EXPRESSION OF KERATINS, EPIDERMAL PROTEINS AND INFLAMMATORY CELLS IN SUPERFICIAL PEMPHIGUS DOGS

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Summary

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Superficial pemphigus in dogs is an autoimmune skin disorder. The disease is characterised by the binding of auto-antibodies to desmosomal molecules including desmocollin-1, a major auto-antigen in dogs. However, data on the expression of epidermal proteins in dogs are still limited. Therefore, the aim of this study was to investigate the keratins and epidermal proteins in dogs with superficial pemphigus using immunohistochemistry. Skin biopsies were performed on dogs with pemphigus foliaceus (n=10), pan-epidermal pustular pemphigus (n=4) and pemphigus erythematosus (n=1). Immunostaining for keratin 5 and keratin 10 showed a premature and discontinuous pattern in the suprabasal layers of all pemphigus skin samples compared to the control group (P<0.05). Filaggrin, desmocollin-1 and claudin-1, but not involucrin, were significantly reduced (P<0.05). Inflammatory cells markedly infiltrated in the dermis and the lower epidermis of all pemphigus skins. This is the first report that associates changes in the expression of keratin 5, keratin 10, filaggrin and claudin-1 with reduced desmocollin-1 expression and subsequent loss of the epidermal barrier in superficial pemphigus.

Key words: dog, epidermal proteins, immunohistochemistry, keratins, superficial pemphigus

INTRODUCTION

Superficial pemphigus refers to a group of autoimmune dermatoses in dogs. The affected lesion is generally a separation of the inter-epidermal layers with subcorneal pustules and acantholytic keratinocytes

(Kuhl *et al.*, 1994; Wurm *et al.*, 1994). Pemphigus in dogs can be classified as pemphigus foliaceus (PF), pemphigus erythematosus (PE) and pan-epidermal pustular pemphigus (PPP) based on histo-

pathology (Wurm *et al.*, 1994; Olivry, 2006). Pemphigus disease is thought to be caused by a disruption of the complex desmosome structure which involves desmoglein-1 (DSG-1) in humans (Waschke, 2008) and desmocollin-1 (DSC-1) in dogs (Olivry, 2006). Two major mechanisms of pemphigus pathogenesis in humans have been proposed based on the reaction of auto-antibodies to epidermal cadherin molecules. The first proposal is that auto-antibodies directly block the targeted desmosomal proteins and result in the disruption of their adhesive ability (Shimizu *et al.*, 2004; Waschke, 2008), while the second suggests that the binding of auto-antibodies triggers intracellular signalling pathways that indirectly result in the loss of cell-cell adhesion (Berkowitz *et al.*, 2008; Waschke, 2008).

A study on epidermal cells in human and animal studies reported that the adhesive skin barrier includes desmosome complex proteins connected to keratin intermediate filaments (KIFs) (Waschke, 2008), and that the terminal keratinocyte differentiation is associated with the presence of KIFs in the suprabasal layer of the epidermis where specific keratin-associated proteins such as involucrin, loricrin and filaggrin are present (Bragulla & Homberger, 2009). A loss of this epidermal interaction is thought to be involved in keratin retraction and the formation of skin blisters and erosions (Anhalt, 1999; Shimizu *et al.*, 2004; Hamada *et al.*, 2013). However, keratin expression in dogs with pemphigus remains unknown. Thus, this study aimed to investigate the localisation patterns of keratin and associated proteins in samples obtained from dogs with superficial pemphigus using immunohistochemistry and to compare these with the immunological cell response during superficial pemphigus.

