1. Structure of attachment apparatus of epidermal keratinocytes

Epidermal keratinocytes attach to neighbouring cells to strengthen epidermal tissue integrity. The major apparatus for this adherent system is the desmosome. Desmosomes are discoid junctions with a diameter of 0.2–0.3 µm and are composed of two electron-dense plaques in each of the two adjacent cells, which are separated by an intercellular cleft of 24–30 nm.\(^4\)

Desmosomes contain proteins from at least three protein families. Desmosomal cadherins form the intercellular adhesive interface, while armadillo and plakin family proteins build up these plaques. The intracellular termini of desmogleins (Dsg) and desmocollins (Dsc) interact with plakoglobin that in turn binds to desmplakin. Finally, desmplakin is anchored to the intermediate cytokeratin.\(^6\) These interactions appear to be stabilised by plakophilin.\(^8\)

Desmosomal protein members of the cadherin superfamily (Dsg 1–4) and desmocollins (Dsc 1–3) are single-pass transmembrane glycoproteins, which mediate adhesion in a Ca\(^{2+}\)-dependent manner. The amino-terminal extracellular domain of desmosomal cadherins consists of four cadherin repeats (EC1–4) of approximately 110 amino acids followed by a less related membrane-proximal domain (EC5).

The EC1–4 domains are rigidified by binding up to three Ca\(^{2+}\) ions each.\(^29\) The cytoplasmic domain contains a juxtamembranous anchor (IA) region that possesses a desmplakin-binding element.\(^33\)

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Figure 1: Ultrastructural view of desmosomes

N: nucleus, D: desmosome, cm: cell membrane
2. Definition of pemphigus in human medicine

Pemphigus is a rare autoimmune blistering disease that is characterised by intraepidermal blistering. The disease can be divided into three distinct varieties, pemphigus vulgaris (PV), pemphigus foliaceus (PF) and other variants of pemphigus, depending on clinical features, the level of separation in the epidermis, and immunological characteristics of auto-antigens. Human patients suffering from mucosal-dominant PV typically have antibodies against Dsg3 but not Dsg1, whereas mucocutaneous PV is characterised by the presence of antibodies to both Dsg 3 and Dsg 1. In contrast, PF patients usually possess auto-antibodies against Dsg1, but not Dsg3. Thus, in human medicine, patients with PV should have auto-antibodies against Dsg3, whereas those with PF should have auto-antibodies to Dsg1 that can be demonstrated by indirect immunofluorescence or ELISA.

3. Clinical aspects of pemphigus in dogs

The first case of canine pemphigus foliaceus (PF) was described by Halliwell et al. in 1977, two years after the first report of canine pemphigus vulgaris (PV) by Hurvitz et al. and Stannard et al. Among many publications of sporadic PV and PF cases, Scott et al. compared 11 PV and 7 PF cases from dogs with human pemphigus that revealed numerous analogies between these diseases.

To the date, there have been four articles reviewing a large number of canine PF cases, with two suggesting that there may not be sex predilection, but a breed predilection in the Akita. In fact, the Akita is a breed of Japanese origin and we see few PF cases in Akita; therefore, other factors may exist for breed predilection.

The trunk and inner pinnae are the most frequently affected areas in canine PF, followed by the dorsal muzzle and foot pads. Exanthema in canine PF cases includes crusts, pustules, alopecia and scaling, whereas canine PV usually produces ulcers and erosions in the oral mucous membrane and mucocutaneous junctions.

4. Detection of canine PF autoantibodies in sera by immunofluorescence and immunoblotting

In canine PV and PF, loss of attachment between epidermal keratinocytes, acantholysis, has been suspected to be related to the reaction of antibodies with specific trans-membrane desmosomal proteins, Dsg3 and Dsg1. However, while nearly 100% of dogs with PF that have not been treated with immunosuppressive drugs have IgG deposits in lesional skin, less than 5% of canine cases have circulating antibodies detected by indirect immunofluorescence. Suter et al. demonstrated that a reactive 148 kDa protein in canine lip epithelium was detected by western immunoblot using two canine PF sera. This 148 kDa protein was thought to be Dsg1. Another immunoblotting study showed reactivity to a 160 kDa protein in the extracts of cultured keratinocytes recognised by eight out of 16 sera with canine PF. These 148 and 160 kDa proteins are both thought to be...
canine Dsg1 but represent differentially glycosylation forms. Furthermore, one study also found that canine PV serum detected a 130 kDa protein in the extracted proteins from canine cultured keratinocytes.

5. The relationship between the distribution of desmogleins and lesions in canine pemphigus

The distribution and expression of Dsg1 and Dsg3 has been investigated in humans, and it has been reported that Dsg1 was expressed most intensively in the upper layer of the skin, whereas Dsg3 was expressed in only the supra-basal layer. In contrast, in mucous membranes, Dsg3 expression was most intense throughout the epithelia, whereas the expression of Dsg1 was very low. These results showed that the distribution and expression of desmogleins may relate to the localisation of lesions. Clinically, canine PF affects the skin and is characterised by acantholysis in the upper layer of the epidermis, whereas canine PV patients have mucosal involvement characterised by supra-basal acantholysis. The differences between canine PF and PV in the distribution pattern of lesions and the depth of acantholysis may relate to the distribution and expression of desmogleins, as in humans. In PF, Dsg3 in the lower layer of mucous membrane compensates for the attachment of cells in spite of the paucity Dsg1, whereas Dsg3 in upper epidermis, where the expression of Dsg3 is weak, is not able to support epidermal cohesion. As in canine PV, mucous membrane lesions occur by similar mechanisms.

