Pemphigus foliaceus in dogs: the immune pathogenesis and therapies.

Why are some dogs not responsive to the treatment?

By

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Literature review as part of the
Master’s Dissertation

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Preface

This report is the first part of my master thesis, which I could conduct in the fields of immunology and dermatology. Both have always been of major interest for me so that I felt privileged that I could deepen my knowledge as part of my study.

The realization of my literature study was enabled by professional assistance and familial support.

In the first place I wish to thank my promoter Dr. Elisa Maina for her time, interest, advice and critical proof-reading.

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Abstract

This literature study reviews up-to-date knowledge on the immunopathogenesis, therapies and prognosis of pemphigus foliaceus (PF) in dogs. PF is an auto-immune blistering skin disease described in dogs, humans and other species. The formation of auto-antibodies, primarily IgG4, against keratinocyte desmosomal adhesion proteins (cadherins) results in acantholysis and intraepidermal blister formation. Desmocollin-1 was identified as the major auto-antigen and desmoglein-1 as a minor auto-antigen. PF however appears to be a complex and multifactorial disease where different auto-antigens, auto-antibodies and pathogenic components are involved. The exact pathomechanism is not yet fully elucidated but different theories have been suggested. Those include among others steric hinder by IgG-binding and thereby disabling desmosomal bond-formation; the initiation of multistep mechanisms of intracellular events triggered by IgG-binding and also neutrophils seem to play a pathogenic role. It is proposed that predisposing and inducing factors such as environmental or endogenous factors, e.g. drugs, are necessary for the outbreak of the disease. Drugs that seem to have a potential to provoke acantholysis similar to PF include trimethoprim-sulfonamides, Certifect®, Promeris® and Vectra 3D®. In most dogs remission of the lesions is achieved with an adequate drug regime. The first-line drugs are glucocorticoids. Due to adverse drug reactions or an insufficient response second-line medication such as azathioprine, cyclosporine or chlorambucil are used on a regular basis. Some dogs however do not respond to the treatment. The reason therefore is not yet known.

Key words: Autoimmunity - Blistering - Canine pemphigus foliaceus - Desmosomes - Glucocorticoids
**Samenvatting**

In deze literatuurstudie wordt gepoogd om de huidige kennis omtrent de immunopathogenese en de verschillende therapeutische benaderingen bij canine pemphigus foliaceus samen te vatten. In het bijzonder worden mogelijke hypothesen besproken waarom sommige honden niet op de therapie reageren.

Pemphigus foliaceus is een auto-immune huidaandoening die onder andere bij de mens en de hond is beschreven. Het lichaam vormt auto-antistoffen, voornamelijk IgG4, die zich tegen adhesie proteïnes van de keratinocyten in de epidermis richten. Daardoor ontstaat acantholyse en intra-epidermale blaarvorming. Een aantal jaar geleden kon desmocollin-1 worden aangetoond als het hoofd auto-antigen en desmoglein-1 als een bijkomend auto-antigen. Pemphigus foliaceus bij honden blijkt een multifactoriële aandoening te zijn met verschillende auto-antigenen, auto-antistoffen en pathologische componenten. Een reeks aan pathologische mechanismen werden door onderzoekers voorgesteld. Tot op heden is de exacte immunopathogenese echter nog niet volledig opgehelderd.

Voorgestelde mechanismen verantwoordelijk voor de acantholyse zijn onder andere sterische hindering door auto-antistoffen die ter hoogte van de desmosomen vasthechten en de intercellulaire verbinding tussen naburige cellen inhiberen. Ook werd de initiatie van een cascade aan intracellulaire mechanismen, uitgelokt door IgG-binding, voorgedragen waarbij een reeks aan componenten betrokken zou zijn. In canine pemphigus foliaceus werd bovendien de betrokkenheid van neutrofielen aangetoond. In de klassieke humane vorm van pemphigus foliaceus is dit niet het geval.

Onderzoekers vonden daarboven indicaties dat de uitbraak van PF door endogene of exogene factoren uitgelokt kan worden. Het best beschreven voorbeeld is een uitbraak na toediening van een beperkt aantal geneesmiddelen. Bij de hond werden uitbraken beschreven na de toediening van trimethoprim-sulfonamides, Certifect®, Promeris® en Vectra 3D®. Men vermoedt dat genetische factoren eveneens een rol spelen in de pathogenese, omdat sommige rassen gepredisponeerd zijn.

De therapie berust voornamelijk op immunosuppressie. Hierbij zijn glucocorticoid zoals prednisolone de meest gebruikte farmaceutica. Door ernstige bijwerkingen of een onvoldoende respons kunnen tweedelijns geneesmiddelen (onder andere azathioprine, cyclosporine of chlorambucil) als vervanger of in combinatie met glucocorticoiden worden gebruikt. In een beperkt aantal gevallen treedt volledige remissie op, in de meeste gevallen is levenslange behandeling echter noodzakelijk. Een ander klein deel van de patiënten reageert niet op de behandeling. De exacte oorzaak hiervoor is niet bekend. Een aantal hypothesen zijn door de auteur voorgesteld. Hierbij behoren het ontwikkelen van resistentie tegen glucocorticoid, een foutieve diagnose, het gebruik van een ongeschikt geneesmiddel protocol of een geïnduceerde vorm van pemphigus in plaats van de spontaan ontstane aandoening.

**Sleutelwoorden:** Autoimmuniteit – Blaarvorming - Canine pemphigus foliaceus – Desmosoom – Glucocorticoiden
Pemphigus foliaceus in dogs: the immune pathogenesis and therapies.

Why are some dogs not responsive to the treatment?

1. INTRODUCTION

This literature study reviews up-to-date knowledge regarding the immunopathogenesis and available therapies for the canine form of pemphigus foliaceus (PF). PF is a complex disease with many components involved. The interactions of these components as well as the exact pathologic mechanisms are not yet identified. It is also not known why some dogs do not respond to a (glucocorticoid) treatment. This literature study strives to give a better insight in already conducted research and proposed hypotheses.

Pemphigus foliaceus (PF) is an auto-immune skin disease described in humans,\(^1\) cats,\(^2,4\) dogs,\(^2,3\) horses,\(^3,5\) goats\(^6\) and a Barbary sheep (single case)\(^7\). The word pemphigus originates from the Greek word for blister \(^8\), which is one of the major characteristics describing this medical condition.

PF is part of the pemphigus complex, which comprises pemphigus vulgaris, pemphigus vegetans, pemphigus erythematosus, pemphigus foliaceus, panepidermal pustular pemphigus, paraneoplastic pemphigus, and drug-related pemphigus.\(^9\) The pemphigus diseases have in common that the body produces auto-antibodies against the adhesion molecules of keratinocytes, resulting in the separation of the keratinocytes, called acantholysis.\(^10,11\) This becomes clinically visible as pustules, which are very fragile, transient, and leave crusts and erosions.\(^11\)

1.1. THE DIFFERENT FORMS OF THE PEMPHIGUS COMPLEX

Diseases included in the group of pemphigus can be differentiated based on the specific auto antigens. The various forms of pemphigus recognize different auto antigens, located in different layers of the skin.\(^12\) Thus, depending on where the acantholysis takes place, the clinical signs such as distribution, severity and location of the lesion changes.\(^13\)

![Figure 1](image.png): The figure shows the location of the different pemphigus diseases within the epidermis.
1.1.1. Pemphigus vulgaris

Pemphigus vulgaris (PV) is the most severe and rarest form of the pemphigus complex.\textsuperscript{10, 12} The auto-antibodies bind to antigens in the deepest layer of the epidermis, close to the dermal-epidermal junction. Acantholysis therefore occurs right above the basal cell layer, forming suprabasilar vesicles.\textsuperscript{11, 12}

PV is divided in both, humans and dogs, in a mucosa-dominant type and a mucocutaneous type. In the mucosal-dominant type oral lesions dominate with little or no skin involvement, whereas in the mucocutaneous form oral as well as skin lesions can be seen.\textsuperscript{14}

The clinical signs are primarily bullae, erosions and ulcers of the skin and can be found, depending on the type, on the mucosae, at the mucocutaneous junctions and on the trunk, especially in areas of skin-to-skin-contact such as in the groin and axillae. Oral lesions often cause increased salivation, halitosis and difficulties to eat.\textsuperscript{9, 10, 11, 12} PV shows varying degrees of pain and pruritus.\textsuperscript{9} Systemic symptoms, such as fever, depression or anorexia are frequently seen.\textsuperscript{10}

Desmoglein-1 was identified as the major-autoantigen in the cutaneous form, thus causing skin-lesions. Desmoglein-3 on the other side is the target-antigen in the mucosal form, resulting in mucosal lesions.\textsuperscript{14} Logically, both auto-antigens (anti-Desmoglein-1 IgG and anti-Desmoglein-3 IgG) are present in the mucocutaneous form.\textsuperscript{15}

The diagnosis is made by histopathological examination and by immunofluorescence. Histology is characterized by suprabasilar clefts and vesicles filled with acantholytic cells.\textsuperscript{12} Acantholytic cells are round, the cytoplasm is dense and the nucleus hyperchromatic. Depending on the stadium (acute or chronic), neutrophils and eosinophils can also be found in the vesicles.\textsuperscript{16} Another diagnostic tool is immunofluorescence. In affected patients antibody-deposition in the intercellular spaces can be found on the biopsy after staining with anti-IgG fluorescein.\textsuperscript{12}

1.1.2. Pemphigus vegetans

Pemphigus vegetans is extremely rare in domestic animals.\textsuperscript{9, 17} It is seen as a localized and benign variant of pemphigus vulgaris.\textsuperscript{18} On histology acantholysis is visible in the middle of the epidermis\textsuperscript{11}, causing pustules and erosions.\textsuperscript{17} The lesions are generalized rather than mucocutaneous.\textsuperscript{9}