MATERIALS AND METHODS

Dogs

Fifteen dogs with pemphigus were used in this study, of which five were female (age range 2–7 years, mean 6.6 years) and ten were male (age range 4–15 years, mean 7.2 years), as shown in Table 1. Fifteen healthy dogs (age range 2–5 years, mean 4.3 years) were also enrolled in this study and served as the control. Pemphigus in the dogs was classified based on previously reported criteria (Olivry, 2006) that included the foci lesion distribution of vesicles, pustules, crusts and erosions. Lesions were observed on the face around the dorsal muzzle, on the nasal planum and in the periocular area as well as on the trunk and the extremities. Other tests, comprising fungal culture, bacterial culture and skin scraping, were performed to rule out other skin diseases. All systemic clinical signs such as anorexia, depression, fever and weight loss were also recorded. A cytological impression smear was done to detect the presence of free-floating acantholytic keratinocytes. The presenting features of pemphigus skin lesions included erosions and crusts, but not transient pustules. A 2% lidocaine infiltration was used to provide local anaesthesia before performing skin punch biopsy (6 millimeters in diameter). This study was approved by the animal ethics procedure of the Kasetsart University, Bangkok, Thailand (Id number: ACKU 011357), and informed consent was obtained from each dog owner.

Histology

Collected skin samples were fixed in 10% neutral-buffered formalin, histologically processed, and embedded in paraffin. Formalin-fixed, paraffin-embedded tissue was cut into 3–4 µm thick sections and

Table 1. History and type of pemphigus in all affected dogs in this study

No	Breed	Sex	Age, years	Collected area	Type	Systemic signs	Fungal and bacterial infection
1	Beagle	Female	7	Face	PF	No	No
2	Border collie	Male	8	Right shoulder	PF	No	No
3	Mixed breed	Male	15	Face	PF	No	No
4	Golden Retriever	Female	10	Face	PF	No	No
5	Golden Retriever	Male	9	Cranial left mammary gland	PPP	No	No
6	Poodle	Male	4	Upper lip	PPP	No	No
7	Golden Retriever	Male	5	Dorsal lumbar	PF	No	No
8	Labrador Retriever	Male	7	Face	PPP	No	No
9	Mixed breed	Female	8	Left flank	PPP	No	No
10	Labrador Retriever	Male	9	Nasal planum	PE	No	No
11	Beagle	Male	7	Left thorax	PF	No	No
12	Husky	Female	6	Face	PF	No	No
13	Husky	Male	4	Dorsal feet	PF	Yes	No
14	Husky	Male	4	Right cervical area	PF	Yes	No
15	Husky	Female	2	Face	PF	No	No

PE = Pemphigus erythematosus; PF = Pemphigus foliaceus; PPP = Pan-epidermal pustular pemphigus

stained with haematoxylin and eosin. The definitive diagnosis of all samples was verified by the presence of histological changes characteristic of pemphigus skin prior to enrollment in the study (Kuhl *et al.*, 1994; Wurm *et al.*, 1994; Olivry, 2006). Tissue sections stained with Periodic acid-Schiff (PAS) were used to exclude the presence of fungi and bacteria in

post-infectious, acantholytic keratinocytes, while Masson-trichrome staining was performed to observe the collagenous connective tissue (CNT) distribution in the dermis.

Protein expression by immunohistochemistry

The tissue sections were deparaffinised, rehydrated and incubated with 3% H₂O₂ to

Table 2. Primary antibodies, secondary antibodies, and visualisation system used for immunohistochemistry

