to standard protocol. BP skin biopsies contained one-third of a blister, and NBP skin biopsies were taken from lesional erythematous skin.

NanoString gene expression profiling

The nanoString nCounter [30] Myeloid Innate Immunity Panel (nanoString Technologies, Seattle, WA) was used to quantify the expression of 180 genes associated with innate and adaptive immune responses. RNA was isolated from four 5 µm thick formalin-fixed and paraffin-embedded skin sections of 12 confirmed NBP and 12 confirmed BP patients using the RNeasy mini kit (Qiagen) according to suppliers instructions. RNA (100ng as measured by Qubit (ThermoFisher)) was hybridized with the nanoString reporter and capture probes overnight at 65 degrees Celsius. Each probe set (reporter and capture probe) is designed to bind a unique mRNA target, and has a unique color-coded molecular tag. The RNA-probe complexes were loaded on an nCounter cartridge, and washed and read on a SPRINT digital counter, using digital photography.

Statistical analysis

nSolver Analysis Software (nanoString Technologies, Seattle, WA) was used for normalization of counts using the arithmetic mean of spiked-in reference gene

transcripts as well as the geometric mean of the hybridization controls. Gene expression data was visualized with the nSolver advanced analysis module. Heatmaps were created with unsupervised clustering. Binomial regression models were used to estimate the differential expression of genes in BP versus NBP patients. To control the rate of type I errors occurring when conducting multiple comparisons, the Benjamini-Yekutieli procedure was applied controlling for the false discovery rate, and providing an adjusted p-value.[31]

Results

Patients characteristics

Two BP samples were excluded from the study, due to a low quality of the transcript data. Gene expression levels were successfully measured in 10 BP, and 12 NBP patients. Patient characteristics, and the results of gene expression involving complement activation, T helper (Th1), and T helper 2 (Th2) responses are presented in table 1.

Complement activation

High expression of genes related to complement activation was observed in six BP patients (figure 1). The patients with a lower expression of complement activation related genes included four BP patients, and 12 NBP patients.

C3c positivity in direct immunofluorescence microscopy

Complement C3c in the skin is routinely stained in NBP and BP patients for diagnostic purposes by DIF. Three of the six BP patients with high expression of complement related genes showed linear C3c along the basement membrane zone (BMZ) by DIF (table 1). Moreover, five of the 16 biopsies with lower expression of complement related genes did show linear C3c along the BMZ by DIF.

Table 1. Patient characteristics and gene expression results

Patient characteristics						Gene expression*		
NBP vs. BP	Age	Gender	DIF IgG	DIF C3c	Antigen recognition**	Complement activation	Th1 response	Th2 response
NBP 1	67	female	2+	neg	BP180 only	Low	Low	Low
NBP 2	41	male	+/2+	neg	BP180 only	Low	Low	Low
NBP 3	95	male	+/2+	neg	BP180 only	Low	Low	Low
NBP 4	101	female	2+	neg	BP180 + BP230	Low	High	High
NBP 5	88	female	2+	neg	BP180 + BP230	Low	Low	Low
NBP 6	78	female	dub	3+	BP180 + BP230	Low	High	High
NBP 7	82	female	dub	neg	BP180 + BP230	Low	Low	Low
NBP 8	79	male	neg	neg	BP230 only	Low	Low	Low
NBP 9	79	male	neg	neg	BP230 only	Low	Low	Low
NBP 10	91	female	neg	neg	BP230 only	Low	High	Low
NBP 11	92	female	neg	neg	BP230 only	Low	Low	Low
NBP 12	86	female	neg	neg	BP230 only	Low	High	Low
BP 1	76	male	3+	2+	BP180 + BP230	Low	High	High
BP 2	53	male	3+	2+	BP180 only	Low	High	High
BP 3	87	male	2+	3+	BP180 + BP230	High	High	High
BP 4	71	female	2+	+	BP180 + BP230	High	High	High
BP 5	80	male	+	3+	BP180 only	High	High	High
BP 6	87	female	+	2+	BP180 only	Low	High	High
BP 7	74	female	+	+/-	BP180 + BP230	High	High	High
BP 8	80	female	+	neg	BP230 only	High	High	High
BP 9	89	female	neg	2+	BP180 + BP230	Low	High	High
BP 10	61	male	neg	neg	BP180 + BP230	High	High	High

** Whether gene expression was scored low or high was determined based on unsupervised clustering and visualization of the data by heatmaps (see figure 1, 2 and 3). ** Antigen recognition is based on results of immunoblot and/or enzyme-linked immunosorbent assay. NBP, nonbullous pemphigoid; BP, bullous pemphigoid; DIF, direct immunofluorescence microscopy; IgG, immunoglobulin G; C3c, complement component C3c; Th1, T helper 1; Th2, T helper 2.*