In humans two types of pemphigus vegetans are described: The Neumann type and the Hallopeau type. Both are sought to be a localized form of Pemphigus vulgaris. The Neumann type is more common. Lesions include large bullae and erosions, which heal by forming granulation tissue. The Hallopeau type is less aggressive and on clinical examination pustules instead of bullae can be seen. When they heal, verrucous hyperkeratotic vegetation develops.\textsuperscript{18}

1.1.3. Pemphigus erythematous

Pemphigus erythematous (PE) and pemphigus foliaceus are more superficial.\textsuperscript{11} They affect especially the stratum corneum, creating intracorneal or subcorneal pustules that rapidly burst, leaving superficial
erosions bordered by epidermal collarettes, erythema, alopecia, scales and honey-colored to brown crusts. Nasal depigmentation is frequently seen. The main difference between PE and PF is that pemphigus erythematosus is limited to the face and ears. It is primarily found on the bridge of the nose, around the eyes and on the ear pinnae. The disease is most common in Collies, German Shepherds and Shetland sheep dogs. Pemphigus erythematous shows clinical, histological and immunological overlap with characteristics of pemphigus foliaceus as well as characteristics of discoid lupus erythematosus. It is thus still questionable if PE is an own entity, a benign variant of PF restricted to the face or a crossover of PF and discoid lupus erythematosus.

Pemphigus foliaceus and drug-related pemphigus will be discussed more in detail further on.

1.1.4. Panepidermal pustular pemphigus

Panepidermal pustular pemphigus (PPP) was introduced in 1994 by a research team for a group of cases that were previously diagnosed as pemphigus vegetans or pemphigus erythematosus. An important criteria for diagnosing PPP is that the pustules can be found throughout all levels of the epidermis and follicular epithelium. This stands in contrast to PF where pustules stay limited to the granular and upper spinous layers. The pustules rupture easily, leaving a thick crust on the skin. On histology the pustules were found to contain neutrophils, eosinophils and acantholytic cells. Desmoglein-1 could be identified as the major auto-antigen, just as in PF. Also the distribution of the lesions is similar to the distribution of PF. It is apparent that features of PPP overlap with features of PF. It is thus debatable if PPP is an own entity or a variant of PF.

1.1.5. Paraneoplastic pemphigus

At last there is paraneoplastic pemphigus (PNP). This form is very rare and shows blistering skin lesions in association with underlying neoplasms. Envoplakin, periplakin, desmoplakin-I and –II and desmoglein-3 were identified as target-antigens in canines. In humans pemphigus has been associated with a range of neoplasia, such as a lymphoma, chronic lymphocytic leukemia, spindle cell sarcomas, squamous cell carcinoma of the lung or thymomas. In dogs only a limited number of cases have been reported to date. A few were linked to a thymic lymphoma, a sertoli cell tumor and another to a splenic sarcoma. On clinical examination severe stomatitis and polymorphous ulcerative lesions were seen in the oral cavity, nose, vulva, mucocutaneous junctions and haired skin. On histopathology suprabasal epithelial acantholysis typical of PV was found as well as apoptotic keratinocytes with satellitosis that showed similarities with erythema multiforme.
2. PEMPHIGUS FOLIACEUS

2.1. EPIDEMIOLOGY

Pemphigus foliaceus was first described in dogs in 1977 by Halliwell and Goldschmidt, since then the disease was mentioned in different species and further researches were carried out. However, limited information about the epidemiology in dogs can be found. Of the pemphigus complex, PF is the one most frequently seen. A study from 1987 reported an estimated prevalence of 0.3%. More specific, three out of 1000 presented canine patients with skin diseases in an animal hospital in New York were diagnosed with pemphigus foliaceus. Another study showed that canine pemphigus foliaceus (cPF) is probably the most common auto-immune skin disease in dogs, accounting for almost one-third of the cases. Contrary, in humans pemphigus vulgaris is the most frequent variant of the pemphigus group. Authors of a retrospective study carried out in Brazil diagnosed 102 dogs with PF over a 25-year period. That is 4.1 cases per year. All breeds can be affected, but some breeds have a higher risk. Different studies describe a higher prevalence in the Bearded Collie, Akita, Newfoundland, Schipperke, Dachshund, Doberman pinscher, English Springer Spaniel, English bulldog, Finish Spitz, Labrador retriever, English Cocker Spaniels, Chow Chows, Shar-Peis and Collies. There appears to be no age or sex predisposition. However, most dogs are middle-aged between four and six years with 4.2 years as the mean age of onset.

2.2. CLINICAL SIGNS

As mentioned before are PF and PE the more superficial versions of the pemphigus complex, affecting primarily the stratum corneum. The primary lesions are superficial and transient pustules. These are yet hard to find, since being very fragile and hidden under the coat. The pustules rupture easily, leaving erythema, yellowish crusts, erosions, alopecia and peripheral collarettes. The lesions most commonly start on the dorsal part of the muzzle, are bilaterally symmetric and spread gradually. PF shows a typical distribution (Fig. 2,3) and most commonly affects the pinnae, perioral and periorcular region, the nasal planum, bridge of the nose and the footpads. The footpad lesions are present in 1/3 of the dogs and are characterized by hyperkeratosis, cracks, possible erythematous swelling and whitish discoloration. Those lesions can be the only symptom. The nailbed can be involved the nails however are usually unaffected. Lesions in the oral cavity and the mucocutaneous junction are rare. In some patients PF can become generalized, with development of the following symptoms in severe cases: anorexia, fever, depression, lymphadenomegaly and limb edema. Pruritus can only be found in less than half of the dogs with 17 to 36% showing moderate to severe itching. The disease can be waxing and waning.

Figure 2, 3: Typical facial distribution of cPF lesions with scaling, alopecia and pustules.
2.3. PATHOGENESIS

2.3.1. Involved components

2.3.1.1. Desmosomes: Function, ultrastructure and composition

Desmosomes or macula adherens are adhesion molecules found in the lateral cell membrane. They are complex structures that are particularly important in tissues that are subject to mechanical stress, such as the epidermis or the cardiac muscle. Their main function is cell-cell-adhesion and to assure tissue integrity. Besides giving strength to epithelia by linkage of the desmosomes to the keratin intermediate filament of the cytoskeleton, more and more evidence is emerging that desmosomes also play an important role in intracellular signaling pathways.\textsuperscript{14,35,36}

Desmosomes are classified as glycoproteins and contain an intra- and an extracellular part that form a complex. They are spot-like distributed across the lateral cell membrane.\textsuperscript{14}

Two adjacent cells each possess an outer and an inner dense plaque. Those plaques are made of proteins of the plakin (desmoplakin) and armadillo-family (plakoglobin and plakophilin).\textsuperscript{14} The inner dense plaque (IDP) consists of desmoplakin and is linked to intermediate filaments of the cytoskeleton, to stabilize the cell and keep the desmosomes on their place. The outer dense plaque (ODP) serves as an anchor for the cytoplasmic domain of the cadherins. Cadherins are a third involved protein-family, comprising a cytoplasmic and an extracellular component. Their name derives from the circumscription “Ca\textsuperscript{2+} -dependent adhesion” and comprises the glycoproteins desmocollin (Dsc) and desmoglein (Dsg). Those two are intracellular connected to plakophilin and plakoglobin and form extracellular heterophilic and homophilic bonds with the adjacent cells.\textsuperscript{37}

Despite their important role in cell-cell adhesion and cell integrity, the desmosomes are not static but dynamic structures that can change their molecular composition and adhesive properties.\textsuperscript{38} Recently two different adhesion states have been described. One is Ca\textsuperscript{2+} independent, stable and hyperadhesive, the other is dynamic, weaker and Ca\textsuperscript{2+}-dependent. Both are reversible through cell signaling mechanisms which involve protein kinase C and epidermal growth factor receptors.\textsuperscript{38}

It is evident that calcium-ions play an essential role in the induction of desmosome formation. Several studies revealed that cultured keratinocytes do not form desmosomes in low Ca\textsuperscript{2+} concentrations (below 0.1mM).\textsuperscript{39-41} When the Ca\textsuperscript{2+} concentration is however increased desmosomes formed within two hours.\textsuperscript{42,43} An increase in extracellular Ca\textsuperscript{2+} is registered by a Ca\textsuperscript{2+} sensing receptor (CaR).\textsuperscript{39-42} CaR activates phospholipase C and triggers the production of inositol 1,4,5-triphosphate (IP3).\textsuperscript{44} The increase in IP3 results in an elevated intracellular Ca\textsuperscript{2+} concentration in keratinocytes.\textsuperscript{45} Similar findings were made in cPF research. Seven out of seven human PF sera bound to canine footpad epithelium under high calcium conditions, but they failed to bind when calcium was chelated (with EDTA).\textsuperscript{46} Desmosomes are associated with even more proteins, among others accessory proteins.
such as Perp, cornodesmosin or the armadillo protein p0071. The discussion of these goes beyond the scope of this literature study. Considering the complexity and numerous components of desmosomes it is intelligible that several of them are plausible candidate autoantigens.

2.3.1.2. Desmosomal cadherins

2.3.1.2.1. Distribution of desmosomal and non-desmosomal adhesion molecules

In humans there are three isoforms of desmocollin (Dsc 1-3) and four isoforms of desmoglein (Dsg 1-4). Each cell expresses several isoforms and each desmosome contains more than one type of desmocollin and desmoglein. The keratinocytes of the epidermis have different degrees of differentiation which are organized in layers and express a variety of molecular compositions. Desmosomal cadherins for example are expressed in a pattern that is typical for a specific tissue. Except for Dsc-2 and Dsg-2, the expression of the cadherins is restricted to stratified epithelial tissues. That means that only Dsc-2 and Dsg-2 are found, for example in the human cardiac muscle, but all seven (Dsc1-3, Dsg1-4) can be found in the epidermis.