Primary antibody	Dilution	Antigen retrieval method	Staining area	Secondary antibody	Enzyme label/visualisation system
<i>Keratins and epidermal proteins</i>					
Keratin 5	Mouse anti-keratin, RTU ^a	1.0% trypsin, 37°C, 15 min	Cytoplasmic	Envision ^{TM b}	Peroxidase (HRP)/DAB ^c
Keratin 10	Mouse anti-keratin10, 1:300 ^d	1.0% trypsin, 37 °C, 15 min	Cytoplasmic	Envision ^{TM b}	Peroxidase (HRP)/DAB ^c
Involucrin	Mouse anti-involucrin, 1:1,000 ^d	1.0% trypsin, 37 °C, 15 min	Cytoplasmic	Envision ^{TM b}	Peroxidase (HRP)/DAB ^c
Filaggrin	Rabbit anti-filaggrin, 1:2,000 ^d	1.0% trypsin, 37 °C, 15 min	Cytoplasmic	Envision ^{TM b}	Peroxidase (HRP)/DAB ^c
Desmocollin-1	Mouse anti-DSC-1, 1:300 ^e	Citrate (pH 6.0), 95 °C, 40 min	Membranous	Envision ^{TM b}	Peroxidase (HRP)/DAB ^c
Claudin-1	Rabbit anti-claudin-1, RTU ^f	Citrate (pH 6.0), 95 °C, 40 min	Membranous	Envision ^{TM b}	Peroxidase (HRP)/DAB ^c
Ki-67	Mouse anti-KI-67, 1:200 ^b	Citrate (pH 6.0), 95 °C, 40 min	Nuclear	Envision ^{TM b}	Peroxidase (HRP)/DAB ^c
<i>Immunological markers of inflammatory cells</i>					
Mast cell tryptase	Mouse anti-mast cell tryptase, 1:200 ^b	Citrate (pH 6.0), 95 °C, 40 min	Cytoplasmic	Envision ^{TM b}	Peroxidase (HRP)/DAB ^c
CD3	Mouse anti-CD79a, 1:200 ^b	Citrate (pH 6.0), 95 °C, 40 min	Membranous	Envision ^{TM b}	Peroxidase (HRP)/DAB ^c
CD79a	Mouse anti-CD79a, 1:200 ^b	Citrate (pH 6.0), 95°C, 40 min	Membranous	Envision ^{TM b}	Peroxidase (HRP)/DAB ^c
IgM	Goat anti-dog IgM, 1:2,000 ^g	Citrate (pH 6.0), 95°C, 40 min	Cytoplasmic	Rabbit anti-goat IgG, 1:200 ^b	Avidin-biotin complex ⁱ
IgG	Goat anti-dog IgG, 1:2,000 ^g	Citrate (pH 6.0), 95°C, 40 min	Cytoplasmic	Rabbit anti-goat IgG, 1:200 ^h	Avidin-biotin complex ⁱ

Materials sources: ^aDiagnostic Biosystems; Pleasanton, CA, USA; ^bDako; Glostrup, Denmark; ^cInvitrogen; Grand Island, NY, USA; ^dAbcam, Cambridge, UK; ^eLifeSpan Biosciences; Seattle, WA, USA; ^fCell Marque; Rocklin, CA, USA; ^gBethyl Laboratories Inc.; Montgomery, TX, USA; ^hSanta Cruz Biotechnology Inc.; Dallas, TX, USA; ⁱVector Laboratories; Burlingame, CA, USA; DAB=3,3'-diaminobenzidine tetrahydrochloride; HRP=horseradish peroxidase; RTU=ready to use.

block endogenous peroxidase activity. Details of antigen retrieval procedures and primary antibodies and secondary antibodies used are shown in Table 2. Each section was treated with 2% bovine serum albumin in phosphate-buffered solution at room temperature for 30 min, incubated with primary antibodies overnight at 4 °C, then with secondary antibodies added to the section together with 3,3-diaminobenzidine tetrahydrochloride (DAB) as peroxidase substrate, and counterstained with Mayer's haematoxylin. All sections were evaluated under a light microscope (FSX-100; Olympus; Nagano, Japan).

Quantitative analysis

Quantitative image analysis used: cytoplasmic staining for keratin 5, keratin 10, involucrin, filaggrin, mast cell tryptase, IgG, IgM; membrane staining for CD3, CD79a, DSC-1 and claudin-1; and nuclear staining for Ki-67. All quantitative analyses of staining were performed as previously described (Theerawatanasirikul *et al.*, 2012). Briefly, the intensity score was assigned into four different levels: 0, 1, 2, and 3 (for none, mild, moderate, and strong intensity) using the Cell-D digital

image analysis software (Olympus, Japan). The proportion of stained areas of that intensity level was calculated to the staining score. The numerical values of staining score were ranged from 0 to 3. Statistical analyses were performed using Prism software (version 5.0; GraphPad Software; San Diego, CA, USA). The Mann-Whitney U test was used to analyze the staining score. The Student t-test was used to compare the numbers of positive inflammatory cells and positive Ki-67 cells. The results were presented as mean $\pm$ SE. A P-value of less than 0.05 was considered statistically significant.