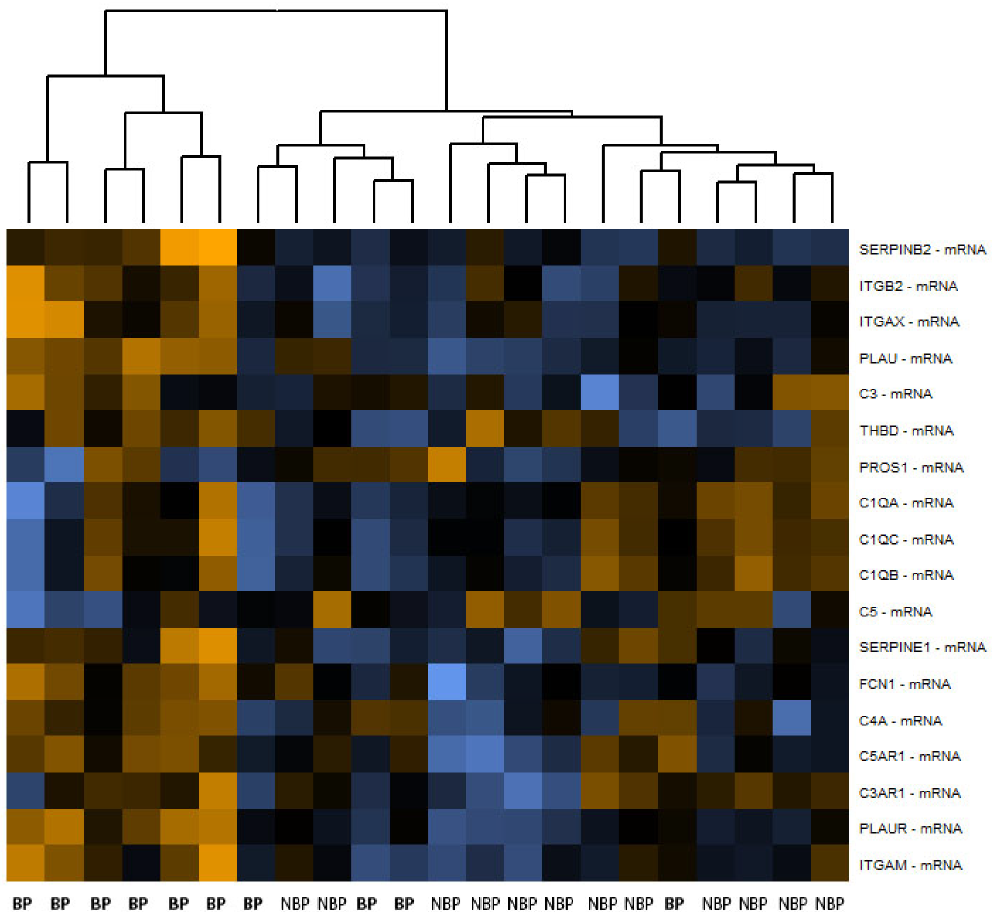

Figure 1. Complement activation. Heat map of expressed genes involved in complement activation. Unsupervised clustering was performed. Highly expressed genes are depicted in orange, whereas genes with lower expression are blue. The phenotype (BP or NBP) is presented per sample.

T helper 1 and 2 responses

In all BP specimens a high expression of both Th1 and Th2 related genes was observed (figure 2 and 3). In NBP specimens, genes involved in Th1 responses were highly expressed in one-third of the cases, while a gene expression signature associated with a Th2 response was observed in 2 out of 12 NBP cases.

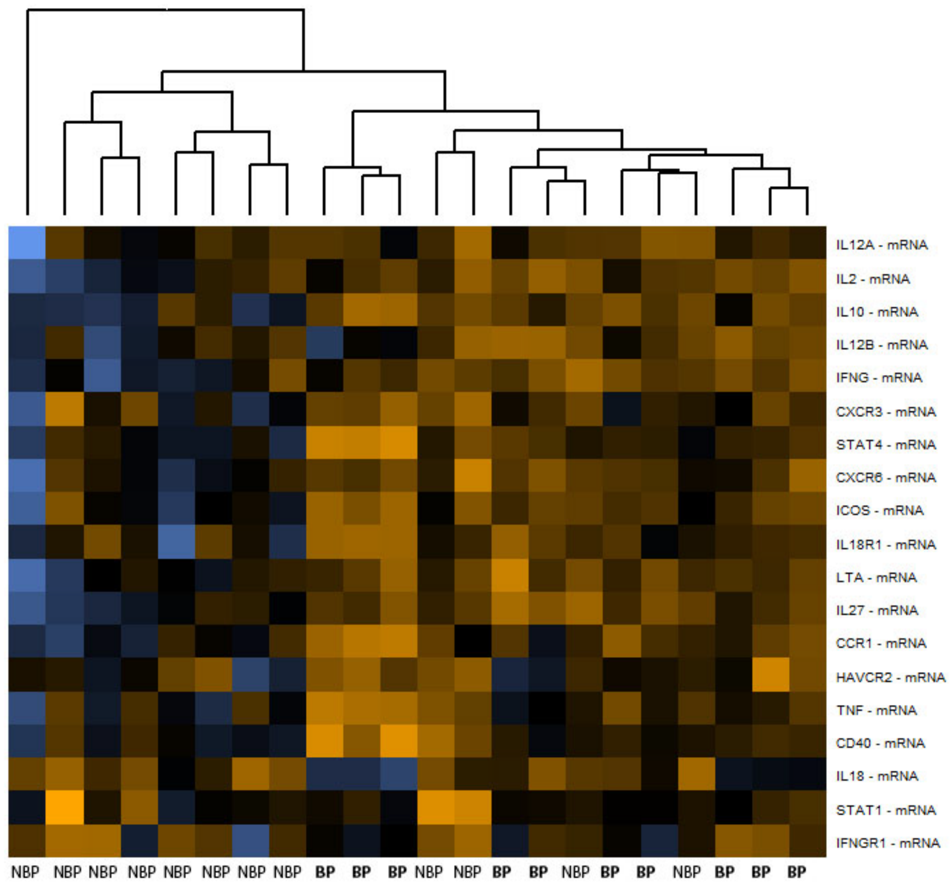

Figure 2. T helper 1 response. Heat map of expressed genes involved in a T helper 1 response. Unsupervised clustering was performed. Highly expressed genes are depicted in orange, whereas genes with lower expression are blue. The phenotype (BP or NBP) is presented per sample.

Gene expression of other immune-response related pathways

No other notable clustering of BP and NBP samples were observed in the heatmaps of genes related to angiogenesis, antigen presentation, cell cycle and apoptosis, cell migration and adhesion, chemokine signaling, cytokine signaling, differentiation and maintenance of myeloid cells, Fc receptor signaling, growth factor signaling, interferon signaling, lymphocyte activation, pathogen response, T-cell activation and checkpoint signaling, and toll-like receptor signaling.