Bizikova et al. carried out a research in 2011 with the goal to identify possible antigens of PF. Therefore the research-team immunomapped the major desmosomal (desmoglein-1, desmoglein-3, desmocollin-1, desmocollin-3, desmoplakin-1/2, plakoglobin and plakophilin-1) and non-desmosomal adhesion proteins (E-cadherin, claudin-1, zona occludens-1 and occludin). The desmosomal immunostaining patterns were then compared to the patterns of IF staining with canine PF sera. Figure 5 compares the staining patterns of desmoglein 1 and 3 in canine footpad and buccal mucosa.

![Figure 5](image_url)

**Figure 5:** Desmoglein-1 and -3 fluorescence patterns in canine footpad and buccal mucosa. Intercellular staining indicates the binding of autoantibodies to extracellular components. The antibody deposition shows a typical honey-comb pattern.

Of the tested cPF sera 88% showed immunofluorescence-staining laterally of the keratinocytes in the stratum spinosum and stratum granulosum, 11% showed additional intercellular staining in the stratum basale, one serum (2%) only bound to the stratum granulosum and 18% also showed intercellular fluorescence of the buccal mucosae. 80% of the sera thus exhibited a restricted staining pattern to the
suprabasal footpad epithelium and very low staining of the buccal mucosa. Several different staining patterns of the various cPF sera were detected, which implies an immunological heterogeneity of cPF IgG auto-antibodies.\textsuperscript{35,48} The figure below (figure 7) shows the distribution of certain desmosomal components in the canine epidermis. The indirect immunofluorescence (IIF) staining patterns and thus the distribution of the most relevant molecules for PF are described more in detail below.

![Desmosomal Cadherins](image)

**Figure 6**: The diagrams represent the staining patterns and immunofluorescence intensity of desmosomal cadherins in the different layers of canine footpad, haired skin and buccal mucosa. The column width is in accordance with the staining intensity.

DSG1: desmoglein-1; DSG3: desmoglein-3; DSC1: desmocollin-1; DSC3: desmocollin-3

**Desmoglein-1 and -3**

In the canine footpad the fluorescence intensity was highest in the stratum spinosum, with a moderate decrease towards the stratum granulosum and the stratum basale. Dsg1 is more evenly distributed in the haired skin with slight decreases towards the basal layer and high fluorescence intensity in the entire stratum spinosum. Dsg1 is furthermore absent in the stratum distendum of the buccal mucosa. Dsg1 and 3 show a reciprocal staining pattern in all examined epithelia. Dsg1 is most present in the superficial layers whereas Dsg3 is highest in the basal layers of the footpad, haired skin and buccal mucosa.\textsuperscript{35}

**Desmocollin**

Desmocollin 1 was exclusively found on the margins of suprabasal keratinocytes in the footpads as well as in the interfollicular epidermis. Interestingly, Dsc1 was not detectable in canine buccal mucosa, with neither of the anti-Dsc1 antibodies. Dsc3 can well be found in all three tissues, is evenly distributed in footpad and haired skin and more present in the deeper layers of the buccal mucosa.\textsuperscript{35} The staining patterns of the human and canine adhesion-molecules showed a more or less identical distribution.\textsuperscript{35} In general it can be said that Dsc 2-3 and Dsg 2-3 are primarily expressed in the lower layers and that Dsc1, Dsg1 and Dsg4 are more present in the upper layers of the human epidermis.\textsuperscript{14}

2. 3. 1. 2. 2. Desmoglein as a minor target auto-antigen

The desmosomal cadherin desmoglein-1 is identified as the major auto-antigen in the human form of pemphigus foliaceus.\textsuperscript{49} Different tests and studies were carried out to determine the homologous
canine target molecule. A study carried out by Iwasaki and his team in 1997 used immunofluorescence and western blotting to detect candidate antigens extracted from canine keratinocytes. By western blotting sera of canine PF patients recognized a 160kDa protein (50% of the sera), a 85kDa protein (25%) and a 120kDa protein (31, 25%). There were indications that the 160kda protein corresponds with the human desmoglein-1. The same study also showed that binding between antibody and antigen could only be seen at the sites of the cell-cell adhesions and not on the entire surface of the cells. Further researches were conducted about the hypothesis of desmoglein-1 being the major auto-antigen using a novel screening strategy to detect conformational epitopes. In this study from 2006 only 6% of the sera from dogs with pemphigus foliaceus contained antibodies that recognized canine desmoglein-1. Another study by Yabuzoe et al. from 2008 showed similar results. All sera (n=3) bound to the extracellular part of the desmosomes where adjacent cells made contact. However, only a limited number of cPF sera bound to Dsg1 specifically. These findings suggest that the target auto-antigen is a desmosomal protein. They furthermore denunciate desmoglein-1 as a minor autoantigen in cPF. What is more, it illustrates that pemphigus foliaceus is a heterogenous disease, with more than one antigen being involved.

In a study that aimed at detecting the main autoantigen in cPF researchers found that calcium depletion and glycosylation have an influence on the recognition of dsg1 in epithelial cells. The role of calcium in expressing epitopes is described more in detail in section 2.3.1.1. To test their hypothesis, the researchers incubated canine footpad epithelium as a substrate in 1mM calcium buffer or 5mM ethylenediamine tetracetic acid (EDTA) for thirty minutes. Ensuing they tested the binding of human PF sera to the substrate with calcium being respectively present or absent. All seven human PF sera bound to canine footpad epithelium under high calcium conditions, but the IgG in the sera did not bind when calcium was chelated. Based on those results they concluded that membrane-bound canine dsg1 was expressed in a calcium-dependent conformation. Another interesting finding is, that the binding of the few canine and human PF sera that contained Dsg1-antibodies, depended on glycosylation. This was investigated by inhibiting protein glycosylation with tunicamycin. 

2.3.1.2.3 Desmocollin as a major target auto-antigen

Desmocollin is an important molecule of the cell-cell adhesion of adjacent keratinocytes. The importance of Dsc1 in cell-to-cell adhesion was demonstrated with the aid of genetically modified mice. Those mice were lacking Dsc1 and showed epidermal fragility and spontaneous blister formation in superficial epidermal layers as aresult. After localizing the major desmosomal adhesion molecules those profiles were compared to the staining profiles of cPF serum IgG. 80% of the tested cPF sera showed a similar staining profile to that of desmocollin-1 (Dsc1). This suggests Dsc1 being a relevant candidate antigen. Desmocollin-1 is additionally not detectable in canine buccal mucosae, which matches the clinical signs of PF, since the pustules typically form in the superficial layers and the oral cavity rarely being involved.
Further researches demonstrated that the sera of most PF affected dogs contain specific IgG antibodies against Dsc1 that are not present in normal dogs. Those findings define Dsc1 (variant “b”) as a major auto-antigen in canine pemphigus foliaceus. The Dsc-variants “a” and “b” differ in the splicing pattern of the mRNA transcript that codes for the Dsc1-protein. In variant “a” an exon containing a stop-codon is removed. The resulting coding sequence is thus translated into the longer Dsc1a protein.14

Similarly to human PF, serum of dogs may contain antibodies against more than one auto-antigen. Sera were found in dogs that contained antibodies against Dsg1 and Dsc1 at the same time.47 Interestingly, although Dsg1 is the major auto-antigen in human PF, desmocollins (Dsc1-3) are identified as auto-antigens in some human PF patients with aberrant forms of superficial pemphigus, especially in those with neutrophil-rich skin lesions.35,54

2.3.1.2.4. Other auto-antigens

So far desmoglein-1 and desmocollin-1 have been specified as auto-antigens in cPF. In humans they also identified the non-desmosomal adhesion protein E-cadherin as a target. The relevance of anti E-cadherin antibodies is not yet clear.36 In a study that involved double-sided immunogold labeling of canine PF sera the researchers detected that more antibodies were bound to the intracellular part of the desmosomes than to the extracellular part. The location of the detected antibodies was similar to that of desmoplakin, an intracellular component of desmosomes. By immunoblot analysis the IgG recognized a 250kDa epidermal protein, which is also conform with desmoplakin.51 In canine pemphigus vulgaris envoplakin and periplakin (besides Dsg1 and -3) are well known as target antigens.55 Another study found two separate auto-antibody staining patterns in PF-dogs as well as in normal dogs. They detected with indirect immunofluorescence one group of antibodies that bound to the superficial layer of the epidermis (predominantly in the stratum granulosum) to peripheral cellular antigens and another group that bound intracellular in the deep layers of the epidermis, especially the stratum basale. Both patterns often co-existed. Most normal and PF dogs contained the superficial antibodies, while only few sera were detected that contained the antibodies that bound to the stratum basale. It is suspected that the cytoplasmic target antigen, just like in humans25, is a plaque protein of the plakin family. The author thinks that the antibodies that bind to the deeper skin layers do not play a pathogenic role, since PF is a superficial skin disease.48 The (possible) pathogenic role of the desmoplakin antibodies is not yet clear. The authors suggest that anti-desmoplakin antibodies can intrude the cell after cell-membrane damage by other factors and that it might act as an accelerator of the disease. They describe this phenomenon as epitope spreading.51

2.3.2. The role of neutrophils

In contrast to the human form of PF, the formed pustules of the canine PF patients contain intense neutrophilic infiltrations.15,56,57 Electron microscopy showed neutrophils next to acantholytic
keratinocytes, as well as in the epidermis next to the pustules. They could be found in the intraepidermal pustules as well as in the superficial ones. Neutrophil chemotaxis might be the result of chemokine secretion by keratinocytes after IgG-binding which can be seen in some human PF-patients that also show neutrophil-rich lesions. Eosinophils were present without pathologic findings. Interestingly, all half-desmosomes were seen at the contact points between the granulocytes and keratinocytes. None were observed on cell surfaces where no neutrophils made contact. The neutrophils featured invaginations that enclosed half-desmosomes of the acantholytic keratinocytes. Remarkably, the half-desmosomes in the invaginations had intact attachment plaques and attached tonofilaments that did not show any retraction. (Retraction of tonofilaments and internalization of desmosomes was described in humans with PV and PF).