RESULTS

Histology of pemphigus skin

Skin biopsies from affected dogs were evaluated along with histological criteria for pemphigus foliaceus (PF) and pemphigus erythematosus (PE). Lesions in some dogs were not confined to the superficial epidermal layers and could not be distinctly classified as either PF or PE entities. Therefore, such lesions, with pustules spanning the thickness of the epi-

Fig. 1. Histopathologic appearance of pemphigus skin: A. hyperplasia of epidermis with subcorneal pustules and acantholytic cells (haematoxylin and eosin); B. collagen bundle destruction (Masson trichrome); e = epidermis; d = dermis. Scale bars = 20 μ m.

Table 3. Quantitative analysis of epidermal protein staining and immunological response markers in healthy control and pemphigus skins. Staining scores and the number of positive cells/mm² are given as mean $\pm$ SEM

Epidermal proteins	Normal control skin (n=15)	Pemphigus skin (n=15)
<i>Keratins and keratin-association proteins (staining score)</i>		
Keratin 5	1.66 $\pm$ 0.07	0.32 $\pm$ 0.03
Keratin 10	1.87 $\pm$ 0.05	0.17 $\pm$ 0.03 ^a
Involucrin	1.56 $\pm$ 0.04	1.85 $\pm$ 0.17 (13 of 15 dogs)
Filaggrin	2.05 $\pm$ 0.05	0.37 $\pm$ 0.04 ^b
<i>Desmosomal and tight junctions (staining score)</i>		
Desmocollin-1	1.54 $\pm$ 0.05	0.59 $\pm$ 0.04 ^b
Claudin-1	1.08 $\pm$ 0.19	0.75 $\pm$ 0.27 ^a
<i>Proliferative marker (positive cells/mm²)</i>		
Ki-67	8.68 $\pm$ 0.54	23.29 $\pm$ 1.22 ^b
<i>Immunological response markers (positive cells/mm²)</i>		
Mast cell tryptase	< 0.01	42.11 $\pm$ 14.92 ^b (10 of PF)
CD3	< 0.01	28.57 $\pm$ 14.55 ^b
IgG	< 0.01	71.97 $\pm$ 18.38 ^b
IgM	< 0.01	46.02 $\pm$ 32.34 ^b
CD79a	< 0.01	97.87 $\pm$ 26.80 ^b

^a P<0.05, ^b P<0.01.

dermal layer were referred to as pan-epidermal pustular pemphigus (PPP).

PF was found in 10 dogs (67%) presenting with epidermal pustules beneath the stratum corneum and follicular involvement. The pustules showed free-floating, clustered, acantholytic keratinocytes in the superficial suprabasal layer with perinuclear eosinophilic rings of keratin filaments. The observed epidermal changes included epidermal hyperplasia, spongiosis, hyperkeratosis and erosion

(Fig. 1). The presentation of PE was similar to that of PF except in one case where the latter was primarily restricted to the face. Four dogs (27%) were classified as PPP as they could not be definitively diagnosed as either as PF or PE.

None of the 15 cases tested showed the presence of PAS-positive dermatophytes, and the sections stained with Masson's Trichrome revealed a superficial, perivascular pattern in the dermis of the

skin and collagen bundle destruction in response to cellular infiltration.

Immunohistochemistry

Quantitative analysis of the staining scores and the immunological response of all antibodies are shown in Table 3 and Figs. 2 and 3.

Keratin 5, keratin 10, involucrin, filaggrin, desmocollin-1 and claudin-1 protein expression in the epidermis

The staining scores revealed that basal and suprabasal keratin expressions were significantly decreased in superficial pemphigus, and that the keratin 5 expression pattern, from stratum basale to stratum granulosum, was strong in the cytoplasm in the basal layers but faint in the upper suprabasal layers. This latter result corresponds to an increase in the number of Ki-67 positive keratinocytes in pemphigus dogs compared to those from normal dogs ($P < 0.01$). Suprabasal keratin 10 showed discontinuous and faint-to-moderate staining in the upper layers of the stratum

spinosum and stratum granulosum and was limited to the stratum corneum. The expression level of keratin 5 increased in acantholytic keratinocytes while expression of keratin 10 decreased.