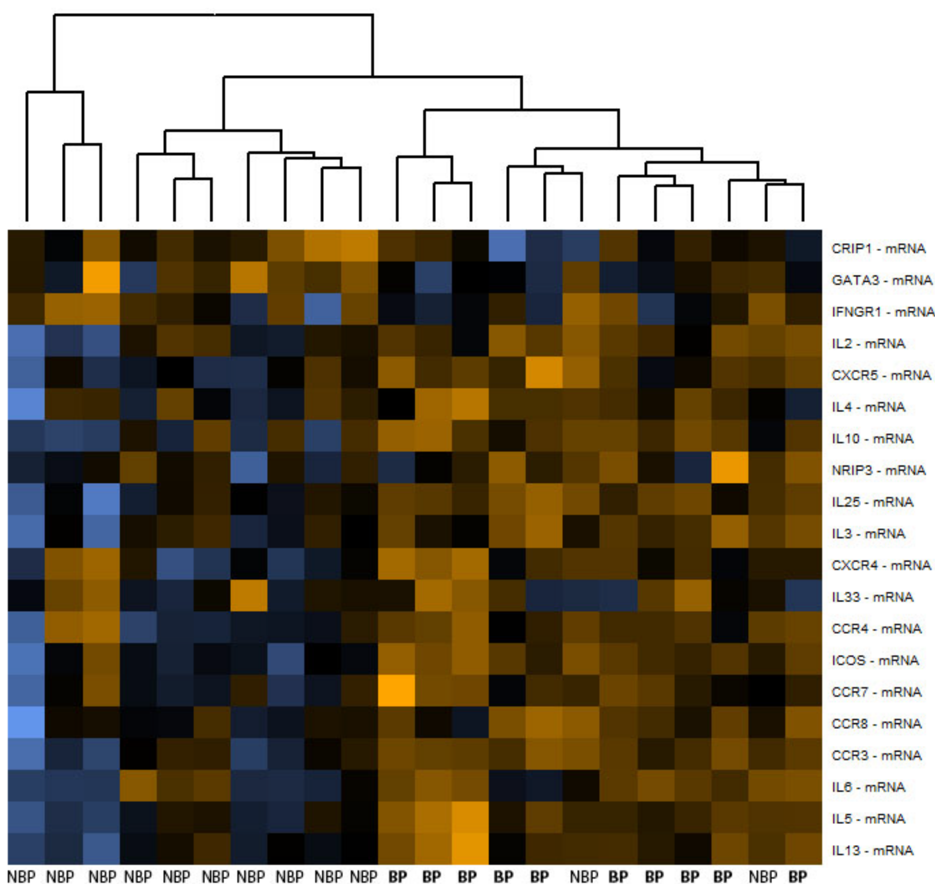

Figure 3. T helper 2 response. Heat map of expressed genes involved in a T helper 2 response. Unsupervised clustering was performed. Highly expressed genes are depicted in orange, whereas genes with lower expression are blue. The phenotype (BP or NBP) is presented per sample.

Differential gene expression

The 10 most statistically differentially expressed genes in the skin of BP and NBP patients are presented in table 2.

Discussion

This explorative study assessed the expression of immune related genes in lesional pemphigoid skin, and is the first to examine immune pathways in patients with NBP. Several previous studies assessed mRNA expression levels of cytokines and

chemokines in BP, but only in single cell types, such as macrophages, eosinophils, or peripheral blood mononuclear cells.[32–35]

Table 2. Top 10 of the most statistically differentially expressed genes in BP versus NBP skin (NBP is baseline).

Gene	Encodes for	Fold change (log2)	Standard error (log2)	95% Confidence interval (log2)	P-value	Adjusted p-value*
HBEGF	Heparin-binding epidermal growth factor-like growth factor	3.47	0.438	2.61 – 4.33	1.38e-07	0.000683
CXCL8	Chemokine IL-8	3.74	0.533	2.70 – 4.79	8.35e-07	0.00194
PTGS2	Prostaglandin-endoperoxide synthase 2	1.49	0.218	1.07 – 1.92	1.18e-06	0.00194
ADAMTS4	A disintegrin and metalloproteinase with trombospondin motifs	2.38	0.429	1.54 – 3.22	1.95e-05	0.0101
AREG	amphiregulin	3.51	0.635	2.27 – 4.76	2.05e-05	0.0101
CCL3	chemokine C-C motif ligand 3	1.45	0.251	0.96 – 1.95	1.14e-05	0.0101
CREM	cAMP (cyclic adenosine monophosphate) responsive element modulator	1.33	0.236	0.87 – 1.79	1.64e-05	0.0101
FOSL1	Fos-related antigen 1	2.1	0.375	1.37 – 2.84	1.78e-05	0.0101
GPR65	G protein-coupled receptor 65	1.1	0.195	0.72 – 1.48	1.6e-05	0.0101
IL1RL1	Interleukin-1 receptor-like 1	1.78	0.307	1.18 – 2.38	1.16e-05	0.0101

** P-values were adjusted for multiple testing by applying the Benjamini-Yekutieli procedure, controlling for the false discovery rate. BP, bullous pemphigoid; NBP, nonbullous pemphigoid.*

By using a broad explorative approach, our results indicated involvement of complement activation in 6 out of 10 BP patients but not in NBP patients (0 out of 12). Moreover, all BP patients showed strong Th1 and Th2 responses, whereas NBP patients did not (high expression of Th1 in 33%, Th2 in 17%). These observations support the hypothesis that blister formation involves Th1 and Th2 responses, and complement activation.

Genes related to complement activation

Six BP patients showed higher expression of genes related to complement activation compared to 4 BP and all 12 NBP patients. In line with these findings, mice studies on the pathogenesis of BP demonstrated that complement activation via the classical pathway and alternative pathway might be essential for blister development.[25,36,37] Complement independent disease mechanisms might have played an more important role in the pemphigoid patients with a lower expression of complement related genes.[29] Surprisingly, expression of genes related to complement activation did not correspond with positivity of C3c linear along the BMZ by DIF in several patients. C3c deposits in perilesional skin apparently do not reflect on lesional complement activation.

Interestingly, several patients (4 BP, and 7 NBP) had IgG deposits along the BMZ by DIF, but did not show an high expression of complement related genes. We hypothesize that IgG visualized by DIF in these patients might consist of IgG2 or IgG4 subclasses, whom have low complement activating capacity.[38] In line with this hypothesis, it was previously shown that NBP patients were IgG4 subclass dominant, and showed less complement deposits in the skin compared to BP patients.[4,39,40] Our observations underscore the complexity of the complement system, and its various activation routes.