In the early stages of acantholysis, neutrophils developed pseudopodia that were brought in between the half-desmosomes of two adjacent keratinocytes. In a later phase, the same study showed that the granules of the neutrophils were secreted to the surface of the acantholytic keratinocytes, resulting in disassembly of the half-desmosomes. Those findings suggest a supplementary pathogenic role of the neutrophils regarding the degeneration of cell-adhesion and separation of adjacent keratinocytes.

2.3.3. Antibodies

2.3.3.1. Antibody profile

In different studies IgG was found as the main immunoglobulin deposited in a net-like manner around the keratinocytes of canine PF patients. Also, in the immunomapping study from Bizikova et al, all cPF sera (n=66) contained anti-keratinocyte IgG in the serum and IgG-depositions primarily suprabasal and membrane-associated. Yabuzoe et al found IgG deposition on the keratinocytes perilesionally and in the serum, but not that of IgA, IgM or C3. Bizikova and her team conducted a study in 2014 to investigate the serum antibody profiles (IgG, IgA, and IgM) of anti-keratinocyte, anti-Dsc1 and anti-Dsg1 immunoglobulines in cPF patients. They detected the auto-antibodies by indirect immunofluorescence (IIF). More sensitive methods might however result in higher positive numbers. Conversely, they found that 18% (n=6) of the sera contained auto-reactive IgA, around 3% (n=1) contained (anti-desmocollin1) IgE and none of the sera contained IgM. An IgA pemphigus without IgG-antibodies, similar to certain human PF forms, was not discovered. Those findings suggest that canine PF is a predominantly IgG-mediated disease and that IgE and IgA can only be detected in rare cases.

In humans two forms of pemphigus are described that feature anti-Dsc1 IgA in a higher extent than in the classical form. These are called atypic pemphigus and IgA pemphigus. In the latter IgA is the dominant antibody isotype. Remarkably both of them, contrarily to the classic human PF form and similarly to canine PF, also feature neutrophilic pustules.
2.3.3.2. IgG subclasses

Four IgG subclasses (IgG1-4) are described in the dog. IgG are composed of four peptide chains; two identical light chains and two identical heavy chains. The subclasses are defined primarily by the amino acid composition and the structure of the hinge region. Furthermore, they differ in abundance in the canine serum. The predominant subclasses of antikeratinocyte antibodies present in canine PF sera are IgG1 and IgG4. In normal healthy dogs those two autoantibodies are present in relatively lower titers, with IgG1 being the most prominent one of the IgG subclasses. In normal and cPF dogs no significant differences in the titers and seroprevalence of IgG1, IgG2 and IgG3 could be detected. IgG4 however was almost exclusively found in dogs with PF (80%) and in only 7% of normal dogs. The same author described furthermore decreasing or stable IgG4 titers when the skin lesions resolved, whereas such development could not be seen in IgG1. IgG4 might thus be used as a marker for disease activity since it is higher in severe cases and decreases when the skin lesions diminish. Based on those findings the author suggests that IgG4 antibodies is most likely the pathogenic isotype in cPF.

2.3.3.3. Titer and severity

Different studies suggest that the serum titers are positively correlated with the severity of the clinical signs. When skin lesions improved antikeratinocyte IgG4 titers stayed constant or decreased. The maximum reduction was seen when the disease improved or reached remission. The titer of IgG1 was not significantly affected by the degree of severity. Bizikova and colleagues conducted a study in 2012 to investigate the relationship between anti Dsc1 IgG titers and disease severity. They selected ten dogs with PF, applied a scoring system to score the severity of the disease and took serum samples before and during treatment. In all dogs a noticeable clinical improvement could be seen. The antibody titre decreased in 70% of the dogs (n=7) together with the decrease in severity.

2.3.3.4. Specificity of the antibodies

As mentioned earlier, more than one auto-antigen and antibody are involved in the pathogenesis of PF. So far anti-Dsg1 and anti-Dsc1 antibodies have been identified. The detection of auto-antibodies is, however, apparently strongly depending on the substrate. In normal bovine esophagus antibodies were detected in 65% of the cPF-patients, indirect IF on cultured canine keratinocytes was positive for all tested cPF sera, when however other substrates were used the frequency of the antibody detection decreased significantly.
2.3.4. Pathophysiology

2.3.4.1 Introduction

The underlying mechanisms and factors that induce the production of pathogenic antibodies are not fully understood yet. Nevertheless, several theories and involved components are proposed. Besides a genetic predisposition, different exogenous factors are proposed to play an important role in the pathogenesis. Drugs are the best-recognized triggers. Other hypotheses include a suppressor T-cell dysfunction or bypass; a modification of self-antigen; a cross-reaction with exogenous antigens; abnormalities of the major histocompatibility complex II and access of T-cells to previously hidden self-antigens.11

2.3.4.2 Role of the auto-antibodies

Intradermal injections of PF IgG in neonatal mice resulted in the formation of pustules that were histologically similar to those of human and canine PF patients. The location of the lesions in the skin were similar to those of PF as well. Passive transfer of the serum of normal dogs to the mice did not lead to blister formation. This proves the pathogenic role of the antikeratinocyte antibodies.48 An important difference between the induced PF-lesions in the mice and natural ones is however that the induced pustules lack eosinophil and neutrophil granulocytes that predominate in natural cPF.48,56

2.3.4.3. Proposed mechanisms

The mechanism between the auto-antibodies and the induction of acantholysis is not satisfyingly explained yet. A logic and simple explanation would be, that the auto-antibodies interfere with the extracellular adhesion by steric hinder. By this means the bond between the extracellular cadherins would be disabled, resulting in the separation of the keratinocytes.14 A research conducted by Waschke et al in 2005, however, has revealed that pemphigus foliaceus IgG from patients was not able to break a homophilic Dsg1-Dsg1 bond. 70

Conversely, evidence is increasing that PF is a more complex disease with several pathogenic components involved. Several papers suggest that serum IgG can trigger a multistep mechanism of intracellular events.31 Research in humans proposes numerous mechanisms of acantholysis after the antibody bound to desmosomal antigens. These include the internalization of the antibody-antigen complex and fusion with intracellular lysosomes; an increased amount of urokinase-type plasminogen activator in the affected epithelium, resulting in high concentrations of extracellular plasmin and thus destruction of the adhesion molecules.71-73 Another possible (supplementary) pathologic mechanism includes the activation and fixation of complement.74 It is proven in humans that the binding of IgG-antibodies induces the following changes in the keratinocytes. IgG binding activates the Fas/Fas ligand cell death pathway and the mitogen activated protein (MAP)-kinase pathway. It also induces phospholipase-C activation, inositol 1, 4, 5-triphosphate
generation, increases intracellular calcium concentration and a redistribution of protein kinase C (PKC) inside the cell. Additionally, it interferes with RhoA signaling.\textsuperscript{75-78} Rhoa stands for RAS homolog family member A and plays a role in the regulation of cytoskeletal dynamics, transcription, cell cycle progression and cell transformation.\textsuperscript{79} Another study regarding human PF suggests as well that IgG binding to desmoglein or other surface-molecules activates a variety of mechanisms resulting in the disassembly of the desmosomes, tonofilament retraction and internalization of desmosomal structures in cultured keratinocytes.\textsuperscript{14,58} A part of this mechanism could be due to the phosphorylation of desmoglein and thus its dissociation from plakoglobin.\textsuperscript{36} Additionally, desmoglein could disappear from the surface by endocytosis.\textsuperscript{58,65} However, those findings are not totally in accordance with the findings in cPF. In cPF a study examined more than twenty half-desmosomes of keratinocytes from canine PF patients. All of them had intact intracytoplasmic dense plaques and no remarkable tonofilament retraction. That might suggest that in cPF IgG does not trigger an alteration of the intercellular pathways and interference with the cell-cell adhesion.\textsuperscript{56}

As described previously, it is also likely that neutrophils play a pathogenic role by forming pseudopodia and release enzymes to break adhesion bonds.\textsuperscript{54, 56}

A different article describes that the onset and course of PV is based on predisposing and inducing factors. The inducing factors can be environmental or endogenous factors. That also implies that a predisposed genetic background alone is not sufficient for the diseases to break out but also requires a trigger.\textsuperscript{31} Endogenous factors might be hormone disorders, emotional stress, immune suppression etc. Environmental factors starting the disease could be drug intake (see “Drug related pemphigus” below), viral infections, diet, contact allergens etc. The same principle might apply to cPF. A genetic background that predisposes certain breeds to cPF has already been mentioned.\textsuperscript{19} The disease was furthermore found in two Shetland sheepdog littermates.\textsuperscript{80}

In humans there is an endemic form of PF described in Brazil, called “fogo selvagem”.\textsuperscript{81} Different authors suggest a genetic predisposition together with an environmental trigger to develop clinical signs.\textsuperscript{15, 31} Additionally, in specific pathogen free (SPF) dogs lower frequencies and titres of antikeratinocyte antibodies were detected, compared to normal outbred client-owned dogs. It is not known if those findings are relevant, but it might indicate that environmental factors might play a role in the pathogenesis.\textsuperscript{48} Pilot studies were conducted in dogs with PF. Flea infestation was one of the exogenous factors associated with the disease\textsuperscript{82}, whereas UV-rays might induce new skin lesions.\textsuperscript{83}