Keratin-associated proteins, filaggrin and involucrin were localised to the cytoplasm in a discontinuous pattern in the upper layers of both stratum spinosum and stratum granulosum, apart from displaying a clumped pattern around the nuclei in epidermis. The expression of filaggrin was significantly reduced in the pemphigus skin compared to normal skin ($P < 0.01$). Increased involucrin expression was found in two cases of PF corresponding to a reduction in the expression of keratins and filaggrin; however, there was no significant difference in involucrin expression between the two groups ($P = 0.12$).

DSC-1 is a major desmosomal glycoprotein in canine PF. While normal skin (except in the stratum corneum) showed the presence of fine granules with enhanced staining along the cytoplasmic membrane in the suprabasal layers, skin

Fig. 2. Immunohistochemistry of protein localisation in pemphigus skin: keratin 5 (A); keratin 10 (B); filaggrin (C); involucrin (D); desmocollin-1 (E); claudin-1 (F), DAB and Mayer's haematoxylin counterstain; *e* = epidermis; *d* = dermis. Scale bars = 20 µm.

Fig. 3. Cellular infiltration and inflammatory response in pemphigus skin: CD3 positive T-cells (A); CD79a positive B-cells (B); Tryptase-positive mast cells (C); Immunoglobulin M (D); Immunoglobulin G (E) and Ki-67 positive cells (F); DAB and Mayer's haematoxylin counterstain, *e* = epidermis; *d* = dermis. Scale bars = 20 μ m.

from dogs with superficial pemphigus had coarse pericytoplasmic granules (40%, $n=6$). Claudin-1 is an epidermal tight junction protein, which, in normal skin, shows a strong, pericytoplasmic-staining pattern in the suprabasal layer; however, its expression was diminished in pemphigus-affected skin and in some acantholytic areas ($P<0.05$). The results of epidermal protein localisation are shown in Fig. 2.

Immunological cell infiltration and response in the dermis

Anti-canine antibodies against immunoglobulin M, immunoglobulin G were used to ascertain the immunological response of the dermis and the inter-epidermal areas, either in the subcorneal pustules or in the acantholytic areas (Fig. 3). IgG and IgM accumulated mostly in the extracellular matrix of the dermis and reached the superficial layer of the stratum granulosum; IgG deposition was greater than that of IgM in the dermis. CD3-positive T-cells were significantly less in number compared to CD79a-positive B-

cells. Tryptase-positive mast cells were significantly higher in all PF dogs. They were abundant in the upper dermis and also infiltrated into the lower part of the epidermis. Tryptase-positive mast cells were not found in either PE or PPP dogs.

DISCUSSION

This study investigated the expression of keratins and keratin-associated proteins in the epidermis of dogs with superficial pemphigus and compared this to changes in the expression of major auto-antigens, immunoglobulins and other immunological response cells.

In humans, PF is characterised by the presence of auto-antibodies against adhesion molecules such as, desmoglein-1 (DSG-1), desmoglein-3 (DSG-3), and desmoplakin which directly lead to desmosomal dissociation (Waschke, 2008). Consequently, these phenotypic molecular defects disrupt epidermal organisation and keratin expression, which in turn weaken