6. Production of recombinant Dsg1 and Dsc1 and its reactivity to PF sera

Since the first description of canine Dsg1, recombinant proteins expressing the extracellular domains of canine Dsg1 have been produced and serum autoantibodies in human PF mainly recognized conformational epitopes rather than continuous epitopes. However, several sera from patients with canine PF did not recognise recombinant canine Dsg1. In 2006, Olivry et al. reported that Dsg1 is likely to be a minor autoantigen for canine PF by showing that only five of 83 canine patient sera with PF recognised this antigen at a titre of >50.

Other major desmosomal adhesion molecules, canine Dsc1 & 2, were cloned and used to produce recombinant proteins by baculovirus expression. As human subcorneal pustular dermatosis (SPD) type IgA pemphigus has similar histopathological characteristics to canine PF, and a target protein of IgA pemphigus is Dsc1, the reactivity of canine PF sera and Dcs1 was examined. However, all five canine PF sera recognised the recombinant Dsc1. Thus, clinically and histopathologically diagnosed canine PF may be a group of multiple diseases in which only some exhibit autoantibodies against Dsg1.

Electron microscopy of canine PF revealed two distinct findings in acantholytic and non-acantholytic keratinocytes. Half-separated desmosomes were observed in acantholytic cells that were in contact with numerous neutrophils in pustules. Furthermore, aggregation of tonofilaments around the nucleus with detachment of the tonofilament from desmosomes was observed, similar to individual cell keratinisation. These findings suggest that neutrophils play a role in the pathogenesis of canine PF and that impaired intracellular signal transduction by the binding of autoantibodies may affect the loss of cell-cell attachment.

Ultrastructural localisation of PF antigen was investigated by the post- and pre-embedding techniques; however, serum did not react with recombinant canine Dsg1 deposited on intra- and extracellular regions of desmosomes. From this result, we can conclude that some cases of PF produce auto-antibodies that recognise desmosomal proteins other than Dsg1.

7. Production of recombinant canine Dsg3 and its reactivity to canine PV sera

Canine Dsg3, the extracellular portion of the PV antigen was cloned, sequenced and its homology to human and mouse Dsg3 was determined. Canine Dsg3 was determined to be 81% homologous to human Dsg3. Recombinant canine Dsg3 was produced and evaluated for its reactivity with human and canine PV sera. Nine human PV sera and one from canine PV recognised recombinant canine Dsg3. This result suggested that canine PV has autoantibodies against canine Dsg1. Olivry et al. also published a report that demonstrated that the autoantigen of canine PV is Dsg3, as determined by immunoblotting and immunofluorescence.

8. Present perspectives for the diagnosis of pemphigus

Canine PV is an autoimmune skin disease whose target protein is Dsg3. The diagnosis of PV is able to be performed by the combination of findings as follows: 1) clinically, mucous membrane lesions are predominant; 2) histologically, separation of the epidermis at a supra-basal layer with acantholytic cells; 3) IgG deposition at the intercellular space of the epidermis by direct and indirect IF; and 4) a non-invasive test to detect circulating autoantibodies by ELISA using recombinant canine Dsg3. The pathogenesis of the majority of canine PF is not known except that some PF sera can recognise canine Dsg1. Therefore, it is possible that canine PF might be a group of diseases caused by different
mechanisms. However, canine PF shows common clinical signs, histopathology, and IgG deposition in the intercellular space of epidermis. Therefore, we may be able to diagnose canine PF by classical clinical signs, subcorneal pustules with numerous neutrophils/eosinophils and acantholytic cells.

The change in autoantibody titre and clinical severity were compared in a dog with PF over the course of a year. When the titres were assessed by IIF using bovine oesophagus as a substrate, disease severity scores and IIF titres were correlated. Furthermore, in some canine PF cases, the titre of anti-keratinocyte antibodies reflected the disease activity.

9. Exotoxins of *Staphylococcus pseudintermedius* cause dissociation of keratinocytes by the digestion of desmoglein 1 resulting in blister formation.

Amagai et al. demonstrated that *Staphylococcus aureus* exotoxins can digest human Dsg1 exclusively and may cause a similar histopathological finding to bullous impetigo. Likewise, Nishifuji et al. have found that exotoxins produced by *S. hyicus*, which elicits porcine exudative epidermitis. Recently, Iyori et al. discovered new exotoxins of *S. pseudintermedius* recovered from pustules of canine pyoderma. These toxins were all able to cleave canine Dsg1 exclusively to produce blisters in the epidermis by the intradermal injection of these toxins. However, this cleavage activity was not observed for canine Dsg3.

References