Genes involved in T helper 1 and 2 responses

High expression of both Th1 and Th2 related genes was found in skin of all BP patients, but in a minority of NBP patients. Previous studies in BP detected autoreactive T cells producing both Th1 and Th2 cytokines, and found elevated levels of Th1 and Th2 chemokines in serum.[12,41–43] More evidence for a Th2 response in BP includes increased serum levels of total IgE, and IgE against the NC16A domain of BP180, which correlates with disease activity.[17,44–46] IgE was also observed bound to the cell surface of mast cells and eosinophils in the dermis of BP

skin, while others found IgE deposits in a linear pattern along the BMZ.[28,47–49] Peripheral eosinophilia was present in approximately half of BP and NBP patients, and dermal infiltrates commonly include eosinophils in both disease phenotypes.[20,50,51] All data above point towards a strong Th2 footprint in BP. Only limited data are published about the immune response in NBP. In chapter 6 of this thesis we detected IgE in serum and skin of NBP patients, suggesting a role for a Th2 immune response in its pathogenesis (unpublished data). However, based on our gene expression data, we may conclude that Th2 responses likely play a less prominent role in the disease pathogenesis of NBP compared to BP.

Differential gene expression

Several genes that were most differentially expressed in BP compared to NBP skin are discussed below.

Epidermal growth factors

The HBEGF gene encodes for heparin-binding epidermal growth factor-like growth factor, and was most differentially expressed, with high expression in BP. HBEGF is an epidermal growth factor produced by monocytes and macrophages, and plays a role in many physiological and pathological processes. No publications link HBEGF gene expression to pemphigoid so far. In rheumatoid arthritis (RA), single cell sequencing identified a population of HBEGF+ inflammatory macrophages in inflamed synovial tissues.[52] These macrophages produced a defined subset of inflammatory products, such as IL-1, HBEGF, and epiregulin, and promoted pathologic fibroblast mediated joint destruction. In a lupus nephritis mouse model, high expression of the HBEGF gene was found in kidney tissue and kidney macrophages, suggesting that HBEGF might be important in the SLE pathogenesis.[53]

A second epithelial growth factor gene that was highly expressed in BP compared to NBP skin is amphiregulin (AREG). Interestingly, AREG is an important paralog of the HBEGF gene, meaning the genes descent from the same ancestral gene.[54] AREG is a transmembrane protein, and in RA its expression seems to be related to fibroblasts proliferation, and increased levels of IL-8.[54,55] AREG and HBEGF were significantly upregulated in bone marrow mononuclear cells, and peripheral blood mononuclear cells of RA patients, but only AREG was significantly upregulated in synovial fluids.[55]

HBEGF and AREG expression is dependent on ADAM17 activation.[56] ADAM17 belongs to the family of disintegrins and metalloproteases, and is best known for processing tumor necrosis factor α (TNF-α). While HBEGF and AREG expression were not previously studied in BP, ADAM17 was highly expressed in the epidermis of BP patients, and was suggested to deplete BP180 from keratinocytes.[56,57]

Pro-inflammatory chemokines, cytokines and enzymes

The CXCL8 gene encodes for the pro-inflammatory chemokine IL-8 and is highly expressed in BP. IL-8 is a potent chemoattractant and activator of leukocytes. It was shown that keratinocytes *in vitro* release IL-8 and IL-6, after binding with anti-BP180 IgG.[58] Moreover, multiple studies found elevated IL-8 levels in blister fluid and serum of BP patients compared to controls.[59–62]

The PTGS2 gene encodes for the enzyme prostaglandin-endoperoxide synthase 2, more often referred to as cyclooxygenase-2 (COX-2). COX-2 catalyzes arachidonic acid to prostaglandin H2, and is highly expressed during inflammation. In literature, the only link between BP and COX-2 is a report describing a pemphigoid case possibly induced by selective COX-2 inhibitor celecoxib.[64] Interestingly, COX-2 is naturally inhibited by calcitriol, the active form of vitamin D.[65] Several studies found an association between low levels of vitamin D and BP.[66–69] *In vitro*, calcitriol showed anti-inflammatory effects in keratinocytes that were treated with BP specific autoantibodies.[70]

The CCL3 gene encodes for chemokine C-C motif ligand 3, also termed macrophage inflammatory protein-1-α (MIP-1- α), and is produced by macrophages. CCL3 has a function in recruitment and activation of granulocytes.[74] Two previous studies observed elevated expression of CCL3 in BP serum and blister fluid.[75,76] However, one of these studies did report that Th2 associated chemokines (eotaxin and MCP-4) were higher in the blister fluid of BP patients, than Th1 associated chemokines (CXCL10 and CCL3).[76]

Transcription factors

CREM is a gene encoding for transcription factor cAMP (cyclic adenosine monophosphate) responsive element modulator, and is an important component of the cAMP mediated signal transduction.[77] No data is available on the expression of CREM and its possible function in pemphigoid. However, in T lymphocytes of patients with systemic lupus erythematosus (SLE) a high expression of CREM is

reported.[78] In SLE, CREM binds to the IL-2 promotor to repress IL-2 transcription by T lymphocytes, while enhancing the expression of IL-17 by direct transcriptional mechanisms.[77–79] IL-2 is important for proliferation of T and B lymphocytes, while IL-17 is a pro-inflammatory cytokine described to have a potential role in the pathogenesis of several autoimmune diseases, such as RA, SLE, multiple sclerosis, inflammatory bowel diseases, and also in BP.[60,77–80]

A second gene that influences gene transcription is the FOSL1 gene, encoding for the fos-related antigen 1 (FRA1). FOSL1 is member of the FOS gene family, that encode leucine zipper proteins that dimerize with proteins of the JUN family to form the transcription factor complex 'activator protein 1' (AP-1).[81] The AP-1 complex regulates a variety of mitogen activated protein kinase (MAPK) pathways. No articles have previously described FOSL1 or AP-1 expression in pemphigoid. Interestingly, CREM expression is regulated by two promotors named P1 and P2, of which the promotor P2 is under tight transcriptional control of AP-1, linking the high CREM and FOSL1 expression found in this study.[77]

Receptor genes

The Interleukin-1 receptor-like 1 (IL1RL1) gene encodes for a receptor that is highly expressed on Th2 cells and mast cells. Binding of IL-33 to IL1RL1 induces the production of Th2 associated cytokines, and IL-8.[83] In BP, IL1RL1 expression and IL-33 levels were measured in serum and blister fluid, but in contrast to our results, IL1RL1 expression and IL-33 protein levels were below the detection limit.[84] Possibly, the difference in patient material en testing method by which IL1RL1 expression was measured could account for these conflicting results.