the cellular stability and promote desmosomal protein dysfunction (Shimizu *et al.*, 2004). Together, these observations imply that epidermal destruction in PF occurs due to poor keratinisation. Dysfunctional keratin expression may lead to blister formation, abnormal keratinisation. Acantholytic mechanisms involving auto-antibody-antigen interaction have been reported to directly induce disruption of cell adhesion via intercellular signaling pathways, such as p38 mitogen activated protein kinase (MAPK), heat shock protein 27 (HSP 27), *c-myc* and epidermal growth factor (EGF)-extracellular signal-regulated kinases (ERKs) 1/2 signals (Berkowitz *et al.*, 2008; Getsios *et al.*, 2009). Furthermore, a decrease in DSG-1 epidermal expression has been reported to interfere with DSC-1, keratin 10 and loricrin expression during skin differentiation (Getsios *et al.*, 2009; Mavropoulos *et al.*, 2013). It is known that KIFs retract in acantholytic keratinocytes, which, consequently, leads to the development of PF and pemphigus vulgaris (Bektas *et al.*, 2013; Mavropoulos *et al.*, 2013). Other studies have reported that DSC-1 is a potential auto-antigen in canine PF (Bizikova *et al.*, 2011; 2012); however, reduced DSC-1 expression was found in PE and PPP perhaps because it is possible that subtypes of superficial pemphigus share DSC-1 aberrance as well. Therefore, further studies are required to correlate DSC-1 expression among these three disease subtypes. Furthermore, while the current study found that claudin-1 was significantly reduced in pemphigus skin, Bizikova *et al.* (2011) reported that canine PF serum did not show any reaction to the epidermal cell of normal dogs, which presented an unmatched pattern to the positive area of claudin-1 localisation.

Specific keratin-associated proteins including filaggrin and involucrin gradually form a scaffold along with the suprabasal keratins (keratin 1, 2e, and 10) during the terminal stage of skin differentiation in the epidermis (Candi *et al.*, 2005), and both filaggrin and involucrin use the transglutaminase-1 enzyme via a Ca^{2+} -dependent pathway to become covalently cross-linked to desmosomal plakins, cytoskeletons and the other cornified envelope proteins. The expression of filaggrin and involucrin has been demonstrated in all layers of the epidermis in normal canine skin (Theerawatanasirikul *et al.*, 2012), and a reduction in the expression of filaggrin and corneodesmosome, and the formation of tight junctions with disorganised keratin filaments, which is pathognomonic of ichthyosis vulgaris (Gruber *et al.*, 2011), have also been found in atopic dermatitis in dogs (Theerawatanasirikul *et al.*, 2012).

The current study observed a reduction in filaggrin staining, probably to compensate for involucrin up-regulation. A similar effect was also observed in inflammatory skin lesions of psoriasis where involucrin expression is markedly up-regulated (Chen *et al.*, 2013). As epidermal protein expression can be modulated through the suppression of EGFR and ERK1/2 phosphorylation pathways (Robertson *et al.*, 2012), it is possible that acantholysis and skin blistering may also be affected by the suppression of EGFR–ERK1/2 signalling, thus resulting in the inhibition of terminal keratinocyte differentiation.

The changed epidermal events were compared to the response of the immunological cells in the dermis. Specific markers of active T-cells and B-cells have been commonly characterised in humans with autoimmune blistering diseases (Sitaru & Zillikens, 2005). Furthermore, it is

important to note that an association between epidermal-specific auto-antibodies and complement proteins has been described previously in human pemphigus (Lessey *et al.*, 2008; Qian *et al.*, 2011); a cross-reaction among IgE, IgM, and IgG4 anti-DSG-1 response has been reported in human PF patients (Qian *et al.*, 2011). In contrast, IgM autoreactivity was not detected when canine PF serum was used against either DSG-1 or DSC-1 (Bizikova *et al.*, 2014), and canine PF showed dominant IgG auto-antibody reactivity (Pérez *et al.*, 2002; Olivry *et al.*, 2009). Moreover, a previous study also detected IgM-producing B-cells in the extracellular matrix of the dermis and in the intercellular epidermis in canine PF (Pérez *et al.*, 2002). Therefore, the IgM auto-antibody response may be less predominant than the IgG response or it may correspond to cross-reactivity in individual dogs with pemphigus. Immunostaining for IgG, IgG1 and IgG4 was predominant in the stratum granulosum of PF dogs which probably correlates with disease activity as IgG auto-antibodies have been shown to cause blister formation in the skin of experimental mice by passive transfer of IgG antibodies (Olivry *et al.*, 2009). Similarly, abundant mast cells in the pustules of pruritic skins were observed in feline PF (Preziosi *et al.*, 2003). Thus together, these observations suggest that mast cell tryptase may additively influence PF pathogenesis.