All the mechanisms mentioned above do not exclude each other. It might be possible that several mechanisms occur simultaneously or even trigger or enhance each other. Waschke et al describe in their article that the binding of IgG to the extracellular part of the desmosomes causes steric hindrance, impaired signal transduction, keratin retraction, cytoskeleton collapse and of course acantholysis. The study was based on human research and not on dogs.\textsuperscript{37}

Kitajima et al proposes in his review article from 2013 the term “Desmosome–remodeling impairment disease”, which shows similarities with some of the mechanisms proposed above. This theory includes
a mechanism of Dsg3 non-assembly and depletion from desmosomes when in the “weak adhesion state”. In human PV the binding of autoantibodies to Dsg3 results in the activation of intracellular events, more specific in the activation of protein kinase C (PKC). PKC induces subsequently a switch from the Ca^{2+}-independent hyperadhesive state to the weak-adhesion state. This seems to be the more susceptible state, creating keratinocytes that are more sensitive to blistering processes.\textsuperscript{38,42} Then endocytosis of Dsg3 and thus a depletion of Dsg3 from the cell surface takes place.\textsuperscript{38}

An article by Ruocco et al from 2013 states that there must be additional auto-antigens in human pemphigus vulgaris (hPV), besides Dsg1 and Dsg3. Otherwise, when both, anti-Dsg1 and anti-Dsg3 antibodies, would be present the epidermis would completely disintegrate. They suggest the keratinocyte acetylcholine receptors as a non-desmoglein pemphigus antigen. The receptors play an important role in the mediation of intercellular adhesion and in particular the expression of desmoglein. Again, similar to the findings of the varying immunostaining-patterns of affected the dogs, it is likely that different subsets of auto-antibodies are involved, which would explain the variety of clinical and biological symptoms.\textsuperscript{31}

As described earlier pemphigus vulgaris in humans has a mucosal-dominant type, caused by anti-Dsg3 autoantibodies and a mucocutaneous form with anti- Dsg1 and -3 autoantibodies. The mucosal-dominant type can evolve into the mucocutaneous form when autoantibodies against Dsg1 develop aswell.\textsuperscript{14} Based on those findings the "desmoglein compensation theory" emerged. This theory states that one isoform of desmoglein can compensate the loss of another.\textsuperscript{84} This theory, or hypothesis, would explain why there is no blister formation in the epidermis in the mucosal-dominant PV form. The auto-antibodies attack Dsg3, but Dsg1 expression in the basal layers can compensate for this loss of function and keeps the epidermis intact. Blistering would, according to this theory, only occur when both, Dsg1 and Dsg3 get attacked.\textsuperscript{14} Thus there are only superficial lesions in PF, since adhesion in the basal epidermal layer, is maintained by Dsg3.\textsuperscript{38} This hypothesis is however not completely conclusive. In November 2014 a case was reported of a human patient with suprabasilar acantholysis, but was at the same time found positive for Dsg-3 and negative for Dsg-1 antibodies.\textsuperscript{85} According to this hypothesis also Dsg4 should be able to compensate for the loss of the other isoforms, which is apparently not the case.\textsuperscript{14}

It is evident that the responsible immunopathogenesis is not yet clearly elucidated. Many investigations offer however valuable clues. The current research shows a huge variety of cell biologic processes that might be involved. Some of them are primarily pathogenic; others only trigger a cascade of mechanisms leading finally to acantholysis. Some components are essential, others add up to the clinical signs.\textsuperscript{38}
2.4 DRUG-RELATED PEMPHIGUS

2.4.1. Introduction

There seems to be a condition of cPF which occurs as a response to drug therapy. Drug related pemphigus may be further divided in drug-induced and drug-triggered PF.9,66 The first one is a transient form of pemphigus in which clinical signs disappear after discontinuing the drug. Conversely, the latter is permanent.19,66 Several drugs have been associated with a PF outbreak in dogs so far. The majority of the reported patients developed localized lesions, whereas only a small percentage showed generalized lesions. More than half of the dogs also developed systemic signs (lethargy, fever, anorexia, pain and lameness). Interestingly, most of the dogs were of large breeds.87 Drug-related PF is clinically, histologically and immunologically similar to the naturally occurring form of canine pemphigus foliaceus. The history helps to differentiate naturally occurring PF from drug-related PF.67 In the drug-related form the dog must have received the inducing drug, there is an unusually fast onset of clinical signs, an unusually early onset and unusual clinical characteristics (e.g. oral lesions).86 Most of the drugs related to cPF outbreaks were insecticides. In humans different pathomechanisms are suggested on how pesticides (including insecticides) induce PF. The proposed pathomechanisms include an alteration of the keratinocyte membrane biochemistry and/or an interaction with the immune-system.67 Many of the described drugs contain a thiol-group, which might be an indication.31 One theory describes the blocking of keratinocyte nicotinic acetylcholine receptors and thus inhibiting signaling which is important for cell-cell adhesion in the epidermis.69 Another possibility could be a modification of the skin by the drug and thus creating neoantigens and secondary auto-antigens.88,89 Other theories include an increased cytokine production or an alteration in enzyme activity that are involved in cell-adhesion.90

2.4.2. Trimethoprim-sulfonamide

Trimethoprim together with sulfamethoxazole, sulfadiazine or sulfamethoxypyridazine may induce pemphigus foliaceus8, 27, 87, 91, 92 as well as other skin diseases, such as erythema multiforme or perforating folliculitis.93 Once the administration stopped the lesions resolved within a few weeks.27

2.4.4. Promeris Duo®

Promeris Duo® (Zoetis Animal Health) is an insecticide, containing metaflumizone94 and amitraz. It is associated with currently 22 cases132 of drug-triggered as well as drug-induced PF.86-88 Promeris is no longer on the market. The lesions strongly resemble those triggered by Certifect®. Systemic signs were reported in both entities, however, it was more frequently described in Promeris-triggered PF (PTPF; 64%) than in Certifect-triggered PF (CTPF; 43%). 91% of the dogs were large breeds and bitches were affected more frequently (68%). Also here the majority of the patients (86%) reached
complete remission. Just as in natural PF (82%), most sera of CTPF (71%) as well as PTPF (75%) dogs contained anti-Dsc1 IgG's.67

No distinct differences between the histopathology of the skin lesions of CTPF, PTPF and natural PF were identified. The exact trigger or component of the drug (amitraz, metaflumizone, fipronil, the vehicles or a combination) causing the eruptions is not yet known but the clinical, histological and immunological similarities imply a common pathogenesis with natural PF.67

Promeris and Certifect both comprise amitraz, but amitraz has been widely used against other ectoparasites such as Demodex or Sarcoptes mites without inducing PF.67 Given the large number of amitraz treated dogs and the relatively low number of PF-outbreaks, it is likely that also other factors influence the disease outbreak, such as the genetic background, environment, immunological or hormonal status.67 Another interesting finding is that lesions developed in 67% of the affected dogs within two weeks. The production of auto-antibodies however takes a minimum of three to four weeks after primary sensitization.67 Moreover, recent articles describe contact-triggered PF also after the application of another antiparasitic drug that does not contain amitraz but even other components (Vectra 3D®).66,87

2.4.3. Certifect®: Fipronil-Amitraz- S-methoprene

Certifect® (Merial) was released in 2011 as an ectoparasiticide which contains fipronil, amitraz and S-methoprene. It has been found to provoke acantholytic pustular dermatitis in some patients.67 21 cases were reported to date.67 It shows a close clinically, histologically and immunologically similarity to the naturally occurring form of canine pemphigus foliaceus.67 Most of the affected dogs were larger breeds and middle-aged or older.67 There were more females affected (71%) than male dogs. Systemic signs were exhibited in 43% (n=9) of the dogs. In 29% (n=9) the lesions were limited to the application site, while the rest of the affected canines also showed distant lesions. In 33% of the dogs one application of the drug was sufficient for clinical symptoms and 29% developed symptoms after the second application. IgG autoantibodies were found in the sera with Desmocollin-1 being the main target antigen (79% of the cases, n=11). Canine desmoglein-1 was found not to be targeted at all. Those findings suggest that this new ectoparasiticide is capable of triggering PF. Complete remission was reported in most of the dogs (81%). Due to its complex composition it is not possible to identify the actual pathogenic agent.67

In humans pesticides are also suggested as PF-triggering factors.68

2.4.5. Vectra 3D

Vectra 3D is used as a flea preventative and contains dinofuran, pyriproxyfen and permethrin.67,86,96 The insecticide has been reported to trigger PF-like lesions in three dogs to date.87,96 Cytology and skin biopsy revealed the same histopathological lesions as in naturally occurring PF.86,96
2.4.6. Others

Other drugs that are associated with a drug-related PF outbreak include cephalexin, oxacillin, and polymixin B. Additionally, a puppy with juvenile cellulitis was treated with amoxicillin-clavulanic acid and topical oxytetracycline and developed ensuing signs of PF. Those symptoms resolved after drug withdrawal.

2.5 THERAPIES

2.5.1. Introduction

Pemphigus foliaceus is based on auto-reactive processes. Consequently most treatments contain a component that suppresses or modulates the immune system. The most popular and first-line choice of drugs are glucocorticoids. Considering the side-effects of corticosteroids, a general consideration should always be to keep the patient on an as low as possible dose, even though that could mean a few remaining lesions. Besides glucocorticoids, azathioprine, cyclosporine, chlorambucil, tetracycline and niacinamide are most commonly used for the treatment, according to Rosenkrantz. There are further drugs that can be used additionally to glucocorticoids or as a substitute. Possible reasons to use second or third-line drugs are unacceptable side-effects, sparing of glucocorticoids or an insufficient response with glucocorticoids.