GPR65 encodes for a G protein-coupled receptor 65, also termed TDAG8 (T cell death associated gene 8). The GPR65 receptor is expressed in several tissue types, as well as in lymphocytes, macrophages and leucocytes.[87] The GPR65 receptor is able to sense the extracellular pH, and in acidic environments its activation results in intracellular cAMP accumulation.[87] GPR65 receptor activation in T cells and macrophages showed a reduced production of pro-inflammatory cytokines IL-6 and TNF-α, and increased production of anti-inflammatory cytokine IL-10 *in vitro*.[88] In line, a GPR65 knockout mice model showed a significant exacerbation of antibody induced arthritis and delayed hypersensitivity.[89] These findings suggest anti-inflammatory effects of GPR65 expression but its function in BP remains elusive

Proteinase

The ADAMTS4 gene encodes for a proteinase that is a member of the "a disintegrin and metalloproteinase with trombospondin motifs (ADAMTS)" family, and is known for degradation of proteoglycans in articular cartilage in osteoarthritis.[90] The role of ADAMTS4 in inflammation is unclear and is not linked to pemphigoid or other autoimmune diseases so far.

Summary differential gene expression

All genes discussed above showed a significant higher expression in BP than in NBP, and therefore might be important in the pathogenesis of BP, and less important in the pathogenesis of NBP. In summary, we may carefully conclude that chemoattractants IL-8 and CCL3 possibly play a role in migration of leukocytes towards the site of inflammation. High expression of epidermal growth factors HBEGF and AREG suggest a role of macrophages in BPs pathogenesis. The high expression of transcription factors CREM and FOSL1 were previously described in T lymphocytes, and possibly enhance the production of pro-inflammatory IL-17 in BP skin. The ILR1RL1 receptor was previously found on Th2 cells and mast cells, and after activation might contribute to a pro-inflammatory environment by releasing Th2 related cytokines and IL-8. In contrast, activation of the GPR65 receptor expressed on macrophages and T cells may have anti-inflammatory effects. The expression of COX-2 is known to be upregulated in inflammation, but its function in BP is not clear. Also the relevance of the observed high expression of the proteinase ADAMTS4 in BP is unknown.

Limitations of the study

The greatest limitations of this study is the limited sample size, combined with performing multiple comparisons, causing a multiple testing problem. By correcting for false discovery rates, the number of type I errors is only partially controlled. Therefore, our explorative genetic data needs further validation.

Another limitation is that a preselected panel of genes was analyzed, which could be seen as a limitation opposed to RNA sequencing. By only focusing on preselected genes, we might have missed differences in genes outside of the tested panel. Besides this limitation, the nanoString technique has some advantages over RNA-sequencing. The nanoString avoids reversed transcription of RNA, and cDNA amplification, two steps in RNA-sequencing that are prone to bias.

Moreover, in RNA-sequencing the use of formalin-fixed paraffin-embedded tissue samples that contain degraded RNA remains a challenge, while nanoString is able to still generate high-quality data from this tissue.

Conclusions

Based on the data of this explorative study, we may carefully conclude that genes related to complement activation, Th1 and Th2 responses showed a higher expression in BP skin compared to NBP skin, suggesting they may be important in blister formation. In BP skin, T-helper responses showed a strong dual character, with both Th1 and Th2 related genes involved, whereas in NBP only few biopsies showed high expression of Th1 and Th2 genes. Further studies for validation of the genetic data are needed, and should assess specific gene and protein expression in a larger patient population so that multiple testing problems are avoided. Moreover, an important next step is to include a series of healthy skin biopsies as control samples to analyze the possible more subtle changes in NBP gene expression.

References

1 Schmidt E, Zillikens D. Pemphigoid diseases. *Lancet (London, England)* 2013; 381:320–32.

2 Hubner F, Recke A, Zillikens D, *et al.* Prevalence and Age Distribution of Pemphigus and Pemphigoid Diseases in Germany. J. Invest. Dermatol. 2016; 136:2495–8.

3 Joly P. Incidence of bullous pemphigoid and pemphigus vulgaris. BMJ. 2008; 337:a209.

4 Meijer JM, Diercks GFH, de Lang EWG, *et al.* Assessment of Diagnostic Strategy for Early Recognition of Bullous and Nonbullous Variants of Pemphigoid. *JAMA dermatology* 2019 Feb 1;155(2):158-165. doi:10.1001/jamadermatol.2018.4390.

5 Della Torre R, Combescure C, Cortés B, *et al.* Clinical presentation and diagnostic delay in bullous pemphigoid: A prospective nationwide cohort. *Br J Dermatol* 2012; 167:1111–7.

6 Di Zenzo G, Della Torre R, Zambruno G, Borradori L. Bullous pemphigoid: from the clinic to the bench. *Clin Dermatol* 2012; 30:3–16.

7 Zhang Y, Luo Y, Han Y, *et al.* Non-bullous lesions as the first manifestation of bullous pemphigoid: A retrospective analysis of 181 cases. *J Dermatol* 2017 Jul;44(7):742-746. doi:10.1111/1346-8138.13782.

8 Sun C, Chang B, Gu H. Non-bullous lesions as the first manifestation of bullous pemphigoid: a retrospective analysis of 24 cases. *J Dermatolog Treat* 2009; 20:233–7.