In conclusion, three subtypes of superficial pemphigus were investigated having minimal macroscopic and microscopic differences and, for the first time, demonstrated considerable overlap in the expression of epidermal proteins associated with the strength of the skin barrier and the accompanying immunological response among these three subtypes. A compari-

son of the expression of a major auto-antigen molecule (DSC-1) also corresponded to skin changes in individual samples. The data suggest that PE and PPP may share some similarities such as altered expression of epidermal proteins and an immunological response that is similar to that observed in PF. Even though the current study could not accurately differentiate PE and PPP from PF (probably due to the small sample size), it provides evidence for the use of certain epidermal proteins (keratin 5, keratin 10, filaggrin and claudin-1) as potential markers for the accurate classification of canine superficial pemphigus subtypes for adjunctive diagnosis.

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REFERENCES

- Anhalt, G. J., 1999. Making sense of antigens and antibodies in pemphigus. *Journal of the American Academy of Dermatology*, **45**, 763–766.
- Bektas, M., P. S. Jolly, P. Berkowitz, M. Amagai & D. S. Rubenstein, 2013. A pathophysiologic role for epidermal growth factor receptor in pemphigus acantholysis. *Journal of Biological Chemistry*, **288**, 9447–9456.
- Berkowitz, P., M. Chua, Z. Liu, L. Diaz & D. S. Rubenstein, 2008. Autoantibodies in the autoimmune disease pemphigus foliaceus induce blistering via p38 mitogen-activated protein kinase-dependent signaling in the skin. *The American Journal of Pathology*, **173**, 1628–1636.
- Bizikova, P., K. E. Linder & T. Olivry, 2011. Immunomapping of desmosomal and non-

- desmosomal adhesion molecules in healthy canine footpad, haired skin and buccal mucosal epithelia: Comparison with canine pemphigus foliaceus serum immunoglobulin G staining patterns. *Veterinary Dermatology*, **22**, 132–142.
- Bizikova, P., G. Dean, T. Hashimoto & T. Olivry, 2012. Cloning and establishment of canine desmocollin-1 as a major auto-antigen in canine pemphigus foliaceus. *Veterinary Immunology and Immunopathology*, **149**, 197–207.
- Bizikova, P., T. Olivry, L. B. Mamo & S. M. Dunston, 2014. Serum auto-antibody profiles of IgA, IgE and IgM in canine pemphigus foliaceus. *Veterinary Dermatology*, **25**, 471–475.
- Bragulla, H. H. & D. G. Homberger, 2009. Structure and functions of keratin proteins in simple, stratified, keratinized and cornified epithelia. *Journal of Anatomy*, **214**, 516–559.
- Candi, E., R. Schmidt & G. Melino, 2005. The cornified envelope: A model of cell death in the skin. *Nature Reviews. Molecular Cell Biology*, **6**, 328–340.
- Chen, J. Q., X. Y. Man, W. Li, J. Zhou, L. Landeck, S. Q. Cai & M. Zheng, 2013. Regulation of involucrin in psoriatic epidermal keratinocytes: the roles of ERK1/2 and GSK-3 β . *Cell Biochemistry and Biophysics*, **66**, 523–528.
- Kuhl, K., F. S. Shofer & M. H. Goldschmidt, 1994. Comparative histopathology of pemphigus foliaceus and superficial folliculitis in the dog. *Veterinary Pathology*, **31**, 19–27.
- Getsios, S., C. L. Simpson, S. I. Kojima, R. Harmon, L. J. Sheu, R. L. Dusek, M. Cornwell, & K. L. Green, 2009. Desmoglein 1-dependent suppression of EGFR signaling promotes epidermal differentiation and morphogenesis. *The Journal of Cell Biology*, **185**, 1243–1258.
- Griffiths, G., R. G. Parton, J. Lucocq, B. van Deurs, D. Brown, J. W. Slot & H. J. Geuze, 1993. The immunofluorescent era of membrane traffic. *Trends in Cell Biology*, **3**, 214–219.
- Gruber, R., P. M. Elias, D. Crumrine, T. K. Lin, J. P. Hachem, R. B. Presland, P. Fleckman, A. R. Janecke, A. Sandilands, W. H. I. McLean, P. O. Fritsch, M. Mildner, E. Tschachler & M. Schmuth, 2011. Filaggrin genotype in ichthyosis vulgaris predicts abnormalities in epidermal structure and function. *The American Journal of Pathology*, **178**, 2252–2263.
- Hamada, T., D. Tsuruta, S. Fukuda, N. Ishii, K. Teye, S. Numata, T. Dainichi, T. Karashima, C. Ohata, M. Furumura & T. Hashimoto, 2013. How do keratinizing disorders and blistering disorders overlap? *Experimental Dermatology*, **22**, 83–87.
- Lessey, E., N. Li, L. Diaz & Z. Liu, 2008. Complement and cutaneous autoimmune blistering diseases. *Immunologic Research*, **41**, 223–232.
- Mavropoulos, A., T. Orfanidou, C. Liaskos, D. S. Smyk, V. Spyrou, L. I. Sakkas, E. I. Rigopoulou & P. Bogdanos, 2013. p38 MAPK signaling in pemphigus: Implications for skin autoimmunity. *Autoimmune Diseases*, **2013**, 1–11.
- Olivry, T. 2006. A review of autoimmune skin diseases in domestic animals: I - Superficial pemphigus. *Veterinary Dermatology*, **17**, 291–305.
- Olivry, T., S. M. Dunston, R. H. Walker, Z. Alhaidari, L. Messinger & Z. Liu, 2009. Investigations on the nature and pathogenicity of circulating antikeratinocyte antibodies in dogs with pemphigus foliaceus. *Veterinary Dermatology*, **20**, 42–50.
- Pérez, J., C. Arce, A. Moreno, E. Mozos, F. Rodríguez & D. Llanes, 2002. Comparison of three monoclonal and three polyclonal antibodies in the immunohistochemical diagnosis of canine autoimmune skin diseases. *Veterinary Dermatology*, **13**, 231–236.
- Preziosi, D. E., M. H. Goldschmidt, J. S. Greek, J. G. Jeffers, K. S. Shanley, K. Drobatz & E. A. Mauldin, 2003. Feline pemphigus foliaceus: A retrospective analysis