2.5.2. Most commonly used drugs in the treatment of cPF

2.5.2.1. Corticosteroids

Historically the standard treatment for cPF was an immunosuppressive therapy with oral glucocorticoids at daily dosages from 2 to 6.6mg kg\(^{-1}\). When the lesions diminish the dose and/or administration frequency is reduced, preferably to an alternate day intake. In some patients however, immunosuppression alone did not lead to the desired remission. In those cases corticosteroids are combined with cytotoxic or other alternative drugs. Frequently used cytotoxic drugs are azathioprine, cyclophosphamide or chlorambucil.

Topical glucocorticoids can be used in localized lesions and if necessary in combination with systemic therapies. Glucocorticoids with different potencies are available. It is advised to initially use an immunosuppressive dosage, for example 2.2-4.4mg kg\(^{-1}\) of prednisone or prednisolone daily. If adequate response is achieved after two weeks it is possible to switch to a less potent glucocorticoid and/or gradually reduce this dosage over a period of 30 to 40 days. If no significant improvement or unacceptable adverse reactions appear after ten to 14 days it is possible to add another immunosuppressive drug to the treatment regime. The final goal is to reach a dosage of 1mg kg\(^{-1}\) on an alternate day basis or less.
A systemic glucocorticoid therapy is however the most used approach for cPF. Rosenkrantz describes in his article that he prefers to use methylprednisolone due to less mineralocorticoid effects and thus less polyuria and polydipsia. Furthermore, some patients respond better to the latter. Much more potent glucocorticoids (six to ten times stronger) are triamcinolone and dexamethasone. Both are administered orally. Those can be used in more persevering cases, in dogs with extensive polyuria and polydipsie or changed behavioral patterns. The starting immunosuppressive dosage for triamcinolone is $0.2 \text{ to } 0.6 \text{mg kg}^{-1}$ daily and $0.2 \text{ to } 0.4 \text{ mg kg}^{-1}$ for dexamethasone. For maintenance it is sufficient to give the drugs every third day, since they suppress the hypothalamic-pituitary-adrenal axis for 24 to 48 hours. The maintenance dosage for triamcinolone lies between $0.1 \text{ to } 0.2 \text{mg kg}^{-1}$ every second or third day, whereas for dexamethasone only $0.05 \text{ to } 0.1 \text{mg kg}^{-1}$ is advised every second or third day as well.

Glucocorticoids affect many cells and tissues of the body, thus initiate a wide range of changes that involve different cell types simultaneously. Their main effect is anti-inflammatory as well as immunosuppressive. Long-term administration can influence the blood count. More specifically it results in neutrophilic leukocytosis together with eosinopenia, monocytopenia, and lymphocytopenia. One of the main anti-inflammatory mechanisms is the hindrance of the neutrophils and monocytes to migrate to the inflammation site. Granulocyte function is relatively less impeded in comparison to the monocyte-macrophage function which seems to be particularly sensitive to corticosteroids. Another article also describes the inhibition of inflammatory mediators and suppression of autoantibody levels.

Another observed side-effect is a transient lymphocytopenia of all detectable lymphocyte subpopulations. That is explained by a redistribution of the circulating lymphocytes. In some species (mouse, rat, rabbit) the lymphocytopenia was also caused by induced cell-death. In humans that phenomenon was only seen in immature or activated T-cells, but not in resting T-cells. Glucocorticoids are steroid hormones that can easily diffuse through the cell membranes when in their free form. In the cytoplasm they bind, with high affinity, to cytoplasmic glucocorticoid receptors. The formed ligand-receptor complexes migrate into the nucleus where it can modify the transcription of specific genes that encode proteins responsible for the action of the glucocorticoids. In normal T-cells, when antigens bind to the T-cell receptor, a phosphate group is added to the tyrosine amino acid of several intracellular proteins. As a result protein kinase C (PKC) is activated and also the intracellular calcium concentration rises. These two events (PKC and calcium increase) are necessary for the transcription factors to bind to the IL-2 gene promoters and initiate the transcription. The messenger RNA (mRNA) then translocates to the cytoplasm where it is either translated by ribosomes to the IL-2 protein or degraded by RNAases. Glucocorticoids are capable of intervening with several of these steps of the T-cell cycle. They inhibit the tyrosine phosphorylation; they inhibit calmodulin kinase II, an enzyme of the calcium pathway and they inhibit the binding of transcription factors to transcript and later translate IL-2 mRNA to effective proteins. Furthermore there is an increase in mRNA degradation.
Cytokines are essential for the proper functioning of T-cells. IL-2 in particular promotes T-cell proliferation and generation of effector, suppressor, and cytotoxic functions. Consequently, the generation, proliferation, and function of helper and suppressor T cells are depressed by these drugs.105 Cytotoxic T-cell responses are also impeded by moderate-to-high doses of glucocorticoids because of the blockade of cytokine expression and to a lesser extent because of the lysis of reactive T-cell clones.106

Generally speaking the cellular immunity is more susceptible than the humoral immunity, which means that the B-cells are relatively resistant to the immunosuppressive effects of glucocorticoids. These drugs inhibit the proliferation of B-cells but have a minimal effect on the transformation of B-cells into active immunoglobulin-secreting plasma cells. Their major effect on B-cells is on the antibody titre. Low doses of glucocorticoids have no significant effect on the titre, however, daily high-doses result in decreasing immunoglobulin levels with the greatest suppression after two to four weeks.107 The suppression is the result of an initial elevated catabolism followed by reduced production. Another reason is the impeded production of cytokines and the decreased activity of helper T-cells that play an essential role in the activation of B-cells.100

Side-effects are commonly seen in long-term treatments with corticosteroids (daily for >14days) and more frequently with the more potent derivatives. Described side-effects of topical treatment include atrophy, alopecia and localized pyoderma. By licking or dermal absorption systemic effects are also possible. In systemic therapies with strong glucocorticoids iatrogenic hyperadrenocorticism is described 27 with cushingoid and diabetic effects. Due to those hormonal alterations, polyphagia, muscle atrophy, a poor hair coat, weight gain, calcinosis cutis, hepatomegaly, and panting can be seen regularly. 102 The glucocorticoids have varying mineralocorticoid effects resulting in polyuria and polydipsia. Since being an immunosuppressive drug, patients are also more prone to infections, such as secondary bacterial skin infections, cystitis or demodicosis and experience delayed healing.102 Reported are also gastrointestinal side-effects, such as ulcerations, pancreatitis, vomiting and/or diarrhea.27 Besides the physical side-effects, also behavioral changes are observed. Some dogs are described to be lethargic, others become more aggressive or restless. 108 Due to the side-effects especially on the blood count, monitoring including complete blood counts, chemistry profiles, urinalysis and urine cultures should be performed every six months.27

The prognosis after a glucocorticoid therapy alone is moderate. Several studies have been conducted to analyze the treatment outcome and gained similar results. Ihrke et al registered a positive outcome with corticosteroids alone in 39% of the examined PF patients33. Rosenkrantz and colleagues documented 35% of the PF cases to be adequately controlled with only glucocorticoid therapy27 and Mueller and his team reported complete remission with only glucocorticoids in 38% of the patients within 1.5-12months after treatment-begin (average 7months).99
2.5.2.2. Azathioprine

Azathioprine is an immunosuppressive drug and can be administered solely, in combination with glucocorticoids or with other immunosuppressive drugs.\textsuperscript{27} It is the first choice to be added to the glucocorticoid treatment regimen as a glucocorticoid-sparing agent.\textsuperscript{11} Azathioprine is a prodrug that inhibits DNA and RNA-synthesis by blocking purine-biosynthesis. Especially fast growing cells such as B- and T-cells are affected because of their lack of a salvage pathway which is necessary for purine biosynthesis. As a result the drug decreases lymphocyte proliferation, lymphocyte numbers and T-cell dependent antibody synthesis.\textsuperscript{109} The main side effects are myelosuppression\textsuperscript{110} and gastrointestinal symptoms such as vomiting, diarrhea and hepatotoxicity.\textsuperscript{111} Bone marrow suppression occurs after one to two weeks after therapy onset and is reversible. Rare side-effects comprise hepatic necrosis and pancreatitis.\textsuperscript{110} The advised doses are 1.5-2.5mg kg\textsuperscript{-1} every 24 to 48 hours. It shows a delayed onset and takes normally one or two months until clinical effects become visible.\textsuperscript{27} It is thus important to not stop or reduce the therapy too fast.\textsuperscript{11} Complete blood counts are recommended every two to three weeks for the first three months after therapy onset, subsequent monitoring should be carried out semi-annually.\textsuperscript{27} The success rate of the treatment with prednisolone and azathioprine together was reported in 55% of the cPF patients\textsuperscript{99}. Five dogs in this study did not react on prednisolone alone but underwent complete remission when both drugs were administered. Nevertheless, more adverse drug effects were described with this combination. The benefit is thus controversial.\textsuperscript{99}

2.5.2.3. Chlorambucil

Chlorambucil is an alkylating agent that interferes with the DNA-synthesis and is thus cytotoxic. It acts primarily in on B-cells and is considered a slow-acting immunosuppressive drug that takes two (to six)\textsuperscript{11} weeks before therapeutic effects can be seen.\textsuperscript{109} Reported side-effects include bone marrow suppression, hepatotoxicity and gastrointestinal distress which shows through vomiting, diarrhea and anorexia. Myelosuppression is nevertheless rather mild and occurs one or two weeks after the therapy onset.\textsuperscript{109} Chlorambucil is used as an alternative to azathioprine, solely when other therapies are not tolerated or in combination with corticosteroids and azathioprine in difficult cases.\textsuperscript{27} It is the first choice for cats together with glucocorticoids.\textsuperscript{11}

2.5.2.4. Chrysotherapy

To prevent extensive side-effects by the long-time administration of corticosteroids an alternative approach is described by administering goldsalts, such as aurothiomalate and aurothioglucose.\textsuperscript{27,33,34} Aurothioglucose is however no longer commercially available. Rosenkrantz found it effective for feline pemphigus, but not for canine pemphigus.\textsuperscript{27} The exact mechanism of action is not fully elucidated, but it works immune-modulating and anti-inflammatory\textsuperscript{10,27} A disadvantage is a long lag phase of ten to 16 weeks.\textsuperscript{27} It can be used solely or adjunctive with glucocorticoids, and orally as well as in form of an
injection. Adverse reactions to the therapy include bone marrow suppression, oral ulceration and glomerulonephropathy. Furthermore are the injections very painful and deep. One case is reported where two dogs died from a toxic epidermal necrolysis after a sudden change from azathioprine to aurothioglucose. Regular monitoring is advised. The monitoring should contain a complete blood count every two to three weeks, and biochemistry and urinalysis every four to six weeks for the first few months after start of the therapy and then repeated every three to six months.