9 Delgado JC, Turbay D, Yunis EJ, *et al.* A common major histocompatibility complex class II allele HLA-DQB1* 0301 is present in clinical variants of pemphigoid. *Proc Natl Acad Sci U S A* 1996; 93:8569–71.

10 Setterfield J, Theron J, Vaughan RW, *et al.* Mucous membrane pemphigoid: HLA-DQB1*0301 is associated with all clinical sites of involvement and may be linked to antibasement membrane IgG production. *Br J Dermatol* 2001; 145:406–14.

11 Amber KT, Zikry J, Hertl M. 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* 2017; 89:127–34.

12 Budinger L, Borradori L, Yee C, *et al.* Identification and characterization of autoreactive T cell responses to bullous pemphigoid antigen 2 in patients and healthy controls. *J Clin Invest* 1998; 102:2082–9.

13 Lo Schiavo A, Ruocco E, Brancaccio G, *et al.* Bullous pemphigoid: etiology, pathogenesis, and inducing factors: facts and controversies. *Clin Dermatol* 2013; 31:391–9.

14 van Beek N, Schulze FS, Zillikens D, Schmidt E. IgE-mediated mechanisms in bullous pemphigoid and other autoimmune bullous diseases. *Expert Rev Clin Immunol* 2016; 12:267–77.

15 Goletz S, Zillikens D, Schmidt E. Structural proteins of the dermal-epidermal junction targeted by autoantibodies in pemphigoid diseases. *Exp Dermatol* 2017; 26:1154–62.

16 Dopp R, Schmidt E, Chimanovitch I, *et al.* IgG4 and IgE are the major immunoglobulins targeting the NC16A domain of BP180 in Bullous pemphigoid: serum levels of these immunoglobulins reflect disease activity. *J Am Acad Dermatol* 2000; 42:577–83.

17 van Beek N, Luttmann N, Huebner F, *et al.* Correlation of Serum Levels of IgE Autoantibodies Against BP180 With Bullous Pemphigoid Disease Activity. *JAMA dermatology* 2017; 153:30–8.

18 Ishiura N, Fujimoto M, Watanabe R, *et al.* Serum levels of IgE anti-BP180 and anti-BP230 autoantibodies in patients with bullous pemphigoid. *J Dermatol Sci* 2008; 49:153–61.

19 Iwata Y, Komura K, Kodera M, *et al.* Correlation of IgE autoantibody to BP180 with a severe form of bullous pemphigoid. *Arch Dermatol* 2008; 144:41–8.

20 Lamberts A, Meijer JM, Pas HH, *et al.* Nonbullous pemphigoid: insights in clinical and diagnostic findings, treatment responses and prognosis. *J Am Acad Dermatol* 2019 Aug;81(2):355-363. doi:10.1016/j.jaad.2019.04.029.

21 Tanaka M, Hashimoto T, Dykes PJ, Nishikawa T. Clinical manifestations in 100 Japanese bullous pemphigoid cases in relation to autoantigen profiles. *Clin Exp Dermatol* 1996; 21:23–7.

22 Hayakawa T, Teye K, Hachiya T, *et al.* Clinical and immunological profiles of anti-BP230-type bullous pemphigoid: Restriction of epitopes to the C-terminal domain of BP230, shown by novel ELISAs of BP230-domain specific recombinant proteins. *Eur J Dermatol* 2016; 26:155–63.

23 Dainichi T, Chow Z, Kabashima K. IgG4, complement, and the mechanisms of blister formation in pemphigus and bullous pemphigoid. *J Dermatol Sci* 2017; 88:265–70.

24 Edwards G, Diercks GFH, Seelen MAJ, *et al.* Complement Activation in Autoimmune Bullous Dermatoses: A Comprehensive Review. *Front Immunol* 2019; 10:1477.

25 Nelson KC, Zhao M, Schroeder PR, *et al.* Role of different pathways of the complement cascade in experimental bullous pemphigoid. *J Clin Invest* 2006; 116:2892–900.

26 Hammers CM, Stanley JR. Mechanisms of Disease: Pemphigus and Bullous Pemphigoid. *Annu Rev Pathol* 2016; 11:175–97.

27 de Graauw E, Sitaru C, Horn M, *et al.* Evidence for a role of eosinophils in blister formation in bullous pemphigoid. *Allergy Eur J Allergy Clin Immunol* 2017; 72:1105–13.

28 Dimson OG, Giudice GJ, Fu CL, *et al.* Identification of a potential effector function for IgE autoantibodies in the organ-specific autoimmune disease bullous pemphigoid. *J Invest Dermatol* 2003; 120:784–8.

29 Iwata H, Ujiie H. Complement-independent blistering mechanisms in bullous pemphigoid. *Exp Dermatol* 2017 Dec;26(12):1235-1239. doi:10.1111/exd.13367.

30 Tsang H-F, Xue VW, Koh S-P, *et al.* NanoString, a novel digital color-coded barcode technology: current and future applications in molecular diagnostics. *Expert Rev Mol Diagn* 2017; 17:95–103.