- of 57 cases. *Veterinary Dermatology*, **14**, 313–231.
- Qian, Y., P. Prisayanh, E. Andraca, B. F. Qaqish, V. Aoki, G. Hans-Filho, E. A. Rivitti & L. A. Diaz, 2011. IgE, IgM and IgG4 anti-desmoglein 1 auto-antibody profile in endemic pemphigus foliaceus (Fogo Selvagem). *Journal of Investigative Dermatology*, **131**, 985–987.
- Robertson, E. D., L. Weir, M. Romanowska, I. M. Leigh & A. A. Panteleyev, 2012. ARNT controls the expression of epidermal differentiation genes through HDAC- and EGFR-dependent pathways. *Journal of Cell Science*, **125**, 3320–3332.
- Shimizu, A., A. Ishiko, T. Ota, K. Tsunoda, M. Amagai & T. Nishikawa, 2004. IgG binds to desmoglein 3 in desmosomes and causes a desmosomal split without keratin retraction in a pemphigus mouse model. *Journal of Investigative Dermatology*, **122**, 1145–1153.
- Sitaru, C. & D. Zillikens, 2005. Mechanisms of blister induction by auto-antibodies. *Experimental Dermatology*, **14**, 861–875.
- Theerawatanasirikul, S., A. Sailasuta, R. Thanawongnuwech, T. Nakbed, K. Charngkaew & G. Suriyaphol, 2012. Differential expression patterns of proteins involved in epidermal proliferation and differentiation in canine atopic dermatitis. *The Thai Journal of Veterinary Medicine*, **42**, 287–296.
- Waschke, J., 2008. The desmosome and pemphigus. *Histochemistry and Cell Biology*, **130**, 21–54.
- Wurm, S., A. W. Mattise & R. W. Dunstan, 1994. Comparative pathology of pemphigus in dogs and humans. *Clinics in Dermatology*, **12**, 515–524.
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