2.5.2.5. Tetracycline and niacinamide

A few cases are also described where PF patients responded to tetracycline and niacinamide treatment. Tetracycline is a broad-spectrum antibiotic with anti-inflammatory properties. Niacinamide is part of the vitamin B-group, inhibits mast-cell degranulation and also phosphodiesterase. A common side-effect is an upset gastrointestinal tract that might result in diarrhea, increased liver enzymes, vomiting and anorexia. In those cases decreasing or discontinuing niacinamide can relieve the symptoms. The drugs have a delayed effect. Clinical benefits are thus not visible before one or two months after therapy onset. The dosage or the frequency of administration may then be reduced. The advised dosage for both are 250mg three times a day for dogs under 10kg and 500mg every 8hours for dogs above 10kg.

2.5.2.6. Mycophenolate mofetil

Mycophenolate mofetil is the prodrug of mycophenolic acid and interferes with the synthesis of de novo purine. T- and B- lymphocytes are especially sensitive since they cannot use the salvage synthesis pathway. This feature enables the drug to selectively inhibit lymphocyte proliferation and the production of antibodies. Further mechanisms include a suppression of dendritic cell maturation and decreased monocyte recruitment to the inflammation site. Adverse drug reactions include bone marrow suppression, being more prone to infections and gastrointestinal problems. Most studies on drugs are executed retrospectively and the drugs were originally developed for humans. A prospective clinical trial was, however carried out in a 16-week pilot study with mycophenolate mofetil (20-40mg kg⁻¹, 3 times daily). Of the eight examined dogs only three improved. Dosages between 22-39mg kg⁻¹ per day were used spread over three applications per day. Adverse drug reactions were minimal. Most commonly were pyoderma, Malassezia infestations, diarrhea and leukocytosis. Due to high costs and a lack of proven efficiency, this drug is not recommended as a treatment by the researchers.
Cyclosporine and Tacrolimus are immunosuppressive drugs that are extensively used in human medicine, especially in patients that received organ transplants. Both drugs have the same proposed mechanism of action and immunomodulating properties. Tacrolimus is however more potent and more toxic for dogs.

Both drugs bind to intracellular receptors called immunophilins. More specifically, cyclosporine binds to cyclophilin, while tacrolimus binds to FK506-binding proteins. Thereby, they inhibit calcium-dependent pathways including that of calcineurin. Calcineurin is a calmodulin-dependent protein phosphatase. This enzyme activates nuclear factor of activated T-cells (NF-AT) by dephosphorylation. NF-AT is a transcription factor of T-cells that is responsible for the initiation of interleukin synthesis. The activated NF-AT is translocated into the nucleus and upregulates interleukin-2 synthesis.

IL-2 is important for the proliferation and differentiation of T-cells. As a consequence cyclosporine causes a decrease in canine lymphocytes and immunosuppression. It also affects different cytokines, such as IL-2, IL-3, IL-4 and tissue necrosis factor alpha (TNFα). IL-2 is also responsible for lymphocyte proliferation. It furthermore modifies the function of granulocytes, macrophages, natural killer cells, eosinophils and mast cells. In a study from 2004 good results were achieved in two dogs within four to six weeks with 25mg kg⁻¹ per day. Another patient received 15mg kg⁻¹ whereupon the clinical signs subsided. When the severity however got worse the patient did not respond to higher doses.

A pilot study on cyclosporine carried out by Olivry investigated whether cyclosporine A (CsA) monotherapy (microemulsified CsA) would be effective for the induction of treatment in cPF. Of the five tested dogs only one showed mild side-effects (intermittent diarrhea). The used induction doses was 5-10mg kg⁻¹ administered daily. None of the dogs showed complete remission of the lesions. Four of the five dogs were even withdrawn due to worsening of the score based on the Pemphigus foliaceus extent and severity index (PEFESI). Based on those results cyclosporine is not recommended as a sole therapy in the proposed doses. It might nevertheless be effective in higher doses or in combination with oral glucocorticoids.

Cyclosporine can be administered orally in combination with ketoconazole or oral glucocorticoids. Ketoconazole inhibits the hepatic microsomal isoenzyme P450 system, resulting in increased blood cyclosporine levels, allowing lower dosages and thus reduced costs.

The reported side-effects of cyclosporine include anorexia, emesis and diarrhea, especially during the first days of treatment. More severe side-effects are rare and appear to be dose-dependent. Weight loss, nephrotoxicity, gingival hyperplasia, papillomatosis, hirsutism, hepatotoxicity, opportunistic infections, lymphoproliferative disorders and involuntary shaking are reported. Tacrolimus shows more extensive side effects than CsA when administered orally alone or in combination. Those adverse reactions include anorexia, vomiting, diarrhea, weight loss, impaired glucose metabolism, marked hepatotoxicity and infections. Nevertheless no side-effects and good
results have been achieved in discoid lupus erythematosus and pemphigus erythematosus when tacrolimus was administered topically (0.1%).

2.5.2.8. Cyclophosphamide

Cyclophosphamide is also an alkylating agent. It interferes with DNA replication by adding an alkyl-group to DNA. It is used, among others, as a cancer therapy agent and against auto-immune disorders. It can be used solely or in combination with other drugs. The drug is very potent and shows thus severe side-effects such as hemorrhagic cystitis, anorexia, vomiting, diarrhea and weight loss. Because of those undesired reactions and sufficient alternative therapies it is not used by default. The recommended dosage is 1.5mg kg\(^{-1}\) every alternate day.

2.5.2.9. Dapsone and sulfasalazine

Dapsone is an antibiotic with anti-inflammatory features. It inhibits neutrophil chemotaxis, reduces complement activation, antibody production and lysosomal enzyme synthesis. As described earlier are the lesions in dogs, contrary to the human form, infiltrated with neutrophils and is dapsone thus more effective in dogs. Dapsone should be given 1mg kg\(^{-1}\) every eight hours. The drug shows a delayed effect after one to two months. The following adverse reactions are described in dogs: anemia, neutropenia, thrombocytopenia, hepatotoxicity, gastrointestinal signs, neuropathies and cutaneous drug eruptions. Therefore close and regular monitoring of the thrombocytes, chemistry profiles and urinalyses are recommended.

Sulfasalazine metabolites have anti-inflammatory features as well. It is dosed at 10-40mg kg\(^{-1}\) spread over the day, thus one dose every eight hours. A big disadvantage is the risk on developing keratoconjunctivitis sicca. Therefore it is recommended to monitor tear production as well as blood counts and chemistry profiles regularly. Tear production is best monitored every two to four weeks, blood counts and chemistry profile initially (first 1.5 months) every two weeks and then reduced to every two to four months.
3. WHY ARE SOME DOGS NOT RESPONSIVE TO THE TREATMENT

3.1 TREATMENT OUTCOME AND LONG-TERM PROGNOSIS

Even after a verified diagnosis of cPF there seems to be quite some variation regarding the treatment outcome and the long-term prognosis. Different studies reported different outcomes. It is assumed that this might be due to different treatment approaches as well as due to a variation of clinical signs and disease severity.

In a study from 1985, 37 dogs with PF were examined and followed-up for one to seven years. The dogs were treated with different drug regimens with the following outcome: 39% responded to corticosteroids solely, 50% responded to prednisone and cytotoxic drugs and 55% reacted to prednisone in combination with aurothioglucose. 47% of the dogs died in less than one year.

A more recent study that was carried out between 1994 and 2000 with 43 cPF patients aimed at identifying the factors that can influence the outcome of cPF. The researchers recorded a case fatality rate of 60.5%. The majority (60%) of the dogs that passed away died within the first year of treatment. Interestingly, the survival rate increased when antibiotics were administered in the beginning of the immunosuppressive therapy and also lowered the number of patients with adverse drug effects. That could be explained by the patients being less susceptible to secondary bacterial infections. What is more, the researchers detected a positive correlation between the duration of the treatment and the survival rate. 92% of the dogs from the non-survival group had died within one year after treatment initiation, but only two died after twelve months. That proposes a better prognosis for survival after a certain period of time. The median of death in the non-survival group was ten months. Also the number of complications shows a significant association with the survival rate.

Of the dogs that died 69% (n=18) were euthanized, four dogs for causes unrelated to PF. Most of the euthanized patients underwent two to four changes in treatment protocol, due to unsatisfying results or unacceptable adverse drug effects, before deciding to euthanize the patient.