31 Benjamini BY, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. *Ann statitistics* 2001; 29:1165–88.

32 Fang H, Shao S, Cao T, *et al.* Increased expression of NLRP3 inflammasome components and

interleukin-18 in patients with bullous pemphigoid. *J Dermatol Sci* 2016; 83:116–23.
33 Furudate S, Fujimura T, Kambayashi Y, *et al.* Comparison of CD163+ CD206+ M2 macrophages in the lesional skin of bullous pemphigoid and pemphigus vulgaris: the possible pathogenesis of bullous pemphigoid. *Dermatology* 2014; 229:369–78.
34 Messingham KN, Holahan HM, Frydman AS, *et al.* Human Eosinophils Express the High Affinity IgE Receptor, FcεRI, in Bullous Pemphigoid. *PLoS One* 2014 Sep 25;9(9):e107725.
35 Gambichler T, Tsitlakidon A, Skrygan M, *et al.* T regulatory cells and other lymphocyte subsets in patients with bullous pemphigoid. *Clin Exp Dermatol* 2017; 42:632–7.
36 Heimbach L, Li Z, Berkowitz P, *et al.* The C5a receptor on mast cells is critical for the autoimmune skin-blistering disease bullous pemphigoid. *J Biol Chem* 2011; 286:15003–9.
37 Liu Z, Giudice GJ, Swartz SJ, *et al.* The role of complement in experimental bullous pemphigoid. *J Clin Invest* 1995; 95:1539–44.
38 Tao MH, Smith RI, Morrison SL. Structural features of human immunoglobulin G that determine isotype-specific differences in complement activation. *J Exp Med* 1993; 178:661–7.
39 Lamb PM, Patton T, Deng JS. The predominance of IgG4 in prodromal bullous pemphigoid. *Int J Dermatol* 2008; 47:150–3.
40 Romeijn TR, Jonkman MF, Knoppers C, *et al.* Complement in bullous pemphigoid: results from a large observational study. Br. J. Dermatol. 2017; 176:517–9.
41 Lin MS, Fu CL, Giudice GJ, *et al.* Epitopes targeted by bullous pemphigoid T lymphocytes and autoantibodies map to the same sites on the bullous pemphigoid 180 ectodomain. *J Invest Dermatol* 2000; 115:955–61.
42 Echigo T, Hasegawa M, Shimada Y, *et al.* Both Th1 and Th2 chemokines are elevated in sera of patients with autoimmune blistering diseases. *Arch Dermatol Res* 2006; 298:38–45.
43 Eming R, Budinger L, Riechers R, *et al.* Frequency analysis of autoreactive T-helper 1 and 2 cells in bullous pemphigoid and pemphigus vulgaris by enzyme-linked immunospot assay. *Br J Dermatol* 2000; 143:1279–82.
44 Asbrink E, Hovmark A. Serum IgE levels in patients with bullous pemphigoid and its correlation to the activity of the disease and anti-basement membrane zone antibodies. *Acta Derm Venereol* 1984; 64:243–6.
45 Arbesman CE, Wypych JI, Reisman RE, Beutner EH. IgE levels in sera of patients with pemphigus or bullous pemphigoid. *Arch Dermatol* 1974; 110:378–81.
46 Saniklidou AH, Tighe PJ, Fairclough LC, Todd I. IgE autoantibodies and their association with the disease activity and phenotype in bullous pemphigoid: a systematic review. *Arch Dermatol Res* 2018; 310:11–28.
47 Freire PC, Munoz CH, Stingl G. IgE autoreactivity in bullous pemphigoid: eosinophils and mast cells as major targets of pathogenic immune reactants. *Br J Dermatol* 2017; 177:1644–53.
48 Kamata A, Kurihara Y, Funakoshi T, *et al.* Basement membrane zone IgE deposition is associated with bullous pemphigoid disease severity and treatment results. *Br J Dermatol* 2019 Jul 22. doi: 10.1111/bjd.18364. [Epub ahead of print]
49 Yayli S, Pelivani N, Beltraminelli H, *et al.* Detection of linear IgE deposits in bullous pemphigoid and mucous membrane pemphigoid: A useful clue for diagnosis. *Br J Dermatol* 2011; 165:1133–7.
50 Kridin K. Peripheral eosinophilia in bullous pemphigoid: Prevalence and influence on the clinical manifestation. *Br J Dermatol* 2018 Nov;179(5):1141-1147.
51 Lamberts A, Meijer JM, Jonkman MF. Nonbullous pemphigoid: A systematic review. *J Am Acad Dermatol* 2018 May;78(5):989-995.e2.
52 Kuo D, Ding J, Cohn IS, *et al.* HBEGF(+) macrophages in rheumatoid arthritis induce fibroblast invasiveness. *Sci Transl Med* 2019 May 8;11(491). pii: eaau8587.

doi:10.1126/scitranslmed.aau8587.
53 Qing X, Chinenov Y, Redecha P, *et al.* iRhom2 promotes lupus nephritis through TNF-alpha and EGFR signaling. *J Clin Invest* 2018; 128:1397–412.
54 Singh B, Carpenter G, Coffey RJ. EGF receptor ligands: recent advances. *2016 Sep 8;5. pii: F1000 Faculty Rev-2270.* doi:10.12688/f1000research.9025.1.
55 Yamane S, Ishida S, Hanamoto Y, *et al.* Proinflammatory role of amphiregulin, an epidermal growth factor family member whose expression is augmented in rheumatoid arthritis patients. *J Inflamm (Lond)* 2008; 5:5.
56 Zebrowska A, Wagrowska-Danilewicz M, Danilewicz M, *et al.* Does Adam17 cause the destruction of anchoring fibers via shedding tumor necrosis factor alpha in bullous pemphigoid and dermatitis herpetiformis? J. Cutan. Med. Surg. 2012; 16:149–50.
57 Liu Y, Peng L, Li L, *et al.* TWEAK/Fn14 Activation Contributes to the Pathogenesis of Bullous Pemphigoid. *J Invest Dermatol* 2017; 137:1512–22.
58 Schmidt E, Reimer S, Kruse N, *et al.* Autoantibodies to BP180 associated with bullous pemphigoid release interleukin-6 and interleukin-8 from cultured human keratinocytes. *J Invest Dermatol* 2000; 115:842–8.
59 Tukaj S, Gruner D, Zillikens D, Kasperkiewicz M. Hsp90 blockade modulates bullous pemphigoid IgG-induced IL-8 production by keratinocytes. *Cell Stress Chaperones* 2014; 19:887–94.
60 Kowalski EH, Kneibner D, Kridin K, Amber KT. Serum and blister fluid levels of cytokines and chemokines in pemphigus and bullous pemphigoid. *Autoimmun Rev* 2019; 18:526–34.
61 Engmann J, Rüdrich U, Behrens G, *et al.* Increased Activity and Apoptosis of Eosinophils in Blister Fluids, Skin and Peripheral Blood of Patients with Bullous Pemphigoid. *Acta Derm Venereol* 2017; 97:464–71.
62 Tabatabaei-Panah P-S, Moravvej H, Sadaf Z, *et al.* Proinflammatory Cytokine Gene Polymorphisms in Bullous Pemphigoid. *Front Immunol* 2019; 10:636.
63 Mitchell JA, Warner TD. COX isoforms in the cardiovascular system: understanding the activities of non-steroidal anti-inflammatory drugs. *Nat Rev Drug Discov* 2006; 5:75–86.
64 Apap C, Boffa MJ, Cordina J. Celecoxib-induced bullous pemphigoid. Skinmed. 2013; 11:190–1.
65 Wang Q, He Y, Shen Y, *et al.* Vitamin D inhibits COX-2 expression and inflammatory response by targeting thioesterase superfamily member 4. *J Biol Chem* 2014; 289:11681–94.
66 Marzano A V, Trevisan V, Eller-Vainicher C, *et al.* Evidence for vitamin D deficiency and increased prevalence of fractures in autoimmune bullous skin diseases. *Br J Dermatol* 2012; 167:688–91.
67 Marzano AV, Trevisan V, Cairoli E, *et al.* Vitamin D and skeletal health in autoimmune bullous skin diseases: a case control study. *Orphanet J Rare Dis* 2015; 10:8.
68 Sarre ME, Annweiler C, Legrand E, *et al.* Association between bullous pemphigoid and hypovitaminosis D in older inpatients: Results from a case-control study. *Eur J Intern Med* 2016; 31:25–8.
69 Sarre ME, Riou J, Duval GT, *et al.* Association between serum 25-hydroxyvitamin D concentration and severity of first-diagnosed bullous pemphigoid in older adults. *Arch Gerontol Geriatr* 2019; 83:28–30.
70 Tukaj S, Gruner D, Tukaj C, *et al.* Calcitriol exerts anti-inflammatory effects in keratinocytes treated with autoantibodies from a patient with bullous pemphigoid. *J Eur Acad Dermatol Venereol* 2016; 30:288–92.
71 Agrawal U, Kumari N, Vasudeva P, *et al.* Overexpression of COX2 indicates poor survival in urothelial bladder cancer. *Ann Diagn Pathol* 2018; 34:50–5.
72 Tong D, Liu Q, Wang L-A, *et al.* The roles of the COX2/PGE2/EP axis in therapeutic resistance. *Cancer Metastasis Rev* 2018; 37:355–68.