Remarkably, four of the treated dogs stayed in prolonged remission with no further clinical signs of PF after discontinuation of the treatment. Also, there was no significant link between initial treatment protocol and survival time or rate. The used initial treatment protocols comprised prednisone (n=16), prednisone together with azathioprine (n=23), mycophenolate moftil (n=2), tetracycline and niacinamide (n=1) and prednisone in combination with chlorambucil (n=1). However, due to the high-incidence of corticosteroid-related side-effects and the great amount of deaths within the first year the authors advice to choose a combination treatment to minimalize the required corticosteroid maintenance doses. However, Mueller et al described contradictory that the combination of
glucocorticoids and azathioprine causes more side-effects in cPF patients. The benefit of a combination therapy is thus controversial.

Three other studies, one by Rosenkrantz and colleagues, one by Mueller and colleagues and one by Kummel reported a more positive outcome. Rosenkrantz reported 71% of the patients still being alive after one year of treatment. Mueller and colleagues examined 91 dogs, whereof only 13% (n=11) were euthanized. And Kummel documented a 75% survival rate after treatment with no or few side-effects. Rosenkrantz stated, similar to the findings by Gomez, that there was no significant difference in rate or degree of remission between dogs that were treated with prednisolone alone or together with azathioprine. However, contrary to Gomez Rosenkrantz also reported that there is no association between the survival rate and the use of antibiotics in combination with immunosuppressive drugs.

Prolonged remission is described in a small number of cases after discontinuation of the immunosuppressive therapy. In a study by Olivry et al were either treated with immunosuppressive doses of oral glucocorticoids or with glucocorticoids together with azathioprine. Clinical remission occurred after 1.5 to 5 months after initiation of the therapy. The doses was progressively reduced and finally withdrawn. The total period of administering immunosuppressive therapy ranged between three and 22 months. In six dogs clinical symptoms did not reappear for the duration of the trial (1.5 to 6 years). This study suggests that a relapse of the lesions does not necessarily occur after withdrawal of the drug and that in some dogs immunosuppressive therapy can lead to a long-term remission of the lesions. 51 dogs met the criteria for the research and six of those went into complete and long-term remission (12%). In a study investigating long-term outcome of treatment in humans with PV it was found that patients with mild to moderate PV were twice as likely to undergo complete remission compared to those with severe lesions. A similar research by Mueller and colleagues found that the distribution of the lesions, localized versus generalized, had no effect on resolving of the lesions or death and that 7-22% of the affected dogs stayed in remission after ending the immunosuppressive therapy. Another finding by Olivry et al was that patients with a rapid response to the immunosuppressive therapy were more likely to benefit from prolonged remission. In contrast, in dogs the severity of the disease appears to play no major role in long term remission. The average time to undergo complete remission after initiating the therapy was two months.

3.2 POSSIBLE REASONS WHY SOME DOGS ARE NOT RESPONDING

To the best of our knowledge, there are no studies investigating why a conspicuous number of dogs are unresponsive to the treatment. Nevertheless, research is giving some hints why some cPF patients might not be responsive.
3.2.1. Incorrect diagnosis

A possible cause could be an incorrect diagnosis. As described earlier, pemphigus foliaceus is an heterogeneous disease with a wide variety of clinical features. It is thus important to rule out other differential diagnostics for pustular dermatitis, such as pustular dermatophytosis.\textsuperscript{126}

3.2.2. Naturally or drug-related pemphigus

In those cases where the history is unknown, it is impossible to determine with certainty if PF is naturally occurring or if it is induced or triggered by drugs. To verify the diagnosis a drug provocation test should be done. This provocative test is carried out rarely due to the potential risk for the patient. The diagnosis of drug related PF is thus based on history, clinical signs, histopathology and the response to drug withdrawal.\textsuperscript{126} Rosenkrantz states in a report that many cases that are actually drug induced, have been misleadingly accredited to chronic diseases and have often been treated with long-term drugs.\textsuperscript{128}

3.2.3. Drug regime

In the study from Olivry et al investigating the long-term remission, it becomes obvious that there is no static therapy regime and dosage. Each of the patients received a different drug therapy and dosage depending on the individual response. In the six studied cases patient one did not react to initial prednisone therapy, therefore azathioprine was added and the lesions resolved. Case number two showed marked improvement on prednisone alone. The third and fourth case required prednisone as well as azathioprine before clinical remission occurred. Case five and six were initially treated with only prednisolone and due to unacceptable adverse side-effects prednisolone dosage was reduced and azathioprine added with a positive outcome. Also, each dog received a different dosage of glucocorticoids (and azathioprine) which was adapted if necessary. For a satisfying outcome it is thus important to find the right dosage, frequency of administration and the right combination of drugs (antibiotics, corticosteroids, chrysotherapy, azathioprine etc or combination of those?) for each patient individually.\textsuperscript{126}

Especially for the chrysotherapy, dapsone, azathioprine and tetracycline and niacinamide a lag-phase is described. Depending on the used agent it is important to not stop the therapy after a lack of response too early.\textsuperscript{27, 109}

3.2.4. Glucocorticoid resistance

Glucocorticoids are the first-line therapy for the treatment of pemphigus diseases yet some patients do not respond. That might be due to the development of resistance. To investigate glucocorticoid resistance in PF patients a study evaluated the glucocorticoid sensitivity. The sensitivity was analyzed on the basis of the number of binding sites, the affinity of glucocorticoid receptors to dexamethasone and the cytokine pattern. The number of binding sites, as well as the affinity of the receptors were
found to be significantly higher in pemphigus patients compared to a control group. Furthermore the study showed a higher production of pro-inflammatory cytokines in human PF patients, especially of IL-6 and TNFα. In summary, the study showed a pro-inflammatory cytokine profile and altered glucocorticoid sensitivity in PF patients. That might be an indication that pro-inflammatory cytokines play a role in developing glucocorticoid resistance in human pemphigus patients. 

Another proposed explanation for the insufficient response could be an increased expression and/or function of P-glycoprotein in lymphoid cells. The protein is located in the cell membrane and by being able to pump foreign substances out of cells it can influence the bioavailability of the drugs. These proteins might thus be capable of reducing the intracellular concentration of glucocorticoids, thereby weakening its therapeutic effects. Research however found neither increased p-glycoprotein levels in pemphigus patients that responded poor to steroids, nor differences in p-glycoprotein activity. Consequently, p-glycoprotein does not seem to be involved in the poor response to steroid treatment seen in some pemphigus patients.

3.2.5 Influence of 25-hydroxyvitamin D concentrations

The active form of vitamin D is well known for its properties of regulating calcium levels in the blood and assuring bone health. As described earlier, calcium is also essential for the functioning of glucocorticoids and has an impact on the expression of cell surface molecules. Recent research revealed further effects of vitamin D on the immune system, especially on T-cells. It has been proven in animal models that 1,25-dihydroxyvitamin D3 can prevent or suppress certain autoimmune diseases. A proposed mechanism therefore is an influence on the cytokine profile which might consequently suppress inflammatory T-cell activity. Another study investigated the relationship between 25-hydroxy-vitamin-D serum concentrations and the response to a subsequent glucocorticoid-therapy in dogs with atopic dermatitis. Interestingly, dogs that showed a a good response to the glucocorticoid treatment also had significantly higher pretreatment serum 25(OH)D concentrations compared to dogs with a poor response. That might be an indication that vitamin D has a synergistic therapeutic effect with glucocorticoids and that low vitamin D-serum concentrations might attenuate the response. Further research to confirm or refute this hypothesis is necessary.

3.2.6. Tachyphylaxis to topical glucocorticoids, is it proven?

Tachyphylaxis( the fast onset of tolerance) to topical glucocorticoids has been reported in literature and is an widely accepted belief. A study was conducted reviewing relevant published articles on the internet, to look for evidence to support this concept. Although they found 52 relevant articles they could not find proof that glucocorticoid treatments are susceptible to tachyphylaxis. Although some of the described effects indeed resembled tolerance effects, the clinical significance however is not known nor proven. The authors assume that the described phenomenon of tolerance is due to other factors such as poor adherence of the therapy over time or that the drugs have a maximum effect in the first weeks resulting in remission but only to a certain degree.
4. Discussion

In the last decades much research regarding canine pemphigus foliaceus has been conducted and much knowledge was gained. Nevertheless, compared with the human form of PF little is known about the pathogenesis and even less is proven. Many pathogenic factors have been associated with the disease and many possible pathological mechanisms proposed. However the exact pathogenic role and importance of most of them are still unclear or questionable. Some of the studies mentioned above even described contradictory results.

Not all the findings from human research can furthermore be extrapolated to dogs. For example cPF involves a strong neutrophilic infiltration of the pustules, which can only be found in seldom cases of human PF. The same applies to the therapies. Veterinary immunosuppressive therapeutics were initially adopted from human medication and research, and thus knowledge of their therapeutic efficacy and potential for adverse effects in dogs remains limited. Most of the studies are also retrospective, a blinded prospective study especially regarding treatment protocols could bring more clarity.

The majority of the canine patients reacts well to the treatment protocols and receives remission. A few of the patients however, initially or after a relapse, do not respond to glucocorticoids (anymore). Different reasons have been proposed but little research was conducted to investigate this issue and no satisfactory explanation can be given yet. Thus also here, more research is needed.

In summary, this literature study shows that cPF is a very interesting and complex disease. It has been getting an increasing degree of awareness among scientists and veterinarians in the last decades and much progress has been made. There are however, still many open questions that require further attention. Answers to the remaining questions about especially the immunopathogenesis could help to develop a more specific treatment with minimal side-effects.
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