73 Friedrich M, Reichert K, Woeste A, *et al.* Effects of Combined Treatment with Vitamin D and COX2 Inhibitors on Breast Cancer Cell Lines. *Anticancer Res* 2018; 38:1201–7.

74 Hatano Y, Katagiri K, Arakawa S, *et al.* Successful treatment by double-filtration plasmapheresis of a patient with bullous pemphigoid: effects in vivo on transcripts of several genes for chemokines and cytokines in peripheral blood mononuclear cells. *Br J Dermatol* 2003; 148:573–9.

75 Nakashima H, Fujimoto M, Asashima N, *et al.* Serum chemokine profile in patients with bullous pemphigoid. *Br J Dermatol* 2007; 156:454–9.

76 Gounni Abdelilah S, Wellemans V, Agouli M, *et al.* Increased expression of Th2-associated chemokines in bullous pemphigoid disease. Role of eosinophils in the production and release of these chemokines. *Clin Immunol* 2006; 120:220–31.

77 Rauen T, Hedrich CM, Tenbrock K, Tsokos GC. cAMP responsive element modulator: a critical regulator of cytokine production. *Trends Mol Med* 2013; 19:262–9.

78 Juang Y-T, Wang Y, Solomou EE, *et al.* Systemic lupus erythematosus serum IgG increases CREM binding to the IL-2 promoter and suppresses IL-2 production through CaMKIV. *J Clin Invest* 2005; 115:996–1005.

79 Ohl K, Wiener A, Lippe R, *et al.* CREM Alpha Enhances IL-21 Production in T Cells In Vivo and In Vitro. *Front Immunol* 2016; 7:618.

80 Arakawa M, Dainichi T, Ishii N, *et al.* Lesional Th17 cells and regulatory T cells in bullous pemphigoid. Exp. Dermatol. 2011; 20:1022–4.

81 Vesely PW, Staber PB, Hoefler G, Kenner L. Translational regulation mechanisms of AP-1 proteins. *Mutat Res* 2009; 682:7–12.

82 Trop-Steinberg S, Azar Y. AP-1 Expression and its Clinical Relevance in Immune Disorders and Cancer. *Am J Med Sci* 2017; 353:474–83.

83 Yagami A, Orihara K, Morita H, *et al.* IL-33 mediates inflammatory responses in human lung tissue cells. *J Immunol* 2010; 185:5743–50.

84 Wakatabi K, Komine M, Meephansan J, *et al.* The levels of soluble ST2 in sera and bullous fluid from patients with bullous pemphigoid. *Eur J Dermatol* 2012; 22:333–6.

85 Margiotta DPE, Navarini L, Vadacca M, *et al.* The IL33/ST2 axis in Sjogren syndrome in relation to disease activity. *Eur Rev Med Pharmacol Sci* 2016; 20:1295–9.

86 Mok MY, Huang FP, Ip WK, *et al.* Serum levels of IL-33 and soluble ST2 and their association with disease activity in systemic lupus erythematosus. *Rheumatology (Oxford)* 2010; 49:520–7.

87 Okajima F. Regulation of inflammation by extracellular acidification and proton-sensing GPCRs. *Cell Signal* 2013; 25:2263–71.

88 Onozawa Y, Fujita Y, Kuwabara H, *et al.* Activation of T cell death-associated gene 8 regulates the cytokine production of T cells and macrophages in vitro. *Eur J Pharmacol* 2012; 683:325–31.

89 Onozawa Y, Komai T, Oda T. Activation of T cell death-associated gene 8 attenuates inflammation by negatively regulating the function of inflammatory cells. *Eur J Pharmacol* 2011; 654:315–9.

90 Kelwick R, Desanlis I, Wheeler GN, Edwards DR. The ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs) family. *Genome Biol* 2015; 16:113.

PART 2

Management of pemphigoid diseases